AGROBACTERIUM-MEDIATED GENE TRANSFER: A LAWYER'S PERSPECTIVE

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Whether or not you agree with patent protection for *Agrobacterium*-mediated transformation technology or for other basic platform technologies, "the times, they are a-changing". In the United States, patents are awarded for many types of biotechnology inventions, including nucleic acid sequences, bacterium containing a vector construct, transgenic plants and methods of making transgenic plants. Both companies and non-profit institutes are affected by such patents. Here, some of the impacts of patents are discussed followed by a mini-primer on key points about patents and patent documents. In the final section, we present a patent landscape of *Agrobacterium*-mediated transformation of plants and discuss a number of key patents impacting research and development.

1. INTRODUCTION -WHY SHOULD A SCIENTIST CARE ABOUT A LAWYER'S VIEW OF *AGROBACTERIUM*?

The legal system is often viewed by the public as inaccessible and incomprehensible; in addition, scientists may feel as if patents and other legalities are being foisted on them. Readers of this book may well just want to get on with their research and wish that patents would just "go away"; some may even resent or ignore patents for a variety of reasons. While we empathize, the reality is that patents are not only here to stay, but are increasing in number (*Figure 20-1*) and importance for basic science research and researchers – for better or for worse.

As a scientist in the 1980s and early 1990s, one of us - Nottenburg - knew precious little about patents. Because her appointment was in a non- profit research institution, patents were not on any list of "things to worry about". When confronted with a material transfer agreement from a company for obtaining a reagent, she read it but with a fair bit of uncertainty and lack of understanding. Luckily, her institution had an Office of Technology Transfer (OTT) that could help, especially in negotiating away a clause that endangered independence in controlling the project's outcomes. Even more luckily, a colleague told her about OTT because the administration hadn't even alerted the faculty of its existence, let alone its services.

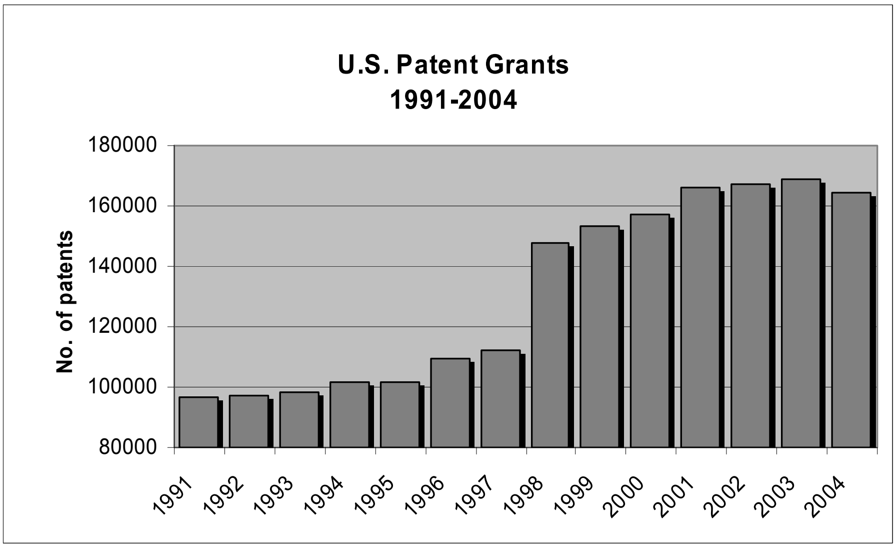

Figure 20-1. U.S. Patent Grants by Year

Since then, a number of substantial and widespread changes has taken place, notably increased patenting and commercialization of biotechnologies and a bigger presence of OTTs in universities and research institutes. Whereas, for most of Nottenburg's scientific career the world of patents and other intellectual properties (IP) in biotechnology belonged primarily to the realm of private sector, now public and private non-profit institutions are increasingly involved in this legal world. For example, during the five-year period from 1981-1985, a mere 0.59 percent (1,887) of United States patents were granted to inventors who assigned to an entity whose name contained "University", subsequent five-year periods saw the percentages (and number) of patents steadily climb to 2.15 percent (13,940) for the latest period from 1996-2000 (Nottenburg et al. 2002).

What hasn't substantially changed though is a scientist's knowledge and understanding of patents and related law areas. Knowledge and understanding are not to be confused with general awareness of patents or the use of legal "buzzwords", which certainly has substantially increased.

> "You can know the name of a bird in all the languages of the world, but when you're finished, you'll know absolutely nothing whatever about the bird... So let's look at the bird and see what its doing – that's what counts. I learned very early the difference between knowing the name of something and knowing something." - Richard Feynman

Our advice to scientists is to learn enough about patents to be able to use the system to your advantage. This doesn't mean that you have to become one of "them", but by participating in the patent system you become empowered to use patents as a tool to help accomplish your goals.

So why should scientists know something of patents? Because more emphasis is being placed on commercial realization of scientific research, because patents are a type of scientific literature, and because infringement can be costly.

1.1 Commercialization of research results

The emphasis on commercial realization of scientific research is readily apparent in both for-profit and non-profit settings. The terms "for-profit" and "non-profit" are used to indicate the main function of the referred-to entity. While "for-profit" institutions are almost always private companies, "non-profit" institutions include both public entities (e.g., state universities) and private entities (e.g., research institutions). In many fields, including agriculture and health sciences, patents play a key role in R&D and product development. Since the Bayh-Dole Act was enacted in 1980, non-profit organizations have increasingly pursued patents and commercial partners for exploiting their patents. Specifically, the Bayh-Dole Act ceded federal interest in ownership and licensing of inventions to institutions receiving federal funding. Analyses have documented that Bayh-Dole was a major contributor to the substantial growth of technology industries, especially in biotechnologies (Mireles 2004; Press and Washburn 2000; Wolf and Zilberman 2001). According to the Association of University Technology Managers (AUTM), from 1980 to 2004, more than 4500 companies total (with 462 companies in 2004 alone) have spun out from research in U.S. universities, hospitals and research institutes (AUTM 2005). Clearly, the Bayh-Dole Act has irrevocably altered the face of universities and moved them into a closer relationship with the corporate world. And part and parcel of this closer relationship are patents. The AUTM Surveys report that since 1993, institutions participating in the surveys have received a total of about 34,500 U.S. patents. In 2004 alone, 183 institutions filed over 10,500 new patent applications, a 16.7 percent increase in filings over 2003 (AUTM 2005).

In addition, some granting agencies now require the grant recipient to provide an analysis of the patents that may affect commercialization of the research subject matter. For example, several of the Research and Development Corporations in Australia (non-profit corporations funded under a Federal Act) request such assurances. For the agricultural R&D Corporations in Australia part of their mandate is to invest in research for the greatest benefit to their stakeholders: the farmers and the Commonwealth. As an exporter of agriculture products, Australian industries must be sensitive not only to domestic intellectual property but also to that of other countries. In Nottenburg's experience at CAMBIA (an Australian non-profit research institute focused on agricultural biotechnology and patent resources), grant applications from two different R&D Corporations required disclosure of any patents that could affect the ability of research results to be commercialized. As well, for certain of its grants, the Gates Foundation also requires due diligence - an investigation into patents that might limit the ability to practice the expected research outcomes.

A third avenue of pressure driving non-profits to patenting is the possibility of financial returns from research back to the organization. Pressure is driven at least in part because of the on-going issues regarding federal funding for science – a mere 2% increase for National Institutes of Health (NIH) from FY 04 to FY 06 (NIH Office of Budget) and no change for National Science Foundation (Meeks 2005, NSF 2005) from FY 04 to FY 06. A continuing climate of funding uncertainty may motivate an increasing emphasis of non-profit organizations to seek financial returns from research, including reimbursement for legal fees (AUTM 2005).

Non-profit institutions that obtain patents typically realize a return on this investment by selling (or licensing) its patent rights to others, such as existing commercial entities, new spin-off companies, or other research institutions. Some highly publicized patents that have been licensed widely have generated a very substantial income for the host institution. For example, Stanford University received over $254 million from licensing the now-expired Boyer-Cohen patent claiming basic recombinant DNA technology; this single patent generated over half of its total licensing income (Feldman et al. 2005).

Furthermore, federal funding for research is shifting to applied research. In a 10-year period from 1993 to 2003, funding for basic research in life sciences (including agriculture) increased 2.4-fold while funding for applied research increased 3.5-fold (NSF

SRS 2004). Applied research is necessarily more commercially oriented, which goes hand-in-hand with filing patent applications.

For commercial entities, patents and determining freedom to operate is not a new story - it's a necessary part of a business plan. Large companies are usually patent-savvy, while small to medium sized businesses may lack the necessary legal skills and often don't avail themselves of publicly available patent information (EPO 2003). Most telling is that larger companies were more likely to have access to patent databases or services. Yet, more than 80% of all companies consider the information in patents to be important or very important. Thus, not only scientists, but also science and business administrators in both public and private sectors, will be well-served to have at least some practical understanding of patents.

1.2 Advantages for scientific research

Scientific research can benefit from patents, apart from providing protection for inventions. An often overlooked benefit is that patent documents comprise a substantial body of scientific literature. Companies especially do not always publish in traditional scientific journals. For example, a search of United States granted patents since 1976 for those owned by "Monsanto" and containing the term "*Agrobacterium*" yielded 208 results, while the same search terms applied to PubMed yielded only 17 results. Furthermore, patent documents contain very detailed descriptions of experimental methods and materials, whereas, journal articles may be very skimpy on the details. Rather than trying to deduce for example the structure of a particular vector construct, look for the corresponding patent document, and with luck, details of experiments and materials will be there. (but, *cf* Dam 1999, arguing that patent disclosure is often insufficient to practice the invention, and Lichtman et al. 2000.)

Unfortunately, recognition and comprehension of the importance of patents do not always coincide; many factors discourage use of patent information, including difficulty of access, amount of time involved, difficulty in reading documents, and inability to fully understand the information.

1.3 The myth of the "experimental use exception"

The right to use a patented invention for research is a concern in both non-profit and commercial settings. Many, if not most, university scientists assume that patent law does not apply to their basic research. Several factors seem to reinforce their perception, including academic culture, which typically is removed from legal concerns, and lack of guidance or even basic information and education from host institutions. Indeed, review of the patent or intellectual property policy documents of a number of United States universities revealed that while they all provided information about invention disclosures, patenting process, revenue sharing mechanisms and similar subjects, none of them discussed infringement of other's patents (or copyrights). Thus, academic researchers are often shocked to discover that, except for some very limited statutory exemptions that rarely apply to them, no general research exemption exists in the United States for using other people's patented technologies. In contrast to patents, the U.S. Plant Variety Protection Act (PVPA, 7 U.S.C. §2321 et seq.) provides for a research exemption. Under the Act, a protected variety may be used and reproduced in plant breeding or other bona fide research. The UPOV Convention (an international agreement that governs plant varieties), has a similar exemption.

In developed countries that have implemented an "experimental use" exemption for patents, such as Germany and Great Britain, the exempt use is for experimenting *on* the invention. Exceptions given for *using* the invention as intended in non-commercial settings are more likely to be found in developing countries (Commission on Intellectual Property Rights 2002). The difference between these types of experimental use are illustrated by two scenarios: "*experimental use of the invention*" – performing PCR (polymerase chain reaction) to verify presence or absence of a specific DNA sequence; and "*experimenting on the invention*" – testing different additives in PCR reactions to find one that markedly improves amplification.

The U.S. Congress has the authority to legislate a general research use exemption, but so far has only enacted a few very narrow exemptions in the medical field. In 1984, the Drug Price and Patent Term Restoration Act allowed drug companies, particularly those that market generic drugs, to proceed with pre-market approval testing of competing drugs or veterinary biological products during the life of the relevant patent. The use of patented herbicides to test new herbicide-tolerant cultivars, however, would not fall within this exemption. Without this exemption, pharmaceutical companies enjoyed an extension of exclusivity after patent expiration while generic manufacturers collected the data necessary for FDA approval. Implementation of the Act was meant to ensure that consumers received the advantages of generic drug prices. In addition, 35 U.S.C. § 287 (c)(1) grants an exemption to medical practitioners performing a medical or surgical procedure that would otherwise be an infringement. The exemption only applies to methods of treating human patients and does not apply to medical instruments or their use.

Except for the few legislated exceptions, the United States has a very proscriptive research use exception, so proscriptive that for all practical purposes, there is no exception for basic research in any kind of setting (e.g., university, non-profit research institute, commercial entity). In the course of the development of patent law in the United States, courts have repeatedly refused to find a general research or experimental use exemption, even in infringement actions against the United States Government, where there is a clear absence of a profit motive for using the patented inventions (*Pitcairn v. United States*).

In this landscape, the researcher at a university or other non-profit organization who uses a patented method or composition research is infringing, even if used without any overt profit motive. This point was hammered home in *Madey v. Duke University*, in which use of a patented laser device for research, academic, or experimental purposes at a non-profit university was held as an infringing use. The decision followed a line of earlier cases in which using an invention for furthering legitimate business purposes is infringing conduct. With respect to a university, its "legitimate business objectives include educating and enlightening students and faculty participating in the projects" and serve "to increase the status of the institution and lure lucrative research grants, students and faculty." Thus, sounded the death knell of an experimental use defense – until such time, if ever, that Congress enacts an exemption.

Do not panic, however. There appears to be a *de facto* exemption (an exemption based on reality rather than based on law) in the United States. The number of patent suits filed in United States District Courts against non-profit organizations is extremely few, so few that Congress does not believe that universities suffer a high or actual risk. In 1990, the House Committee on the Judiciary, which has jurisdiction over patent matters, recommended a broad research exemption (House of Rep. 1990), but in opposing the exemption, one Representative questioned the need for the exemption, challenging universities to come forward to show how the existing patent law was harming them (*ibid*). We assume that the evidence simply was not there because the exemption was never passed. Moreover, in the United States, the 11th Amendment of the Constitution protects State institutions from being sued in federal courts unless they consent to the suit or implicitly waive their immunity. Although Congress has attempted several times to make a law that removes State immunity from patent infringement actions, none of the

Acts has passed muster in the U.S. Supreme Court (*Florida Prepaid 1999*). Although eventually Congress is likely to succeed in passing legislation that will abrogate States' rights and withstand the scrutiny of the Supreme Court.

Even in the absence of a research exemption, non-profit organizations likely have only a very minor risk of patent infringement exposure. It would be poor public relations for a patentee company to sue a non-profit organization for infringement, and it is likely that a jury would sympathize with the defendant. In addition, the type of remedy imposed is unlikely to be severe from the institute's point of view. In *Roche Products v. Bolar Pharmaceutical Co.*, a key experimental use exemption case, the patent owner urged that the data generated during the infringing activity be confiscated and destroyed. The Court however, expressed a preference for monetary damages and admonished that injunctions are an equitable remedy and by no means a mandatory remedy. Although difficult to predict with certainty, damages owed by a non-profit infringer would likely be limited, possibly to the cost of a license, as use of the technology within a non-profit organization would not generally cause a company to lose profits. Thus, weighed against the significant expenses of litigation, a corporation is unlikely to pursue such a suit except for very significant matters. Furthermore, patentee corporations stand to gain some advantages by having researchers do some of their research and widely adopt technologies that the corporation can then license. For example, CAMBIA owns rights to β-glucuronidase (GUS), which was widely used by researchers in non-profit organizations who ultimately moved to corporations and continued using GUS. While CAMBIA grants non-commercial research in non-profit settings a cost-free license, fees are charged for using GUS in commercial research.

Thus, although there is no research exemption for non-profit institutions, it is unlikely that infringement suits will be filed against universities and research institutes in cases where the nature of the research is clearly non-commercial.

1.4 Freedom-to-commercialize and anti-commons problems

Generally, the main concern voiced by scientists and other inventors is whether their great idea is patentable. For someone eating, breathing, and dreaming about the big breakthrough, patenting the invention assumes prime importance. Sad to say, almost always the most important issue is not whether the idea is patentable, but can you practice your own invention?

It often surprises people that someone else's patent may be more important than their own. That importance follows from the nature of patent rights, which are a grant to *exclude* others from making, using, selling, offering for sale or importing the patented invention. Or when the invention is a method, the rights additionally allow the patent holder to exclude others importing at least the product obtained directly by that process for the purposes of using, selling, and offering for sale. (Patent rights are set forth in 35 U.S.C. §271 and article 28 of TRIPs (Agreement on Trade-related Aspects of Intellectual Property Rights), which requires WTO member countries to provide essentially these same rights.) Note that none of these rights actually grants the patent holder a right to practice her own invention.

How is that possible? A trivial example illustrates this conundrum.

Figure 20-2. Pencils

Imagine that Company A owns a patent with this claim: *A pencil comprising No. 2 lead*. The pencil that is made and sold is shown to the left in *Figure 20-2*. Some time after this patent issues, another patent issues to Company B with the claim: *A pencil comprising (i) No. 2 lead and (ii) an eraser attached to one end*. An example of such a pencil is shown on the right in *Figure 20-1*. Company B cannot make, sell, etc. its pencil without permission from Company A because the claim in Company A's patent encompasses pencils with or without erasers. (This statement is true when a claim uses the term "comprising", which means that the elements listed in the claim are the minimum elements required. Additional, unnamed elements (e.g., eraser) are covered under the patent claim.) The reverse however, is not true: Company A does not need permission from Company B as long as A's pencils do not have both No. 2 lead and an eraser. Therefore, A's patent dominates B's patent. The result is that Company B has no freedom-to-commercialize without permission from Company A.

The thicket of patents that capture various aspects of a technology and that are required to practice the technology without infringing is sometimes referred to as "anticommons" (Heller and Eisenberg 1998). Under an anticommons scenario, resources (i.e., technologies) are prone to underuse because the technology is vested in the hands of multiple owners, each of whom have the right to exclude others at the same time that none has consolidated a right to use. This theory is by no means proven. A lively debate centers on whether or not there is an anticommons and, if so, its impact on research and development (Epstein and Kuhlik, 2004; Lichtman 2006; Mireles 2004; Stern and Murray 2005). In any case, the debate is about overall patenting trends and does not look at or define the patent thicket and extent of anticommons for a particular technology. Delineating the patent thicket for a particular technology requires exhaustive patent searching and claim analysis of the key patents. In the next section, we present a primer on patents to lay a foundation for the patent landscape analysis.

2. SOME BASICS ABOUT PATENTS

Remember that the patent owner's right is exclusionary: she may exclude others from making, using, selling, offering to sell, and importing the patented invention and importing a product made by a process that is patented in the importing country. To determine if someone is infringing a patent, the allegedly infringing product is compared to the claims.

2.1 Claims define the "metes and bounds" of protection

The *claims* are the most important part of a patent. Not the title, not the text, not the examples, and not the drawings. It is the claims that define the boundaries of the patent owner's rights. Don't fall into the trap of concluding that the title or the abstract or the general description found in the text of the patent indicates what is patented. For example, United States Patent No. 6,074,877 is titled "Process for transforming monocotyledonous plants". From the title, you might conclude that these patent owners have protected a method for transforming all monocot plants. The claims, however, refer only to transformation of cereal plants, and furthermore an embryogenic callus must be wounded first or treated with an enzyme that degrades cell walls prior to transferring DNA into the cells with *Agrobacterium*. A bit different than what the title implied.

In order to avoid infringement, the meaning of claims must be determined. While the purpose of claims is to clearly demarcate the extent of the patent owner's rights, the meaning and scope of the claimed invention are not always clear from just reading the claim. Proper claim interpretation is achieved by reading the claims in the context of the specification and in the context of the "prosecution history" (the back and forth negotiations of the claim language between the patent applicant and the patent office). In this case above, for example, claim 1 recites:

> A process for the stable integration of a DNA, comprising a gene that is functional in a cell of a cereal plant, wherein said DNA is integrated into the nuclear genome of said cereal plant, said process comprising the steps of:
>
> (a) providing a compact embryogenic callus of said cereal plant;
>
> (b) wounding said compact embryogenic callus or treating said compact embryogenic callus with a cell wall degrading enzyme for a period of time so as not to cause a complete disruption of tissues, and transferring said DNA into the nuclear genome of a cell in said compact embryogenic callus by means of *Agrobacterium*-mediated transformation to generate a transformed cell; and
>
> (c) regenerating a transformed cereal plant from said transformed cell.

Upon first reading this claim most people will think that they understand its meaning. But read the claim again, or several times again, and ask yourself if you are sure what the inventors mean by the terms: "cereal plants", "wounding" "embryogenic callus", and "enzyme that degrades cell walls". Is there only a single meaning for these terms? Has the inventor provided her own definitions? How do you uncover the true meaning of these terms? First, read the text of the patent, especially looking for a section titled "Definitions" or for phrases like "as used herein" or "cereal'plant refers to". Oftentimes, the inventor will directly define a term. If no explicit definition exists, then try deducing a meaning from how the term is used when the invention describes the invention. Clues might also be found in the prosecution history posted online by the U.S. Patent and Trademark Office for patents and pending, published patent applications. Uncovering the meaning may take a bit of detective work. Don't despair; it can be difficult (and time-consuming) to accomplish.

Claim basics. Claims are written in a way peculiar to patents. A claim is always written as a single sentence, composed of two parts – the preamble and the body – with a transition word or phrase between them.

- The preamble is an introductory statement that names the thing that is to be claimed. For example, "A method for making a genetically modified plant."
- The body of a claim defines the elements or steps of the named thing or method.

The transition words or phrases commonly used are "comprising," "consisting of" and "consisting essentially of" and have very distinct meanings ("Consisting essentially of" is rarely, if ever, used in biotechnology and is not discussed here). "Comprising" (also "having") means that the claim encompasses all the elements listed and, moreover, can include additional, unnamed elements. For example, if a claim recites elements (A and B), an individual that uses elements (A and B) or elements (A, B, and C) is infringing, whereas using the single elements (A) or (B) is not infringing. The same rationale applies to the pencils example above, where A = No. 2 lead and B = an eraser.

In contrast, the transition "consisting of" has more limited scope. "Consisting of" means that the device (or method) has the recited elements (or steps) and no more. For example, if a claim recites (A and B) and the individual uses only (A), or (A and C), or even (A, B, and C), the claim is not infringed. (A′ and B) also escapes the claim, where A′ is a modified version of A. With respect to the pencils, C could be a rubber finger grip and A′ could be No. 3 lead.

Claims also come in two flavors: independent and dependent. An independent claim stands alone. It includes all the necessary limitations and can be read without reference to any other claim. A dependent claim refers back to another claim and includes all the limitations of the referred-to claim. Thus, when analyzing claims in a patent for infringement or claim scope, only the independent claims need to be considered.

2.2 A patent application is not a patent

Are claims in a patent *application* important? The answer is both yes and no. Yes, because the claims indicate the invention intended for protection and may indicate the scope of protection that is desired. No, because claims in a patent application have no force. A patent application is NOT the same as a patent. Claims in a published patent application have not been examined by a national patent office and may not be representative of a scope that will ultimately be granted.

During the application process, the patent text is published 18 months after the earliest filing. The publications print the claims as filed. Sometimes the claims are written much broadly than is actually patentable. As the application is examined by a patent office and claim language negotiated, the claims may shrink in scope. In contrast, the specification of a granted patent is nearly always identical as filed; typos and obvious errors may be fixed, material can be deleted, but new matter is not allowed to be added to the text after filing.

Because the claims in an application are what the applicant hopes for and not what she will necessarily receive, it is important to know whether you are looking at a granted patent or a patent application.

2.3 Parts of a patent document

A patent document has three main sections:

1. a cover page which presents bibliographic information,
2. a specification, which describes the invention, and
3. claims, which define the scope of activity from which the patentee has the right to exclude others unless they sign licensing agreements.

The cover page presents mainly bibliographic information. The information provides notice mainly of historical facts and identifying elements, such as application filing date and serial number. None of it, including the abstract, has legal import for interpreting the patent. Nevertheless, in a departure from previous decisions, the Federal Circuit in *Hill-Rom* used the abstract for aid in interpreting the claims.

The specification is also called the disclosure. It contains a description of the invention that must satisfy certain writing requirements. The layout and content of a specification may vary somewhat from country to country.

The specification has particular value as an aid to interpreting the scope of the claims. Thus, a patent specification is drafted both to satisfy the written requirements for patentability as well as to define claim scope. With this in mind, we will examine each major section of the specification and analyze what purpose is being accomplished. For a more detailed guide to "How to Read a Patent" the reader can referee to the tutorial area of the CAMBIA web site (www.patentlens.net) or on the Cougar Patent Law web site (www.cougarlaw.com).

"Background of the Invention" is typically drafted for a jury audience or a patent examiner. Selected art in the field is discussed to emphasize differences with the current invention, and to point out the need for the current invention. "Summary of the Invention", which is distinct from the abstract, is meant to discuss the invention (i.e., the claims) rather than the disclosure as a whole. Often, the summary will discuss advantages of the invention or how it solves the problems existing in the art.

"Detailed Description of the Invention" is the meatiest section of a patent. Its purpose is to adequately and accurately describe the invention. There are generally two sections: (1) a general explanation of the invention and how to practice it; and (2) specific examples of how to practice the invention. Many new readers find the purposes of these two sections confounding and assume that the examples set forth how the invention will be practiced. Rather, examples are meant only "to illustrate, but in no way to limit, the scope of claimed invention."

In the first section the invention is described in its broadest sense, to show that the inventors have a broad view of the scope of the elements. Preferred embodiments of the invention are often described. Such embodiments are generally more limited versions of the broadest concept and are provided for support of the claims. Definitions of key terms are often provided and are extremely important in interpreting the scope of the claims.

Although a patent application does not require examples, in practice, they can often assist in showing patentability (e.g., enablement). The examples may or may not actually have been performed by the inventors. "Working" examples present completed undertakings. "Prophetic" examples are hypothetical undertakings and are always written in the present or future tense. Typically, the examples demonstrate practice of one or more specific embodiments of the invention.

And now armed with the basics on patents and tools to read them, let us enter the patent world of *Agrobacterium* as a vehicle for plant transformation.

3. AGROBACTERIUM-MEDIATED TRANSFORMATION AND PATENT LAW

An analysis of the patent landscape of *Agrobacterium*-mediated transformation begins with determining the scope of the subject matter. Patent documents pertaining to the subject are then obtained by searching on-line databases, such as CAMBIA's Patent Lens (www.patentlens.net), Esp@cenet at the European Patent Office (ep.espacenet.com), patents at U.S. Patent and Trademark Office (www.uspto.gov/patft/index/html), PatBase (www.patbase.com), and Delphion (www.delphion.com). An exhaustive patent search is conducted, typically based on a combination of keywords, scientists' names, organizations, and patent office classification codes. If the number of documents obtained is quite large, a rapid screening method may be used to reduce the pile to a manageable number of key documents. In our rapid screening method, the document text is skimmed, especially the background and the summary of the invention sections and the claims read.

A broad claim , which encompass a relatively large part of the field, is the criterion for identifying a key patent. Especially key patents may dominate others (like the pencil example above). The relationship of patents found can be visualized either as a pyramid, with the broadest patent claims at the apex and each row (tier) moving down the pyramid to the base contains patents with successively narrower claims, or as Venn diagrams, with the broadest claims residing in the outermost circle. For a patent landscape review generally only the top 1-3 tiers are analyzed.

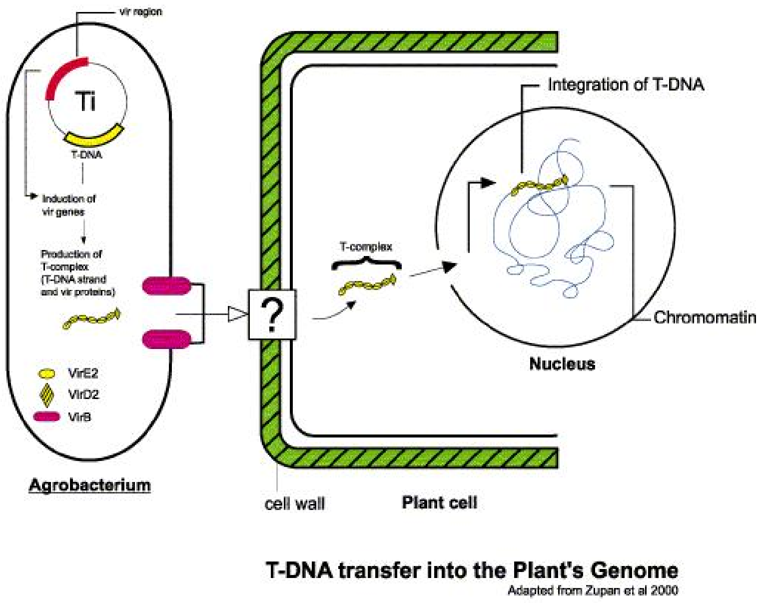

Figure 20-3. A lawyer's view of the transformation process.

Patent analysis focuses on interpreting the meaning of the claims because patent rights are delimited by the scope of the claims. Claim scope analysis requires an understanding of the science and the invention described in the patent text as well as the legal rules that define claim interpretation. Briefly, claims are interpreted by their plain language, by the definitions or context used in the patent text, and by statements made to the patent office during the back and forth negotiations called prosecution (see also discussion in section 2.1 above). Other resources, including dictionaries and experts, can be consulted as needed. For the patent landscape analyses of the sort presented here, the meaning and scope of claims are established only from looking within the "four corners" of the patent. Due to the volume of key document s, time and resource constraints, and the purpose of the landscape paper, we did not consult the prosecution history or other sources.

For the analysis of patent landscape surrounding *Agrobacterium*-mediated transformation of plants, the subject matter of the technology for inclusion was decided on before any patent search. The following analysis is based on Roa-Rodríguez and Nottenburg, 2003. Figure 20-3 illustrates various aspects of transformation as a patent lawyer might draw it out.

In this simplistic scheme, gene vector(s) that contain a *vir* region and a T-DNA with a gene of interest on the same or separate vectors are constructed, *Agrobacterium* containing the vectors are prepared, plant cells or tissues are incubated with the *Agrobacterium*, and transgenic plants are grown. Underneath the main pathway, it is helpful to list alternatives for each component or method step. It is these possibilities that form the contours of the subject matter.

Looking at the diagram, let's examine some pieces that might be patentable (assuming that the requirements of patentability are met):

- Vectors for transforming plants
- Genes of the vectors
- Transgene
- Methods for making vectors
- Methods for making *Agrobacterium* with engineered vector
- *Agrobacterium* containing engineered vectors.
- Improved *Agrobacterium* strains for transformation
- Methods of preparing plant tissue for transformation
- Methods of transforming specific plants
- Transformed plants and plant cells.

Agrobacterium itself is not patentable under patent law. Only inventions are patentable; a naturally-occurring bacterium is not considered an invention even under the most magnanimous patent laws. In addition to that, some countries do not allow patents for plants or native sequence genes or others, but for purposes of this chapter, we consider transgenic plants and gene sequences patentable – especially as initial patenting of *Agrobacterium*-mediated transformation was primarily done in U.S., which considers all of these subject matters patentable.

3.1 Vectors for transformation

The basic elements of vectors designed for *Agrobacterium*-mediated transformation are derived from the native Ti-plasmid. The necessary elements are:

- **T-DNA border sequences**, or at least the right border, which initiates the integration of the T-DNA region into the plant genome;
- ***vir* genes**, which are required for transfer of the T-DNA region to the plant, and
- **a modified T-DNA region** of the Ti plasmid, in which the genes responsible for tumor formation are removed by genetic engineering and replaced by one or more foreign genes of interest.

A few different types of vector systems are used in transformation protocols. The patent landscape presented in this section analyzes two vector systems: binary vectors and co-integrated vectors.

Although not examined here, a third type of system is mobilizable vectors, vectors unable to promote their own transfer without an appropriate conjugation system that is provided by a helper plasmid. Mobilizable plasmids readily transfer genes between bacteria, and between bacteria and fungi, but few results are reported in plant transformation.

3.1.1 Patents on binary vectors and methods

Binary vector systems are the most commonly used systems for *Agrobacterium*-mediated gene transfer to plants. In these systems, the T-DNA region, which contains a gene of interest is located in one plasmid vector and the *vir* region is located in a separate, disarmed (lacking tumor genes) Ti plasmid vector. The plasmids co-reside in *Agrobacterium* and remain independent (*Figure 20-4*).

The basic elements of binary vectors are claimed by Mogen (now called Syngenta Mogen B.V. and part of Syngenta Co.) in two United States patents and one European patent that expired in 2004. (A "European patent" is not a Europe-wide patent but rather a patent that has been examined and granted by the regional patent office, European Patent Office and in order to have force in a particular country, the European patent must be registered in the patent office of the country. Currently, 31 countries have signed on to the European Patent Convention. Alternatively, a patent application may be examined and granted by individual patent offices in European countries.)

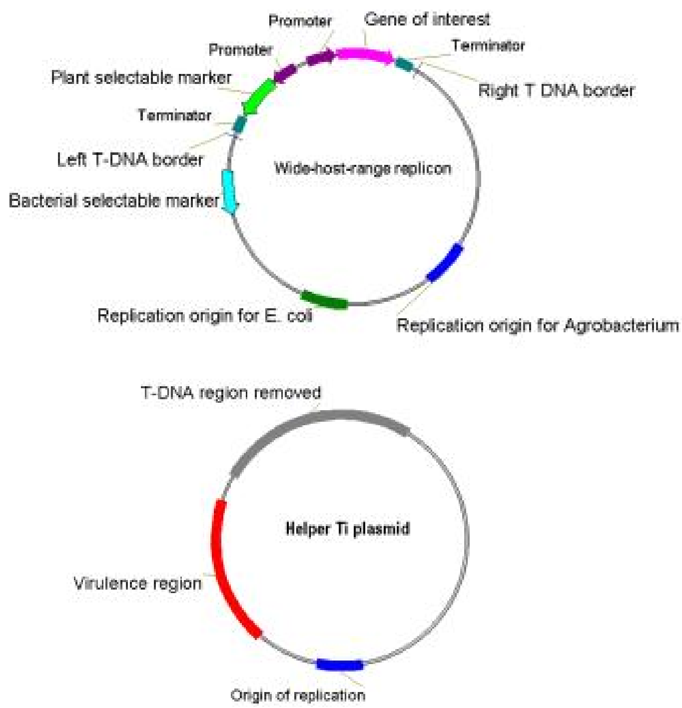

Figure 20-4. Binary vector system

Table 20-1 presents an overview of these three patents.

Table 20-1. Binary vector patents

Patent No:	US 4,940,838	US 5,464,763	EP 120 516 B1
Filing date:	23 Feb 1984	23 Dec 1993	21 Feb 1984
Issue date:	10 July 1990	07 Nov 1995	23 Oct 1991
Expiration date	10 July 2007	07 Nov 2012	21 Feb 2004

Broadly speaking, these patents describe methods for transforming dicotyledonous plants with *Agrobacterium* that contains binary vectors. More specifically, in claim 2 of US 4,940,838, the invention is:

Agrobacterium containing two plasmids, in which

1. one of the plasmids has foreign DNA residing in a T-region but lacks a *vir* gene region, and
2. the other of which has a *vir* gene region but lacks the T-region.

Other claims in this patent recite using this *Agrobacterium* for transforming plants and plant cells. But unlike claim 2, method claims in the patent are limited specifically to transformation of dicot plants and cells. This difference begets an interesting conundrum:

if you make and use the *Agrobacterium* of claim 2 to transform monocot cells, are you infringing this patent?

There is little doubt that *using* the claimed *Agrobacterium* to transform monocots is not infringing. Making the claimed *Agrobacterium*, whether intended or not for transformation of monocots, appears to be literally infringing. (This analysis is based only upon the published specification and claims and as such is preliminary and informal.) An argument can be made however, that the claimed *Agrobacterium,* which is constructed for transforming monocots, is not infringing: in 1984 when the patent application was filed transformation of monocots was not routine and the inventors could not have known that the claimed *Agrobacterium* would successfully transform monocots. This situation is analogous to US 5,561,236, owned by Plant Genetic Systems (now a part of Bayer Crop Science), first filed in 1987, which claims "plants" transformed with an herbicide resistance gene. The term "plants" was interpreted by the Federal Circuit court in *Plant Genetic Systems N.V. v. DeKalb Genetics Corp.* to mean only "dicot plants" because monocot transformation was not routine at the time nor was it adequately taught in the patent. Based on this court decision, it is likely that the *Agrobacterium* of US 4,940,838 is limited to uses in dicots (The Federal Circuit is the only appeals court in the United States that reviews patent cases. Its decisions can only be reviewed by the U.S. Supreme Court).

The claims in US 5,464,763 additionally limit the binary plasmid vectors by requiring that only foreign DNA be contained between the 23 bp borders of the T-region. Furthermore, the two plasmids lack a region of homology, which insures that the two plasmids do not recombine. The now-expired European patent issued with claims equivalent to US 5,464,763.

Other patents are directed to variations of the basic binary vector system. Variations include: *Agrobacterium* with multiple copies of T-DNA and *vir* integrated in the bacterial chromosome (US 5,149,645 owned by Leiden University and Schilperoort, but method claims are limited to transforming plants in either the Liliaceae or Amaryllidaceae families)**;** *Agrobacterium* with more than one binary vector (US 6,265,638 B1, EP 1 117 816 B1, CA 2,344,700 C, and AU 764100 B2 owned by Pioneer Hi-Bred). In other variations, Syngenta Mogen B.V patents claim vectors that can integrate into plant genomes by homologous recombination (US 5,501,967 and EP 436 007 B1). In this scheme, the vector has a region homologous to a part of a target locus in the plant, permitting insertion of a gene of interest or a specific mutation in a particular locus of a plant genome.

Other binary vectors have been devised to suit different needs of plant transformation. For example, some desirable features of binary plasmids include different origins of replication, a large maximum size of the insert a binary plasmid can carry, and the size of the plasmid. These features are claimed in a number of patents (see e.g., US Patent 6,165,780 filed by The National Institute of Agrobiological Resources (Japan) in which the binary plasmid has origins of replication that maintain either a low or high copy number).

In summary, the key patents on basic binary vectors, those that claim the basic elements, were granted to Mogen in the United States (two patents) and in Europe (one patent, which has expired). The claims in these patents encompass essentially any two vector system located in the same *Agrobacterium* strain having (i) a T-region in one vector, and (ii) a *vir* region in another vector.

While it is difficult to form a conclusion that will apply to every reader, overall these patents likely encompass many transformation protocols in common use, with one major exception. The claims are limited to methods of transformation of dicotyledonous **(**dicots**)** plants only; use for transformation of monocotyledonous plants is not covered. Keeping this limitation in mind, users of this binary vector system in the United States should consider these patents when crafting a commercial research strategy.

3.1.2 Patents on co-integrated vectors

A co-integrated plasmid vector is the product of homologous recombination between a small plasmid of bacterial origin and an *Agrobacterium* Ti plasmid. In general, the *Agrobacterium* Ti plasmid lacks tumor-causing genes ("disarmed" Ti plasmid) and the small vector plasmid is engineered to carry a gene of interest between a right and a left T-DNA border of the T-DNA region. Recombination takes place through a single crossover event in a homologous region present in both plasmids.

Although co-integrated vectors have become less popular in recent years due to some difficulties encountered in engineering them, they are still used to a certain extent when modified, for example, to allow site-specific recombination of the plasmids within *Agrobacterium*.

A co-integrated plasmid or hybrid Ti plasmid contains at least (i) a *gene of interest*, located between the left and right T-DNA border sequences, and (ii) a *vir* region, which allows the transfer of the gene of interest located between the two border sequences into the plant genome. Two plasmids are required for the assembly of a co-integrated plasmid. They are (i) a *vector molecule containing a gene of interest* to be transferred into a plant and a homologous region, and (ii) a *Ti plasmid* containing the *vir* region and a homologous region but does not contain tumor-inducing genes.

Max-Planck Society and Monsanto Company have both been granted patents on basic co-integrated vectors. Patents awarded in Europe, Australia, Japan and Russia have all expired. Notably, no patent has been granted in the United States. Most likely any application filed in the U.S. has either been abandoned or is in interference, which is a procedure to determine who was the first inventor in time. Unfortunately, if a patent does issue in the U.S. it will likely have a 17-year term from the *date of issue*.

Be aware that although the patents have expired on the most basic features of co-integrated vectors, other patents on improvements or modifications of co-integrated vector systems may still be in force (see, e.g., US 5,635,381).

3.2 Tissue types for transformation

The efficiency of T-DNA transfer via *Agrobacterium* to a plant varies considerably, not only among plant species and cultivars, but also among tissues. Various protocols for *Agrobacterium*-mediated transformation of plants use leaves, shoot apices, roots, hypocotyls, cotyledons, seeds and calli derived from various parts of a plant. In other methods, the transformed tissue is not removed from the plant but left in its natural environment, thus the transformation takes place *in planta*.

Patents directed specifically to different tissues to be transformed are relatively few, but the scope of their protection is rather broad. Some of the patents referred to in this section are considered key patents for widely used technologies by the research community. With the exception of Japan Tobacco's patents directed to callus and immature embryo transformation of a monocotyledonous plant, claims in these patents are not restricted to the type or species of plant to be transformed.

3.2.1 Callus transformation

Japan Tobacco was granted two patents, one in the United States and the other in Australia (now expired). The United States patent claims a method for transforming a monocot-derived tissue with *Agrobacterium*. Moreover, the tissue must be dedifferentiated or undergo dedifferentiation by culture for at least 7 days.

US 5,591,616 issued 7 Jan 1997 expires 7 Jan 2014

In an allowed United States patent application, Japan Tobacco claims transformation of a dedifferentiated monocot tissue cultured from 1 to 6 days. As filed, the tissue is not limited to any particular plant tissue; it just has to be immature.

US 2002/0178463 when issued, will expire 07 Jan 2014

3.2.2 Immature embryo transformation

Protected by Japan Tobacco in Australia and in Europe, the patents claim transformation of the scutellum of an immature embryo of a monocot plant with *Agrobacterium.* The transformation process takes place before the tissue has differentiated into a callus. To date, no related patent has issued in the United States.

AU 687863 B issued 5 Mar 1998 expires 1 Sep 2014
EP 672 752 B1 issued 26 May 2004 expires 1 Sep 2014

3.2.3 *In planta* transformation

Three different entities have patents in this area. Cotton Inc., Rhobio and Paradigm Genetics claim transformation of a plant tissue *in situ* with *Agrobacterium*. Cotton Inc. claims the injection of *Agrobacterium* into floral or meristematic tissue, whereas the other entities' patents do not limit the type of tissue (*Table 20-2*).

Table 20-2. Patents on *in planta* transformation

Patent No.	Issue Date	Expiry date	Patent Owner
US 5,994,624	30 Nov 1999	20 Oct 2017	Cotton Inc
AU 752717 B2	26 Sep 2002	19 Oct 2018	Cotton Inc
US 6,353,155 B1	5 Mar 2002	30 Jun 2020	Paradigm Genetics
EP 1 171 621	7 Dec 2005	19 April 2020	Rhobio
AU 775949 B2	19 Aug 2004	19 April 2020	Rhobio

3.2.4 Floral transformation

Floral transformation is basically an *in planta* method that is very popular for transformation of *Arabidopsis thaliana* (Brassicaceae), one of the best known model plants in genomic studies, and is also suitable for the transformation of monocotyledonous plants. A U.S. patent assigned to Rhône-Poulenc Agro is described further in section 3.3.1 below. The U.S. patents granted to Cotton Inc. and Paradigm Genetics for *in planta* transformation (discussed above) also include claims for transformation of floral or meristematic tissue. Unlike the Cotton Inc. patent where *Agrobacterium* is injected into the floral tissue, in the Paradigm Genetics patent, the floral tissue is immersed into a diluted suspension of *Agrobacterium* cells (*Table 20-3*).

Table 20-3. Patents on floral transformation

Patent No.	Issue Date	Expiry date	Patent Owner
US 5,994,624	30 Nov 1999	20 Oct 2017	Cotton Inc
AU 752717 B2	26 Sep 2002	19 Oct 2018	Cotton Inc
US 6,353,155 B1	5 Mar 2002	30 Jun 2020	Paradigm Genetics
US 6,037,522	14 Mar 2000	22 June 2018	Rhône-Poulenc Agro

3.2.5 Seed transformation

Two groups have pending patents or patent applications on transformation of plants using seed as target tissue: (1) the Agricultural Biotechnology Research Center (ABRC) of Shanxi (China) in China, the U.S., and Canada, and (2) Scigen Harvest Co. (Korea) in Korea. As filed, the U.S. and Canadian applications of ABRC generally claim applying *Agrobacterium* to germinating seed, without further treatment of the seed. The scope of a related Chinese patent is unknown, as it is only available in Chinese language. The Scigen method uses needle-wounded seed as target tissue in combination with *Agrobacterium tumefaciens*.

In addition, the Protein Research Trust of South Africa has an granted Australian patent and a European patent application directed to transforming seed with a mixture of *Agrobacterium* and a wetting or surfactant agent that enhances or facilitates the penetration of the bacterium into the explant (*Table 20-4*).

Table 20-4. Patents on seed transformation

Patent No.	Issue Date*	Expiry date*	Patent Owner
WO 20/66599 A2	29 Aug 2002	not applicable†	Scigen Harvest Co
US 2002/0184663 A1	5 Dec2002	19 Feb 2022	Agricultural Biotechnology
AU 762964 B2	10 Jul 2003	14 Oct 2019	Protein Research Trust
EP 1 121 452 A1	8 Aug 2001	14 Oct 2019	Protein Research Trust

*Publication date and estimated expiry date (if the application is granted) are given for patent applications.
†WO patent applications do not have an expiry date because they can only become patents if converted to national applications.

3.2.6 Pollen transformation

A patent related to this topic was granted to the United States Department of Agriculture (USDA) in the U.S., Europe and Australia. In this invention, *Agrobacterium* containing a foreign gene is applied to pollen, allowing the pollen to take up the bacteria and germinate. The transformed pollen fertilizes a second plant to obtain transgenic seed, which is then germinated to obtain a transgenic plant (*Table 20-5*).

Table 20-5. Patents on pollen transformation

Patent No.	Issue Date	Expiry date	Patent Owner
US 5,929,300	27 Jul 1999	15 July 2017	US Dept. of Agriculture
EP 996 328 B1	5 Mar 2003	14 July 2018	US Dept. of Agriculture
AU 733080 B	3 May 2001	14 July 2018	US Dept. of Agriculture

3.2.7 Shoot apex transformation

Transformation of an excised shoot apical tissue by inoculating the tissue with *A. tumefaciens* is disclosed by Texas A & M University in a granted United States patent (US 5,164,310; issued 17 Nov 1992; expires 17 Nov 2009). Applications filed in Europe and in Australia have been abandoned.

Additionally, in a U.S. application filed in 2003, Texas A&M University disclosed a transformation method consisting in direct inoculation of *Agrobacterium* into a shoot apex still attached to a plant seedling. The application was abandoned in 2005.

3.2.8 Summary

Transformation of pollen with *Agrobacterium* is fairly broadly protected in the United States and in Australia. Similarly, shoot apex transformation is protected in the United States, except that the bacterium used in this case is specifically *A. tumefaciens.* Thus, use of other species of *Agrobacterium* to transform apical shoots from any plant may fall outside of the scope of the claimed invention.

There appears to be more room to avoid infringing patents on *in planta* and callus transformation. A United States patent owned by Cotton Inc. particularly claims: use of a needleless device to inject *Agrobacterium* into floral or meristematic tissue. Thus, use of a different device or other tissues may bring the method outside the scope of the claimed invention. In the case of Paradigm Genetics' U.S. patent, the suspensions of *Agrobacterium* cells are of specified density. If one uses aqueous solutions that do not conform to the specified dilutions and density, the method may fall outside the terms of the claimed invention.

With respect to callus transformation claimed by Japan Tobacco, at least in the United States, the monocot tissue must be at least seven days old. Sometime in 2006 however, a new Japan Tobacco patent will issue that claims use of any immature monocot tissue with a one-day minimum culture period. With the grant of this patent, Japan Tobacco's protection is expanded in the U.S. for essentially all *Agrobacterium* transformations of immature tissue of a monocot.

Seed transformation with *Agrobacterium* seems to be an area still to be explored. Not only are dominant patents absent in the major jurisdictions, but no patents appear to be either filed or granted in the USA to date.

In addition, there are inventions where the plant tissue or cell type employed in the *Agrobacterium*-mediated transformation protocol is not defined. This is the case of a pending U.S. application filed by Monsanto in 2003 (US 2003/0204875 A1). One limitation in the disclosed invention is the use of a non-specified agent to inhibit the growth of *Agrobacterium* in the co-culture medium with the plant cells or tissue. The addition of such agent is said to facilitate the generation of transformed plants with low number of inserted copies. If granted as filed, avoidance of the patent may be possible by forgoing a growth inhibiting agent.

3.3 Patents on transformation of monocots

Monocots (monocotyledonous) comprise one of the large divisions of angiosperm plants (flowering plants with seeds protected within a vessel). They are herbaceous plants with parallel veined leaves and have an embryo with a single cotyledon, as opposed to dicot plants (dicotyledonous), which have an embryo with two cotyledons.

Most of the important staple crops of the world, the so-called cereals, such as wheat, barley, rice, maize, sorghum, oats, rye and millet, are monocots. Other monocots include food crops such as onion, garlic, ginger, banana, plantain, yam and asparagus.

Agrobacterium-mediated transformation of commercially important monocots was first attained in rice and maize in the mid 1990s. Following these achievements, other monocot crops were successfully transformed and refinements of techniques led to improved regeneration of transformed monocot tissue.

3.3.1 General methods for transforming monocots

Japan Tobacco (in Japan), Rhône-Poulenc Agro (in France), and the National Institute of Agrobiological Resources (in Japan) own patents or pending patent applications directed to methods for *Agrobacterium*-mediated transformation of any monocot with a gene of interest (*Table 20-6*). The main differences among the patent claims lie in:

- the type of plant tissue or explant used for the transformation process, and
- the use of additional treatments, such as vacuum infiltration or adding phenolic compounds, to facilitate the transformation process.

Table 20-6. U.S., European and Australian patents for transforming monocots

Patent No.	Issue Date*	Expiry date*	Patent Owner
US 5,591,616	7 Jan 1997	7 Jan 2014	Japan Tobacco
AU 667939 B	18 Apr 1996	6 Jul 2013	Japan Tobacco
EP 0 604 662 A1	6 Jul 1994	6 Jul 2013	Japan Tobacco
AU 687863 B	5 Mar 1998	1 Sep 2014	Japan Tobacco
EP 0 672 752 B1	26 May 2004	1 Sep 2014	Japan Tobacco
US 6,037,522	14 Mar 2000	23 Jun 2018	Rhône-Poulenc
EP 1 198 985 A1	24 Apr 2002	22 Jul 2019	Nat'l Inst. of Agrobiological Resources
AU 775233 B2	22 Jul 2004	22 Jul 2019	Nat'l Inst. of Agrobiological Resources

*Publication date and estimated expiry date (if the application is granted) are given for pending patent applications

Japan Tobacco is typically considered to have the broadest patent in this area. In two different sets of patents, it claims the transformation of a monocot callus during a dedifferentiation process and the transformation of the scutellum of an immature embryo prior to dedifferentiation. Thus, these patents cover transformation of monocot tissues that are widely and commonly used.

Rhône-Poulenc Agro (now Bayer Crop Science) claims the transformation of a monocot inflorescence via *Agrobacterium*. The inflorescence can be dissected and then transformed. Alternatively, callus formation is induced from an inflorescence in culture, and the derived callus is then transformed with *Agrobacterium* and regenerated into a plant. The invention is thus limited to transformation of a monocot inflorescence. Transformation of other monocot tissues are not claimed.

The National Institute of Agrobiological Resources (Japan) has a PCT application and an Australian granted patent that discloses a method for transforming a monocot by treatment of intact seed with *Agrobacterium* containing a recombinant gene of interest. In the Australian patent, the seed to be transformed is a germinated seed pre-cultured in a medium with 2,4 D for four or five days.

3.3.2 Gramineae and cereals

Gramineae is one of the largest families of monocot plants. Mostly herbaceous, grass-like plants, this family includes several important staple crops (cereals) such as wheat, rice, maize, sorghum, barley, oats, and millet. It also encompasses plants such as bamboos, palms, and foraging grasses (e.g. turf grass, king grass (*Pennisetum purpureum*), and *Brachiaria*). Therefore, patents addressing the Gramineae family embrace cereals, but patents directed to cereals *do not embrace* all Gramineae.

Gramineae transformation. The United States and Australian patents granted to the University of Toledo and the United States patent granted to Goldman and Graves belong to the same patent family (*Table 20-7*). They all claim a method for transforming seedlings of a Gramineae with a vir^+ *Agrobacterium*. Claims of both United States patents limit the inoculation of the bacterium to a particular area in the seedling, where cells divide rapidly and wounding takes place prior the inoculation.

Remarkably, the United States patent granted to Goldman and Graves (US 6,020,539) – and licensed by the University of Toledo (*Table 20-7*) – also contains broad claims to the transformation of Gramineae with *Agrobacterium*. One particular claim (claim 22) encompasses any Gramineae, constituting one of the broadest claims recently issued in the area of plant transformation technologies. United States patents claiming *Agrobacterium* transformation of any tissue of a Gramineae might be dominated by this patent. The grant of this patent has wreaked havoc in the scientific community and multiple parties with interest in *Agrobacterium*-mediated transformation of Gramineae.

Cereal transformation. Plant Genetic Systems (now part of Bayer Crop Science) has granted United States and European patents disclosing the transformation of any cereal with *Agrobacterium* (*Table 20-7*). The most limiting elements in the claims are the wounding of a cereal embryogenic callus and the enzymatic disruption of a tissue cell wall before transformation. The claims of the European patent do not recite enzymatic degradation but do additionally encompass different transformation methods besides *Agrobacterium*.

Table 20-7. Patents directed to transformation of Gramineae and cereals.

Patent No.	Issue Date	Expiry date	Patent Owner
Gramineae			
US 5,187,073	16 Feb 1993	16 Feb 2010	University of Toledo
AU 606874 B2	21 Feb 1991	30 Jan 2003	University of Toledo
US 6,020,539	1 Feb 2000	1 Feb 2017	University of Toledo
US 2002/0002711 A1	3 Jan 2002*	Abandoned	University of Toledo
Cereals			
US 6,074,877	13 June 2000	23 June 2013	Plant Genetic Systems
EP 0 955 371 B1	22 Feb 2006	21 Nov 2011	Plant Genetic Systems

*Publication date

3.4 Patents on transformation of dicots

Dicotyledonous plants (dicots) are the second major group of plants within the *Angiospermae* division (flowering plants with seeds protected in vessels). The mature leaves have veins in a net-like pattern, and the flowers have four or five parts. Apart from cereals and grasses that belong to the monocot group, most of the fruits, vegetables, spices, roots and tubers, which constitute a very important part of our daily diet, are dicots. In addition, all legumes, beverages (e.g., coffee and cocoa), and a great variety of flowers, oil seeds, fibers, and woody plants belong to the dicot group.

3.4.1 General transformation methods

There are fewer patents on general methods for *Agrobacterium*-mediated transformation of dicots than for transformation of monocots. A broad patent directed to transformation of dicots using an *Agrobacterium* strain lacking functional tumor genes was granted a few years ago to Washington University (US 6,051,757; issued 18 Apr 2000; expires 18 Apr 2017)

Although issued in 2000 in the United States, this patent has an initial priority date of 1983. Thus, the prosecution process took approximately 17 years until the patent was finally granted. The patent appears to be one of the broadest in scope granted in the area of *Agrobacterium* transformation. Moreover, the patent rights under this patent may overlap with the rights already granted in previous patents related to transformation of dicots with *Agrobacterium*.

One of the distinctive features in the patent claims is that the cytokinin function in the Ti plasmid is knocked out in order to obtain a non-tumorigenic (disarmed) *Agrobacterium* strain. Disarmed strains lacking functional tumorigenic genes are typically used in protocols of *Agrobacterium*-mediated transformation. The present patent thus may constitute a blow for a widely used and standard procedure carried to transform dicot plants.

Other patent applications in this area may still be in interference proceedings (procedures that determine who is the first inventor-in-time) at the U.S. Patent and Trademark Office. While many press releases from Monsanto Co., Syngenta, Bayer CropSciences, and Max Planck Society, allude to the settlement of interference proceedings, details are notably lacking.

In 2003, Monsanto was granted a U.S. patent that claims the transformation of a dicot cell or tissue with *Agrobacterium* (US 6,603,061 B1; issued 3 Aug 2003; expires 29 July 2019). The distinctive element in the claims of this patent is the use of an antibiotic that inhibits or suppresses the growth of *Agrobacterium* during the inoculation phase. According to the applicants, this procedure promotes the generation of transformed plants with low copy inserts and improves the transformation efficiency. The possible effects of this patent in the U.S. depend on the extent of the practice of using a growth inhibiting agent in the inoculation medium.

Most of the other major patents in this area claim transformation of dicots in conjunction with the use of co-integrated or binary vectors, the vectors being the main subject matter of the claimed inventions. These patents have been reviewed above in sections on “Binary vectors” and “Co-integrated vectors”.

3.4.2 Transformation of cotton

Of the patents directed to transformation of various dicot plants, the patents directed to transformation of cotton present an interesting study. Five different entities have patents and applications in this area.

The most contentious of the patents belong to Agracetus (now owned by Monsanto), in particular, two patents in the United States and one in Europe directed to transformation of immature cotton plants with *A. tumefaciens* (*Table 20-8*).

Table 20-8. Patents owned by Agracetus (Monsanto).

Patent No.	Issue Date	Expiry date	Comments
US 5,004,863	2 Apr 1991	2 Apr 2008	Re-examined twice: in 1992 and 2000 Currently in interference
US 5,159,135	27 Oct 1992	27 Oct 2009	Re-examined once: in 2000 Currently in interference
EP 270 355 B1	16 Mar 1994	2 Dec 2007	Originally opposed by Monsanto, but opposition withdrawn

In the first patent, US 5,004,863, the claims are directed to a method of transforming cotton plants where:

- hypocotyl cotton tissue is used as the target of transformation;
- non-oncogenic *Agrobacterium* contains a Ti plasmid having a T-DNA region containing both a foreign gene and a selectable resistance gene;
- formation of somatic embryo is induced in the transformed tissue; and
- cotton plants are regenerated.

The claims of the second patent, US 5,159,135, are directed to transformed cotton seeds and plants. In its broadest claims, the cotton seed and cotton plants contain a gene of interest whose product – a protein or a negative strand RNA – confers a detectable trait.

The contentious nature of these patents is reflected by requests for re-examinations in the U.S. and opposition in Europe. Nothing changed however as a result of the challenges: the claims were upheld in two re-examinations, and the opposition in Europe brought by Monsanto was dropped when Monsanto bought Agracetus. Currently, the U.S. patents are in an interference proceeding to determine who was the first inventor-in-time of the claimed invention. The other party (or parties) involved in the interference are not known.

Calgene (also owned by Monsanto) has a United States patent, as well as European and Australian patents, claiming transformation of cotton (*Table 20-9*). In both Agracetus' and Calgene's inventions hypocotyl cotton tissue is transformed with *Agrobacterium.* In Calgene's patent, the hypocotyl tissue is cut from a seedling that has been grown in the dark and the transformed tissue is further cultured on callus initiation media that contains a selective agent and is plant hormone-free.

Another strong player in the area of genetically engineered cotton is Mycogen, an affiliate of Dow AgroSciences LLC (*Table 20-9*). It has a large portfolio of patents and applications related to cotton transformation and regeneration, mostly to transgenic herbicide-resistant cotton. Of the several patents granted in the U.S., one of them (US 6,620,990 B1) claims methods to obtain transformed cotton plants from embryogenic callus transformed with *Agrobacterium*. The invention also includes additional tissues for transformation such as cotyledons and zygotic embryos. Mycogen's non-U.S. patents additionally claim other tissues for transformation (i.e., hypocotyl), and specific media elements and conditions (i.e., time length, light and temperature) for the incubation with *Agrobacterium* and recovery of transformed explants.

In contrast to those discussed above, Cotton Inc. and The Institute of Molecular Agrobiology disclose the use of meristematic cells of apical shoot tips of cotton, and cotton petiole and root callus, respectively, as tissues to be transformed with either *Agrobacterium* (Cotton Inc.) or *A. tumefaciens* (Institute of Molecular Agrobiology).

Besides the mentioned U.S. patent application, Cotton Inc. has patents granted in Europe and in Australia with broader claims. These claims recite transformation of meristematic apical shoot tips of *any* plant in addition to cotton, with *any* DNA-based transforming agent. Thus, the patents are not limited to the use of *Agrobacterium* as agent for transformation.

The University of California has filed a U.S patent application describing the transformation of *Gossypium* clusters of cells or callus with *Agrobacterium*. This patent application has the most recent priority date (15 Jul 2002) among the cotton transformation patents.

Finally, patents and applications by Bayer BioScience (*Table 20-9*), which now owns Aventis CropScience, disclose the use of cotton embryogenic callus as target tissue for transformation with *Agrobacterium*. The addition of a plant phenolic compound prior or during the transformation of the cotton tissue for *vir* gene induction constitutes a disclosed improvement of cotton transformation methodology.

Table 20-9 Patents on cotton transformation

Patent No.	Issue Date*	Expiry date*	Patent Owner
US 5,846,797	8 Dec 1998	4 Oct 2015	Calgene (Monsanto)
EP 0 910 239 B1	5 Dec 2001	4 Oct 2016	Calgene (Monsanto)
AU 727910 B2	4 Jan 2001	4 Oct 2016	Calgene (Monsanto)
US 6,620,990 B1	16 Sep 2003	14 Sep 2017†	Mycogen
EP 0 344 302 B1	31 Mar 1999	2005 lapsed	Mycogen
EP 0 899 341 A2	3 Mar 1999	16 Nov 2008	Mycogen
AU 632038 B2	17 Dec 1992	16 Nov 2008	Mycogen
AU 668915 B2	23 May 1996	16 Mar 2013	Mycogen
AU 708250 B2	29 Jul 1999	23 Aug 2016	Mycogen
US 2003/208795 A1	6 Nov 2003	19 Feb 2018	Cotton Inc.
EP 1 056 334 B1	8 Sep 2004	18 Feb 2019	Cotton Inc.
AU 747514 B2	16 May 2002	18Feb 2019	Cotton Inc.

Patent No.	Issue Date*	Expiry date*	Patent Owner
EP 1 159 436 A1	5 Dec 2001	10 Mar 2019	Institute of Molecular Agrobiology
EP 1 194 579 A1	10 Apr 2002	11 Jun 2019	Institute of Molecular Agrobiology
AU 777365 B2	14 Oct 2004	10 Mar 2019	Institute of Molecular Agrobiology
AU 782198 B2	7 Jul 2005	11 Jun 2019	Institute of Molecular Agrobiology
US 2004/009601 A1	15 Jan 2004	15 Jul 2022	University of California
US 6,483,013	19 Nov 1999	19 May 2019	Bayer BioScience
EP 1 183 377 A1	6 Mar 2002	18 May 2020	Bayer BioScience
AU 772686 B2	6 May 2004	18 May 2020	Bayer BioScience

*Publication date and estimated expiry date (if the patent application is granted) are given for pending patent applications.
†U.S. patent subject to a terminal disclaimer. Thus, it is possible that the expiry date is earlier than the date provided here.

Given the thicket of proprietary technology in the area of cotton transformation, those who would like to enter this field and those who already are in it should exercise caution when deciding on the tools and methods to use for R&D on cotton transformation and generation of transgenic plants. An opinion should be sought as to whether and which licenses would need to be obtained.

3.5 *Agrobacterium* and Rhizobiaceae

A review of the key patents and claims presented above reveals an interesting fact: all these inventions are claimed in combination with *Agrobacterium* or *Agrobacterium tumefaciens*. What exactly is *Agrobacterium*? Taxonomy of bacteria is an evolving field. As more biochemical and genetic data of species become available, criteria for classifying organisms changes. Since the time that most all of the above patents were filed, *Agrobacterium tumefaciens* has been reclassified as *Rhizobium radiobacter* (Young et al. 2001). While *Agrobacterium* have long been recognized as belonging to the Rhizobiaceae group, the International Nomenclature Commission believes that *Agrobacterium* is an artificial genus and so have recommended a major overhaul of the genus. This belief is by no means universal. Considerable debate has ensued as to whether or not *Agrobacterium* is an artificial species (Farrand et al., 2003). One rationale for keeping *Agrobacterium* as a separate genus is its pathogenicity (Farrand et al. 2003).

As shown in this chapter and in the technology landscape "*Agrobacterium*-mediated transformation of plants" at the CAMBIA web site (Roa-Rodríguez and Nottenburg, 2003), the key patents lie mostly in the hands of large, international agriculture biotechnology companies. These companies not only actively sue each other for infringement but also cross-license their technologies. For example, in February 2004, Syngenta International AG and Monsanto Co. announced an agreement to cross-license proprietary *Agrobacterium*-mediated transformation technology (Jones 2005). The same month, Bayer CropScience, the Max-Planck Society and Monsanto Co. announced an agreement that ended approximately 12 years of interference. Under the agreement, the entities entered into a cross-licensing situation (Monsanto 2005) (Curiously, no U.S. patent seems to have issued after this settlement, and there is a worrisome aspect that it will have a 17-year patent term from the *date of issue*). Absent though are press releases that "Small Agri-biotech Co." has licensed transformation technology from the multi-national companies.

Rather than submit to the patent stranglehold on transformation technologies, CAMBIA in Canberra, Australia, pursued an invention to "design around" the patents. (In the interest of full disclosure, at the time of the invention, one of us (Carol Nottenburg) was in-house patent counsel at CAMBIA and remains its patent counsel.) To this end, several strains in the Rhizobiaceae family, including *Rhizobium* spp., *Sinorhizobium meliloti,* and *Mesorhizobium loti*, were made competent for gene transfer into plants by acquisition of both a disarmed Ti plasmid and a suitable binary vector. (Broothaerts et al., 2005) These non-*Agrobacterium* strains produced transgenic rice, tobacco and *Arabidopsis* plants. Patent applications for this technology (called TransBacter™) were initially filed in the United States (US 2005/289667 and US 2005/289672). Moreover, licenses for the technology are available.

4. CONCLUSIONS

Intellectual property is often not very well understood by the research community, especially those in the public sector. All too often rumors and misstatements about patents are passed along from researcher to researcher. This is an unfortunate situation; however, it is understandable as scientists are not generally familiar with reading and understanding patents.

Because of increasing importance and emphasis on patents, in the non-profit sector as well as the for-profit sector, scientists are well-advised to become familiar with basics of intellectual property, especially patents. This chapter was prepared to assist and inform researchers using or interested in *Agrobacterium*-mediated transformation of plants.

The information in this chapter is not exhaustive, but consists of selected topics in patent law and selected areas of transformation. To satisfy the myriad questions and issues raised by the research or the interests of each person who reads this chapter requires considerably more space than was allotted. Instead, this paper should open the door into the patent world and furnish platform knowledge from which additional self-directed investigation can be performed.

The patent landscape section touches on portions of the *Agrobacterium*-mediated transformation process that are most widely used. The landscape of only a single crop - cotton - is evaluated. As shown, the private sector holds many of the key patent positions. Although the basic technologies are not widely licensed by companies, many of the key patents have expired or will expire in the next few years. As demonstrated with *Rhizobium*-mediated transformation, creative thinking to come up with alternatives for the patents still in force can be empowering and fruitful.

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