How to Read a Biotech Patent

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ABSTRACT

This chapter provides an annotated description of a sample U.S. patent. The U.S. patent is a convenient model because its format is well laid out and is similar to the required formats of patents granted in other major jurisdictions, including Europe.

INTRODUCTION

A patent is an exclusionary grant of intellectual property (IP) rights, typically awarded by a government through a patent office, and effective for a limited period of time. Article 28 of the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS), binding for member countries of the World Trade Organization (WTO), states that a patent owner has the right "*to prevent third parties … from the acts of: making, using, offering for sale, selling or importing*" the protected product. If the protected invention is a process, the owner can prevent third parties not only from using the process, but also from using, offering for sale, selling, or importing "*at least the product obtained directly by that process.*" It is important to note that under TRIPS the patent owner does not have the right to practice her or his invention, only the right to prevent others from practicing it.

The TRIPS Agreement requires the time limit of the patent (*patent term*) to be at least 20 years. Most countries allow a 20-year term, starting from the date on which the application for the patent was first filed. Extensions of the patent term may be available in cases of regulatory or patent office delays that were imposed before a product is commercialized. Significantly, a patent grant is only legally binding in the country in which it was awarded.

2. PATENT PUBLICATION

Box 1 (at the end of this chapter) contains the front page of U.S. Patent No. 6,551,586,[1] and Box 2 contains extracts of U.S. Patent No. 5,723,765 (hereafter referred to as "the '765 patent").[2] A cursory review of the '765 patent reveals that it has three main sections:

- a front page, which presents bibliographic information (Box 2a, also at the end of this chapter),
- text, which describes the invention (Box 2b), and
- claims, starting in column 35 (Box 2c), which define the limit of the protected invention.[3]

2.1 *Cover Page*

The cover page primarily contains bibliographic information, historical facts about prior patent applications, and identifying elements, none of which has any legal import for interpreting the

Nottenburg C. 2007. How to Read a Biotech Patent. In *Intellectual Property Management in Health and Agricultural Innovation: A Handbook of Best Practices* (eds. A Krattiger, RT Mahoney, L Nelsen, et al.). MIHR: Oxford, U.K., and PIPRA: Davis, U.S.A. Available online at www.ipHandbook.org.

patent. The bracketed number adjacent to each data subsection is used by the patent office for internal identification purposes.

At the top of the cover page is the vital identification of Patent No. 6,551,586 (Box 1):

[12] **nature of the publication.** In this case, United States Patent and, below, the first inventor's name, Davidson et al.[4]

[10] **patent number.** In the United States, the patent number is sequentially assigned by the patent office. Prior to early 2000, the patent number was the only publication number.[5]

[45] **date the patent was issued.** This date (in this case, Apr. 22, 2003) is important for two reasons: (1) if the patent was not published as a patent application, then this is the date it became public knowledge and thus prior art for non-U.S. jurisdictions;[6] and (2) in the case of applications filed in the United States prior to the General Agreement on Tariffs and Trade (GATT) treaty (8 June 1995), as in this example, it is the date that initiates the patent term.[7]

The remainder of the front page presents the main bibliographic data:

[54] **title of the patent.** Should be representative of the content, is written by the inventors or their attorney and has no impact on the interpretation of the patent. In many cases, the title is wishful thinking.

[75] **inventor(s)' name(s) and place(s) of residence**. For patent purposes, the order of the names is not important; the applicant determines the order, not the patent office. In the United States, the inventors and their assignees (see below) can independently practice or license all of the patent rights without the permission of the other inventors. It is important to note that Australia and Europe, among other countries, have the opposite rule: an inventor cannot practice or license patent rights without the permission of the other inventors.

[73] **assignee(s) and his/her/their place(s) of business.**[8] An assignee is an owner of the patent because an inventor or inventors have signed over the rights to the invention. Typically, an inventor who is also an employee in a company or university is obligated to formally assign invention rights to the employer. In the United States, such assignment documents are recorded by the patent office and are publicly accessible, once the patent application is published. The identity of the owner of a patent is public knowledge, but the identity of those who have licensed a patent is not necessarily available to the public.

[21] **application number.** Assigned by the patent office

[22] **filing date of the subject patent application.** If there are no related U.S. application data (see below), this date is used to determine the beginning of the 20-year patent term.

[63] **related applications.** It is from these related applications that the patent claims priority. The United States is unusual in allowing applications to be refiled, either with or without new disclosure. A refiled application is called a *continuation*, or, if it contains new disclosure, a *continuation-in-part.* U.S. Patent 6,551,586 was filed on 27 November 1998 (field 21); however, an earlier application filed on Jan. 29, 1996 (serial number 08/593,006) contained at least some of the disclosure of the subject patent; in other words, this patent is a continuation-in-part of the earlier application.[9] As the patent term begins from the filing date of the earlier application, this patent expires on 29 January 2016.

[60] **provisional applications.** The filing date of a provisional application does not affect the patent term, but it is critical for considering prior art that might affect patentability.

[51] **International Patent Classification (IPC) code.** A combination of letters and numbers.[10] A patent application's IPC code is assigned by the national or regional patent office that publishes it. The IPC is an indispensable tool for patent-issuing authorities, potential inventors, attorneys, and others

concerned with the application or development of technology.

[52] **U. S. Classification Code.** Assigned by the U.S. Patent Office.

[58] **field of search.** Contains the U.S. classification codes that the examiner used to perform searches for prior art.

[56] **references.** Subdivided into U.S. patent documents, foreign patent documents, and other publications that the examiner considered when evaluating the patentability of the claimed invention.[11]

[no number] **examiners.** The names of the primary examiner at the patent office and the assistant examiner (if any).

[74] **attorney, agent, or firm.** Representatives of the inventor or assignee.

[57] **abstract.** A short description of the invention written by the applicant(s). The abstract enables the patent office and the public to quickly determine the content of the patent. Although the "abstract shall not be used for interpreting the scope of the claims," courts have taken it under consideration on one or two occasions.[12]

[no number] **number of claims and drawings**. In this patent, there are eight claims and 13 drawings.

2.2 *Text of the patent*

The text of the patent is also called the *disclosure* (In the United States, it may also be called the *specification*). According to the TRIPS Agreement, the invention must be disclosed "*in a manner sufficiently clear and complete for the invention to be carried out by a person skilled in the art*" (Article 29.1). Each country specifies its own requirements; the U.S. Patent Office requires a written description of the invention, a so-called *enablement*, and a so-called *best mode*.[13]

The layout of the patent varies somewhat from country to country. The United States and Europe have a similar required layout, except that (b) and (c) below are unique to the United States:

a. title of the invention
b. cross-reference to related applications
c. statement regarding federally sponsored research, if applicable
d. background of the invention
e. summary of the invention
f. description of the drawings
g. detailed description of the invention
h. listing of relevant nucleotide and peptide sequences
i. claims defining the scope of the invention

2.3 *Background of the invention*

The *background* is typically drafted for the patent examiner and a jury audience, in case the patent is ever litigated. It compares selected art in the field with the current invention and explains why the current invention is necessary. As one can see from downloading the full patent (and the extract on Box 2b), a large part of the background of the '765 patent explains the technologies of several relevant references.

2.4 *Summary of the invention*

The *summary of the invention* is distinct from the abstract and summarizes the scope of the invention (the claims). It often discusses the advantages of the invention or explains how it solves problems existing in the art.

The summary of the '765 patent discusses the invention as embodied in the claims. It also describes the specific advantages of the invention (see, for example, col. 1, lines 61–64; col. 2, lines 1–6; and col. 2, lines 51–54; not shown here). The inventors believe that the advantages of their invention include: positive control of gene expression by an external stimulus without the need for continued application of the stimulus, the ability to grow plants under various conditions with expression of different phenotypes, and the ability to develop seed where a trait is desirable only in the first or in subsequent generations.

2.5 *Detailed description of the invention*

The *detailed description* of the invention is the most substantial section of the patent. It is made up of two sections: the first section (col. 2, line 58–col. 8, line 40) explains the invention and how to practice it; the second section (col. 8, line 43 to col. 20, line 33) provides specific examples of the invention. Many new readers mistakenly assume that examples are intended to delineate how

the invention must be practiced or used, but this is not the case. The examples are merely meant *"to illustrate, but in no way to limit, the claimed invention."* While examples are not required by the patentability statutes, in practice the enablement requirement is difficult, if not impossible, to satisfy for biotechnology inventions without examples.

Paragraphs 1 and 2 describe the *broadest concept* of the invention, explaining how DNA constructs are used to create transgenic plants and then describing how the invention works to control gene expression.

Paragraphs 3–11 (col. 4, lines–1-39) set forth some *definitions* of key terms. Definitions are extremely important in interpreting the scope of the claims. For example, this patent defines the term *"plant-active promoter"* as *"any promoter that is active in cells of a plant of interest."* The promoter can be derived not only from plants, but also from viruses, bacteria, fungi, and so on. This list only provides examples of sources from which promoters can be derived and the inventors do not intend it to be exhaustive.

The next three paragraphs (col. 4, line 10–col. 5, line 47) describe *preferred embodiments* of the invention. These are usually more limited versions of the broadest concept. They provide a "safety net" for the inventors in case the broader concept is not patentable.

In paragraph 12 (col. 4, line 10), the preferred embodiment is a "*transiently-active promoter*" (active only in late embryogenesis) and a "*gene linked to this promoter"* that is a "*lethal gene."* The next two paragraphs describe an embodiment in which a pair of transgenic plants is crossed to produce progeny that display an altered phenotype, and an embodiment in which the recombinase is linked to an inducible promoter. In addition, the paragraph provides a few examples of inducible promoters.

The next several paragraphs (col. 5, line 48–col. 7, line 48) define and give examples of some of the important elements of the **claim** (transiently active promoters, genes whose expression results in a detectable phenotype, lethal genes, blocking sequences, repressor and repressible promoters, and recombinase/excision sequences). These paragraphs support the scope of the inventors' claim. In col. 6, lines 47-60, the inventors define "lethal gene," then provide a single example (saporin-6, which acts by cleaving the large ribosomal RNA molecule and thus inhibiting protein synthesis). Overall, the disclosure in this patent is relatively thin.

The next four paragraphs (col. 7, line 49–col. 8, line 29) discuss the techniques that can be used to transform the target plant (col. 7, lines 62-65). This is a classic style of patent drafting and clearly indicates that the actual method used for transformation is not critical. Other methods of introducing the DNA constructs are described in paragraphs 21–23.

Finally, paragraph 24 (col. 8, lines 30–40) discusses suitable plant species. The inventors do not believe that the process they describe need be limited to particular species.

The next section presents the **examples**. Typically, the examples show how one or more specific embodiments of the invention could be put into practice. The examples may or may not be based on successful experiments performed by the inventors. If the experiments have been performed, the examples are called "*working"* examples; if not, the examples are called "*prophetic*" examples and are always written in the present or future tense. In the '765 patent, examples 1–6 (Box 2c) describe the cloning of three DNA sequences: (1) a lethal gene, saporin-6, under control of a late embryogenesis promoter, and separated by a blocking sequence, LOX; (2) a tet repressor gene under the control of a CaMV 35S promoter; and (3) a CRE (recombinase) gene under the control of a tetracycline-derepressible 35S promoter. Examples 7–10, which describe the introduction of the constructs into plants and activation of the system are written in a future tense because the relevant experiments were not performed as of the filing date of the application.

2.6 *Sequence listing*

The sequence listing includes all nucleic acid molecules mentioned in the patent application that are comprised of at least 10 nucleotides and all peptide sequences comprised of at least four amino acids.

2.7 *Claims*

The claims must "particularly point out and distinctly claim the subject matter which the applicant regards as his invention."[14] The claims define the boundaries of the patent owner's right against possible infringement.

Each claim must be written as a single sentence. A claim is presented in two parts, the **preamble** and the **body**, with a transition word or phrase between them.

- The **preamble** is an introductory statement that names the subject of the claim. For example, the preamble of claim 1. is: "*A method for making a genetically modified plant."*
- The **body** of the claim describes the elements or steps that compose the claimed subject. In claim one, the body of the claim consists of the steps of "*stably transforming …*" and "*regenerating …*"

The transition words or phrases between the preamble and the body of the claim indicate whether the claim encompasses *at least* the listed elements or steps or whether the claim encompasses *only* the listed elements or steps. The transition word *comprising* means "*including the following elements but not excluding others.*"[15] In claim one of the '765 patent, *comprising* is used in two places: (1) in the preamble ("*A method... comprising …*") and (2) in the body ("*a … DNA sequence comprising …*"). If someone were to use the patented method with small changes—additional steps or a DNA sequence with additional elements, for example—he or she would still be infringing on the claim.

In contrast, the transition "consisting of" limits the claim scope to the recited elements or steps. If the claim were "a DNA sequence consisting of ACGTGC," a person would be able to make the DNA sequence "ACGTGCTA" without infringing on the claim.

The meaning of the transition phrase *consisting essentially of* falls somewhere between the other two. It indicates that the patent does not regulate the use of variables that do not affect the basic and novel characteristics of the method or product. It is not often used in biotechnology patents.

Furthermore, there are two kinds of claims: independent and dependent. An *independent claim* (for example, claims 1, 10, 19, 28, 37, 46, and 55) includes all necessary limitations and does not depend on nor include limitations from any other claim. Curiously, although *dependent claim* is defined in the patent rules of the United States, *independent claim* is not. U.S. patent rules state that a dependent claim must "*refer[s] back to and further limit[s] another claim or claims.*"[16] Moreover, a dependent claim "*shall be construed to include all the limitations of the claim incorporated by reference.*"[17]

Claim 4. of the '765 patent is an instructive dependent claim. Since claim 4. depends upon claim 1., the transiently active promoter is limited to the LEA promoter. All other elements of claim 1. remain intact and are not limited any further.

Dependent claims serve several very important purposes. In the first place, they help with so-called claim differentiation: in patent law, no two claims can have the same scope. Therefore, the transiently active promoter in claim 1. must encompass more than the LEA promoter mentioned in claim 1.; otherwise, claims 1. and 4. would have the same scope. Dependent claims are also written to protect specific embodiments of an invention. Should the main claim fail in a court case, a dependent claim may still stand. In addition, it is easier for a jury to have the alleged infringing activity clearly spelled out.

3. CONCLUSION

Patent documents contain substantial information that has value to researchers, even if infringement isn't an issue. While many patent documents are readily available on the Internet for free—generally from patent offices—they may not always be capable of being understood or appreciated. One reason for inaccessibility is that patent applications are written in a special style that does not follow the conventions of scientific or technical literature. To understand a patent document, a roadmap helps until the route is familiar.

This chapter provides a roadmap for reading a patent document. The various sections of a

document are explained in view of their purposes. The purposes especially delineate the amount and type of reliance that can be made of each of the sections. Each section contains its own set of useful information. The importance of the claims is paramount for knowing the boundaries of the patent right, however, interpreting claims requires more of a roadmap than this chapter provides. Even without a full appreciation of claim boundaries, much information may still be obtained from patent documents. ■

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1 patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=6,551,586.PN.&OS=PN/6,551,586&RS=PN/6,551,586.

2 patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=5,723,765.PN.&OS=PN/5,723,765&RS=PN/5,723,765.

3 More typically, patents contain four sections with drawings comprising the last section.

4 In the United States, a patent application must be filed for in the name of the inventors. In most of the rest of the world, patent applications can be filed for in the name of the inventors or in the name of the assignee(s).

5 Patent applications are generally published 18 months after the earliest priority application date. Depending on the country, the publication number may or may not differ from the patent number. If the numbers are the same, a suffix is usually used to denote the status of the application. For example, in Europe, the publication and patent numbers are the same, but the suffix *A* is used to indicate an application and *B* is used to indicate an issued patent.

6 In the United States, inventions that are disclosed but not claimed are prior art against other U.S. applications and patents, as of their filing date. 35 U.S.C. § 102(e).

7 Before the GATT treaty implementation, the patent term in the United States was 17 years from the date of issuance. Under GATT, the patent term is 20 years from the earliest claimed priority date.

8 An assignee in the United States is called an applicant in the rest of the world.

9 Priority applications determine both patent term and which prior art can be applied in a patent examination. A particular claim has a priority date as of the earliest application that contains the patentable subject matter. Art available after the priority date cannot be cited against the claim. In practice, U.S. examiners rarely determine the priority date of a claim, whereas European examiners frequently review priority applications to determine priority dates of claims.

10 The IPC system is a hierarchical classification system administered by the World Intellectual Property Organization. For more information on international classifications and IPC, see WIPO's Web site at www.wipo.org.

11 In the United States, each individual associated with the filing and prosecution of a patent application (for example, inventor, patent attorney, assignee) has a duty to disclose all material information to the patent office.

12 37 C.F.R. 1.72(b).

13 The written description shows that the inventor has the invention in mind. The enablement describes the invention clearly enough that one skilled in the art can understand it, make it, and use it without undue experimentation. In the best mode, an inventor discloses the most effective method of practicing or using the claimed invention. The patent office does not ask applicants whether or not they have disclosed the best mode, a question which usually only arises during litigation.

14 35 U.S.C. § 112.

15 Equivalent words are *having* and *including*, but most practitioners use *comprising* because it has become a standard term of art.

16 37 C.F.R. 1.75(c).

17 See *supra* note 16.

Box 1: Sample Front Page of Issued U.S. Patent

US006551586B1

(12) **United States Patent**
Davidson et al.

(10) **Patent No.: US 6,551,586 B1**
(45) **Date of Patent: Apr. 22, 2003**

(54) **MALARIA VACCINE BASED UPON THE ADDITION OF A MSA1 PEPTIDE**

(75) Inventors: **Eugene A. Davidson**, Washington, DC (US); **Shutong Yang**, Washington, DC (US)

(73) Assignee: **Georgetown University**, Washington, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/117,415**

(22) Filed: **Nov. 27, 1998**

Related U.S. Application Data

(63) Continuation-in-part of application No. 08/593,006, filed on Jan. 29, 1996, now abandoned.
(60) Provisional application No. PCT/US97/01395, filed on Jan. 29, 1996.

(51) **Int. Cl.**[7] **A01N 63/00**
(52) **U.S. Cl.** **424/93.2**; 514/44; 435/320.1; 435/69.1; 435/325; 435/455
(58) **Field of Search** 514/44; 536/23.1, 536/23.4, 24.1; 435/69.1, 320.1, 325, 455; 424/93.1, 93.2, 184.1

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Primary Examiner—Dave Trong Nguyen
(74) *Attorney, Agent, or Firm*—Henry D. Coleman; Coleman Sudol Sapone P.C.

(57) **ABSTRACT**

The present invention relates to an expression vector which expresses a malaria MSA1 peptide in combination with a signal peptide and anchor peptide in a host animal. The MSA1 peptide is combined with a signal peptide and anchor peptide for expression. Chimeric peptides being expressed with both signal peptides and anchor peptides were the most effective in eliciting an immunogenic response from a vaccinated host.

8 Claims, 13 Drawing Sheets

BOX 2A: FRONT PAGE OF PATENT NO. 5,723,765

US005723765A

United States Patent [19]

Oliver et al.

[11] **Patent Number: 5,723,765**

[45] **Date of Patent: Mar. 3, 1998**

[54] **CONTROL OF PLANT GENE EXPRESSION**

[75] Inventors: **Melvin John Oliver**, Lubbock; **Jerry Edwin Quisenberry**, Idalou; **Norma Lee Glover Trolinder**, Quanah, all of Tex.; **Don Lee Keim**, Leland, Miss.

[73] Assignees: **Delta and Pine Land Co.**, Scott, Miss.; **The United States of America as represented by the Secretary of Agriculture**, Washington, D.C.

[21] Appl. No.: **477,559**

[22] Filed: **Jun. 7, 1995**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 283,604, Aug. 1, 1994, abandoned.

[51] **Int. Cl.**[6] **C12N 15/29**; C12N 15/82; A01H 4/00; A01H 5/00

[52] **U.S. Cl.** **800/205**; 800/250; 536/24.1; 536/23.6; 536/24.5; 435/320.1; 435/240.4; 435/172.3

[58] **Field of Search** 536/24.1, 23.6, 536/24.5; 435/320.1, 240.4, 172.3; 800/205, 250

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[57] **ABSTRACT**

A method for making a genetically modified plant comprising regenerating a whole plant from a plant cell that has been transfected with DNA sequences comprising a first gene whose expression results in an altered plant phenotype linked to a transiently active promoter, the gene and promoter being separated by a blocking sequence flanked on either side by specific excision sequences, a second gene that encodes a recombinase specific for the specific excision sequences linked to a repressible promoter, and a third gene that encodes the repressor specific for the repressible promoter. Also a method for making a genetically modified hybrid plant by hybridizing a first plant regenerated from a plant cell that has been transfected with DNA sequences comprising a first gene whose expression results in an altered plant phenotype linked to a transiently active promoter, the gene and promoter being separated by a blocking sequence flanked on either side by specific excision sequences to a second plant regenerated from a second plant cell that has been transfected with DNA sequences comprising a second gene that encodes a recombinase specific for the specific excision sequences linked to a promoter that is active during seed germination, and growing a hybrid plant from the hybrid seed. Plant cells, plant tissues, plant seed and whole plants containing the above DNA sequences are also claimed.

55 Claims, No Drawings

Box 2b: Extract of Patent No. 5,723,765 Describing the Invention

CONTROL OF PLANT GENE EXPRESSION

This is a continuation-in-part application of application Ser. No. 08/283,604, filed on Aug. 1, 1994, now abandoned.

BACKGROUND OF THE INVENTION

This invention relates to certain transgenic plants and involves a method of creating transgenic plants with controllable genes. More particularly, the invention relates to transgenic plants that have been modified such that expression of a desired introduced gene can be limited to a particular stage of plant development, a particular plant tissue, particular environmental conditions, or a particular time or location, or a combination of these situations.

Various gene expression control elements that are operable in one or more species of organisms are known. For example, PCT Application WO 90/08826 (Bridges, et al.) discloses an inducible gene promoter that is responsive to an exogenous chemical inducer, called a "gene switch." This promoter can be linked to a gene and introduced into a plant. The gene can be selectively expressed by application of the chemical inducer to activate the promoter directly.

PCT application WO 94/03619 (Bright, et al. discloses a gene cascade consisting of a gene switch linked to a repressor gene and a repressible operator linked to a disrupter protein capable of disrupting plant development. Growth of the plant can be controlled by the application or withholding of a chemical inducer. While the inducer is present, the repressor is expressed, the promoter attached to the disrupter gene is repressed, the disrupter protein is not expressed, thereby allowing the plant to grow normally. If the chemical inducer is withheld, the gene switch is turned off, the repressible promoter is not repressed, so the disrupter protein is expressed and plant development is disrupted. This system is said to be useful for controlling the escape of plants into the wild by making their continued growth and development dependent on the continued application of a chemical inducer, and to mitigate the problem of preharvest sprouting of grains by withholding the chemical inducer at the last stages of seed development.

Gatz and Quail (1988) and Gatz, et al. (1992), (Hoppe-Seyler), 372:659-660 (1991), disclose a plant-active repressor-operator system that is controlled by the application of tetracycline. The system consists of the Tn10 tet repressor gene, and a cauliflower mosaic virus (CaMV) 35S promoter, modified to contain two tet operons and linked to the chloramphenicol acetyltransferase (cat) gene (Gatz and Quail, 1988), or modified to contain three tet operons and linked to the beta-glucuronidase (gus) gene (Gatz, et al., 1992). So long as the Tn10 tet repressor gene is active, the modified promoter is repressed by the interaction of the repressor with the tet operons, and the cat or gus gene is not expressed. The presence of tetracycline inhibits repressor binding, enabling expression of the cat or gus gene.

SUMMARY OF THE INVENTION

The present invention involves, in one embodiment, the creation of a transgenic plant that contains a gene whose expression can be controlled by application of an external stimulus. This system achieves a positive control of gene expression by an external stimulus, without the need for continued application of the external stimulus to maintain gene expression. The present invention also involves, in a second embodiment, the creation of transgenic parental plants that are hybridized to produce a progeny plant expressing a gene not expressed in either parent. By controlling the expression of genes that affect the plant phenotype, it is possible to grow plants under one set of conditions or in one environment where one phenotype is advantageous, then either move the plant or plant its seed under another set of conditions or in another environment where a different phenotype is advantageous. This technique has particular utility in agricultural and horticultural applications.

In accordance with one embodiment of the invention, a series of sequences is introduced into a plant that includes a transiently-active promoter linked to a structural gene, the promoter and structural gene being separated by a blocking sequence that is in turn bounded on either side by specific excision sequences, a repressible promoter operably linked to a gene encoding a site-specific recombinase capable of recognizing the specific excision sequences, and a gene encoding a repressor specific for the repressible promoter whose function is sensitive to an external stimulus. Without application of the external stimulus, the structural gene is not expressed. Upon application of the stimulus, repressor function is inhibited, the recombinase is expressed and effects the removal of the blocking sequence at the specific excision sequences, thereby directly linking the structural gene and the transiently-active promoter.

In a modification of this embodiment, the sequences encoding the recombinase can be introduced separately into the plant via a viral vector.

In an alternative embodiment, no repressor gene or repressible promotor is used. Instead, the recombinase gene is linked to a germination-specific promotor and introduced into a separate plant from the other sequences. The plant containing the transiently-active promotor, blocking sequence, and structural gene is then hybridized with the plant containing the recombinase gene, producing progeny that contain all of the sequences. When the second transiently-active promotor becomes active, the recombinase removes the blocking sequence in the progeny, allowing expression of the structural gene in the progeny, whereas it was not expressed in either parent.

In still another embodiment, the recombinase gene is simply linked to an inducible promoter. Exposure of the plant to the induce specific for the inducible promoter leads to the expression of the recombinase gene and the excision of the blocking sequence.

In all of these embodiments, the structural gene is expressed when the transiently-active promoter becomes active in the normal course of growth and development, and will continue to be expressed so long as the transiently-active promoter is active, without the necessity of continuous external stimulation. This system is particularly useful for developing seed, where a particular trait is only desired during the first generation of plants grown from that seed, or a trait is desired only in subsequent generations.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to a method of creating transgenic plants wherein the expression of certain plant traits is ultimately under external control. In one embodiment the control is achieved through application of an external stimulus; in another embodiment it is achieved through hybridization, in still another embodiment it is achieved by direct introduction of a recombinase or recombinase gene into a plant. The transgenic plants of the present invention are prepared by introducing into their genome a series of functionally interrelated DNA sequences, containing the

Box 2c: Extract of Patent No. 5,723,765 Containing the Claims

-continued

(i i i) HYPOTHETICAL: NO

(i v) ANTI-SENSE: NO

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GATCCATAAC TTCGTTATAA TGTATGCTAT ACGAAGTTAT 40

We claim:

1. A method for making a genetically modified plant comprising

stably transforming a plant cell with a first DNA sequence comprising a first gene whose expression results in an altered plant phenotype, and a transiently-active promotor, the first gene and the transiently-active promotor being operably linked to one another, but separated by a blocking sequence that is flanked by specific excision sequences, such that the presence of the blocking sequence prevents the expression of the first gene, a second DNA sequence comprising a second gene that encodes a recombinase specific for the specific excision sequences flanking the blocking sequence of the first DNA sequence, and a repressible promotor operably linked in functional relation to the second gene, and a third DNA sequence comprising a third gene that encodes a repressor specific for the repressible promotor of the second DNA sequence, the third sequence being linked to a plant-active promoter;

regenerating a whole plant from the plant cell.

2. A method according to claim **1**, wherein the blocking sequence comprises the third DNA sequence.

3. A method according to claim **1** or claim **2**, wherein

the transiently-active promotor is selected from the group comprising a promotor active in late embryogenesis, in seed development, in flower development, in leaf development, in root development, in vascular tissue development, in pollen development, after wounding, during heat or cold stress, during water stress, or during or after exposure to heavy metals,

the first gene is selected from the group comprising a lethal gene, an insecticidal gene, a fungistatic gene, a fungicidal gene, a bacteriocidal gene, a drought resistance gene, a protein product gene or a gene that alters secondary metabolism,

the specific signal sequences are selected from the group comprising LOX sequences and se quences recognizable by either flippase, resolvase, FLP, SSV1-encoded integrase, or transposase,

the second gene encodes a specific recombinase selected from the group comprising CRE, flippase, resolvase, FLP, SSV1-encoded integrase, and transposase,

the third gene encodes a repressor selected from the group comprising the Tn10 tet repressor, and the lac operator-repressor system,

the repressible promotor is selected from the group comprising a 35S promotor modified to contain one or more tet operons, a modified ubiquitin promotor, a modified MAS promotor and a modified NOS promotor.

4. A method according to claim **3**, wherein the transiently active promotor is the LEA promotor.

5. A method according to claim **3**, wherein the first gene encodes ribosomal inhibitor protein (RIP).

6. A method according to claim **3**, wherein the specific excision signal sequences are LOX sequences and the second gene encodes CRE.

7. A method according the claim **3**, wherein the third gene encodes the Tn10 tet repressor.

8. A method according to claim **3**, wherein the repressible promotor is a 35S promotor modified to contain three tet operons.

9. A method according to claim **2** wherein the plant is cotton, the transiently active promotor is a LEA promotor, the specific excision signal sequences are LOX sequences, the first gene encodes ribosomal inhibitor protein (RIP), the repressible promotor is a 35S promotor modified to contain three tet operons, the second gene encodes CRE, and the third DNA sequence is the Tn10 tet repressor gene.

10. A method for producing seed that is incapable of germination, comprising

stably transforming a plant cell with a first DNA sequence comprising a lethal gene and a promotor that is active in late embryogenesis, the lethal gene and the late embryogenesis promotor being in functional relation to one another, but separated by a blocking sequence that is flanked by specific excision sequences, such that the presence of the blocking sequence prevents the expression of the lethal gene, a second DNA sequence comprising a gene that encodes a recombinase specific for the specific excision sequences flanking the blocking sequence of the first DNA sequence, and a repressible promotor linked in functional relation to the specific recombinase gene, and a third DNA sequence comprising a gene that encodes a repressor specific for the repressible promotor of the second DNA sequence, third sequence being linked to a plant-active promoter;

regenerating a whole plant from the plant cell;

allowing the regenerated whole plant to produce a first generation seed;

exposing the first generation seed to a stimulus that blocks the function of the repressor, such that the repressor element no longer inhibits expression of the specific recombinase gene, thereby allowing expression of the specific recombinase and excision of the blocking sequence of the first DNA sequence at the specific excision sequences, resulting in the direct functional linkage of the late embryogenesis promotor with the lethal gene;

germinating the first generation seed to produce a first generation plant expressing the late embryogenesis promotor/lethal gene sequence;

allowing the plant to produce second generation seed, whereby in the course of embryogenesis, the late embryogenesis promotor becomes active, permitting expression of the lethal gene in the second generation seed, thereby rendering the second generation seed incapable of germination.

11. A method according to claim **10**, wherein the blocking sequence comprises the third DNA sequence.

12. A method according to claim **10** or claim **11**, wherein the seed is cotton seed;

the late embryogenesis promotor is selected from the group comprising a LEA promotor and a promoter other than LEA that is active in late embryogenesis,