

# Resistance to Phosphinothricin

DISCLAIMER  
THE CONTENTS OF THESE PAGES ARE INFORMATIONAL ONLY  
AND SHOULD NOT BE SUBSTITUTED FOR LEGAL ADVICE

Authored by:  
Jorge Mayer, Jade Sharples, and Carol Nottenburg

Production by "The Team"

CAMBIA Intellectual Property Resource

GPO Box 3200  
Canberra ACT 2601  
AUSTRALIA

Phone: +61 2 6246 4505  
Fax: +61 2 6246 4533  
Email: [cambialP@cambia.org](mailto:cambialP@cambia.org)

© 2004 CAMBIA

Permission is granted to download and copy for personal use and to redistribute without charge, provided that this is not done for profit, no text is changed, and the copyright notice remains with the text.

CHAPTER 1 .....	3
Introduction: Herbicide resistance.....	3
CHAPTER 2 .....	7
Introduction: The <i>bar</i> gene .....	7
Patent issues surrounding the <i>bar</i> gene .....	8
Who owns the dominant patents on the <i>bar</i> gene? .....	9
Dominant <i>bar</i> gene patents.....	10
A <i>bar</i> gene from <i>Streptomyces</i> sp. ....	15
A <i>bar</i> gene from <i>Streptomyces viridochromogenes</i> .....	19
A <i>bar</i> gene from <i>Alcaligenes</i> sp and other gram-negative bacteria ...	23
A <i>bar</i> gene in combination with a virus resistance gene.....	25
APPENDIX .....	29
1. Dominant <i>bar</i> gene patents.....	30
EP 242 236 B2 .....	30
US 5,561,236 .....	32
2. <i>Bar</i> genes from <i>Streptomyces</i> sp. ....	32
EP 242 246 B1 .....	32
US 5,646,024 .....	34
US 5,648,477 .....	35
3. <i>Bar</i> genes from <i>Streptomyces viridochromogenes</i> .....	36
EP 257 542 B1 .....	36
EP 275 957 B1 .....	36
US 5,273,894 .....	37
US 5,276,268 .....	37
US 5,637,489 .....	38
US 5,879,903 .....	39
4. <i>Bar</i> genes from <i>Alcaligenes</i> sp and other gram-negative bacteria	39
EP 290 986 B1 .....	39
US 5,077,399 .....	40
5. <i>Bar</i> genes in combination with a virus resistance gene .....	41
EP 513 054 B1 .....	41
EP 899 340 A2 .....	41
EP 714 237 B1 .....	41
US 5,633,434 .....	42
US 5,792,926 .....	42
US 5,908,810 .....	43
US 5,739,082 .....	43

# CHAPTER 1

## Introduction: Herbicide resistance

Genetically modified crops are the most rapidly adopted technology in agricultural history due to the social and economic benefits these crops may offer. Crops that are genetically altered to be tolerant to herbicide, followed by crops that are resistant to insects, represent the very first advance in agricultural biotechnology that has been successfully commercially exploited worldwide.



Weeds are one of the major problems encountered in crop management. Weeds compete with crops for water and nutrients and, as a result, decrease farming yields and productivity. Given the harmful economic implications of poor weed management, it is hardly surprising that the production of herbicides constitutes the majority of the agrichemical industry.

Until the emergence of genetically modified crops, selective herbicides (i.e. herbicides that only kill a specific weed therefore allowing it to safely come into contact with a planted crop) dominated the market. The development of selective herbicides is not an easy task and for this reason only a few common weed species could be contained. Given that each weed requires a different herbicide, herbicide application has been frequent, in large volumes and very costly.

The advent of herbicide resistant crops has caused a major shake-up in the agrichemical industry. Selective herbicides are no longer in high demand. Instead, herbicide resistant crops are planted and otherwise non-selective (broad spectrum) herbicides are used for weed management. Provided that the field crops are genetically modified to carry the gene/s for herbicide resistance, these broad-spectrum herbicides will not harm the crop. Broad-spectrum herbicides, such as glufosinate and glyphosate, are comparably biodegradable, display low levels of toxicity, and to date, weeds have shown minimal resistance to repeated applications.

Resistance to these broad-spectrum herbicides depends upon the genes that have been inserted into the crop plant. This white paper focuses primarily on the patent landscape surrounding the *bar* gene which confers resistance to the broad-spectrum herbicide, glufosinate.

The table below shows the most commonly used broad-spectrum herbicides and the genes that are inserted in crop plants in order to confer resistance.

<b>Herbicide</b>	<b>Resistance Gene</b>	<b>Gene Source</b>
Glufosinate, phosphinothricin, bialaphos	<i>bar</i> , PAT (phosphinothricin acetyl transferase)	<i>Streptomyces</i> sp. <i>Alcaligenes</i> sp.
Glyphosate	<i>aroA</i> , EPSPS (3-enoyl pyruvyl shikimate 5-phosphate synthase) gene	<i>Agrobacterium</i> sp.
Bromoxynil	BXN (Bromoxynil nitrilase)	<i>Klebsiella pneumoniae</i>
Sulfonamides	DHPS (dihydropteroate synthase), <i>sul</i>	Broad host range plasmid
Sulfonylurea	ALS (acetolactate synthase)	<i>Nicotiana tabacum</i>

In 2003, the global area planted with transgenic crops increased for the seventh consecutive year. According to statistics released by the International Service for the Acquisition of Agri-biotech Applications (ISAAA), the total global area of transgenic crops was 67.7 million hectares, a 40-fold increase from 1996 and a 15% increase since 2002.

Approximately six million farmers in 18 countries chose to grow transgenic crops in 2003. The majority of the growth occurred in the United States (63%), Argentina (20.5%), Canada (6.5%) and Brazil (4.4%). Almost one third (30%) of the global acreage was grown in developing countries. While the number of countries growing transgenic crops is constantly increasing, so too are the varieties of transgenic crops being approved for growth. In 2003, Brazil and the Philippines approved the planting of specific varieties of transgenic crops for the first time.

In 2003, herbicide resistant crops made up 73% of the total genetically-modified (GM) crop growing area, while insect resistant crops constituted 18%. GM crops containing genes for both herbicide resistance and insect resistance comprised 8% of the total GM crop growing area.

The table below compares the total area farmed worldwide by the dominant herbicide resistant crops in 2003 and the area farmed by the same crop regardless of its genetic constitution.

<b>Crop</b>	<b>Planted Crop Area (mha)*</b>	<b>Herbicide Resistant Crop Area (mha)*</b>	<b>Herbicide resistant crop area as % of total crop area</b>
Corn	15.5	3.2	20.6%
Soybean	41.4	41.4	100.0%
Cotton	7.2	1.5	20.8%
Canola	3.6	3.6	100.0%

\*mha = million hectares

While the statistics for 2004 have not been released at the time of writing, it is expected that the overall global area of transgenic crops and the number of countries growing transgenic crops will increase.

Currently, the agricultural GM market is dominated by a single company, Monsanto. Monsanto produces approximately 90% of genetically engineered crops worldwide. This most likely reflects the ownership by Monsanto of patents on the *bar* gene which confers herbicide resistance as well as patent ownership of various *Bt* toxin genes for insect resistance. It also indicates that Monsanto not only has ownership of technology, but also has access to enabling technologies that facilitate the development and commercialization of transgenic crops. Another four companies, Syngenta, Bayer Crop Science, Dow and DuPont produce the remaining 10% of transgenic crops.

The table below shows the major manufacturers of the most common commercially grown herbicide resistant crops.

<b>Crop</b>	<b>Glyphosate Resistance</b>	<b>Glufosinate Resistance</b>	<b>Sulfonylurea Resistance</b>	<b>Bromoxynil Resistance</b>
Sugar beet ( <i>Beta vulgaris</i> )	Mon	BCS		
Argentine canola ( <i>Brassica napus</i> )	Mon	BCS	PHB*	BCS
Rapeseed/Canola ( <i>Brassica napus</i> )	Mon			
Carnation ( <i>Dianthus caryophyllus</i> )			Florigene	

Soybean ( <i>Glycine max</i> )	Mon	BCS		
Cotton ( <i>Gossypum hirsutum</i> )	Mon		DuPont	Calgene
Flax, linseed ( <i>Linum ussitatum</i> )			US	
Tobacco ( <i>Nicotiana tabacum</i> )				SNETA
Rice ( <i>Oryza sativa</i> )		BCS	BASF	
Wheat ( <i>Triticum aestivum</i> )			BASF	
Corn ( <i>Zea mays</i> )	Mon	PHB, DK, Syn, BCS, Myc	PHB <sup>SC</sup> , Syn	
Chicory ( <i>Chicorium intybus</i> )		BZ		

**BCS**, Bayer Crop Science; **BZ** Bejo Zaden; **DK**, DeKalb Genetics; **Mon**, Monsanto; **Myc**, Mycogen (Dow AgroSciences); **PHB**, Pioneer Hi-Bred (DuPont); **SNETA**, Societe Nationale d'Exploitation des Tabacs et Allumettes; **Syn**, Syngenta; **US**, University of Saskatchewan Research Center; \*, obtained through chemical mutagenesis; <sup>SC</sup>, obtained through somaclonal variation (data obtained from <http://www.agbios.com/>).

## CHAPTER 2

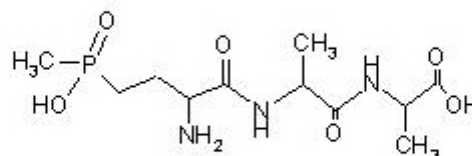
### Introduction: The *bar* gene

Members of the genus *Streptomyces* (Actinobacteria: Actinomycetales) produce hundreds of antibiotics, one of which is bialaphos (also known as bilanafos or PTT). The chemical structure of bialaphos can be seen below. It consists of a glutamic acid analogue moiety, called phosphinothricin [PTC or glufosinate = 2-amino-4-(hydroxymethylphosphinyl) butanoate] and two alanine residues.

Bialaphos is an inhibitor of the key enzyme in the nitrogen assimilation pathway, glutamine synthetase (GS). It becomes active after removal of the alanine residues by intracellular peptidases. The remaining glufosinate

compound inhibits GS and as a result, leads to accumulation of toxic levels of ammonia in both bacteria and plant cells. The biochemical and toxicological characteristics of glufosinate have made it a popular, non-selective herbicide, which has been commercialized as Basta® and Liberty® by Bayer Crop Science (formerly Aventis). Meiji Seika, Japan has also commercialized it as Bilanafos.

#### Bialaphos



#### phosphinothricin-alanyl-alanine

Some microorganisms can detoxify glufosinate by producing an enzyme that causes acetylation of the amino group. The gene encoding the acetylating enzyme has been isolated from *Streptomyces hygroscopicus* (Thompson et al., 1987) and from *S. viridochromogenes* (Wohlleben et al., 1988). It has been referred to as *bar* (for bialaphos resistance) and PAT gene, respectively. The *bar* gene encodes a phosphinothricin acetyl transferase (PAT). In this paper we will use *bar* when referring to the gene and PAT when referring to the enzyme.

A *bar* gene has also been isolated from *Alcaligenes faecalis* (Proteobacteria: Beta subdivision). Thanks to multiple ongoing genome annotation projects, the homologous (i.e. sharing common ancestry) genes have also been identified in the genomic sequences of many other microorganisms, including archaeobacteria. The *Alcaligenes*, *Streptomyces* and archaeobacterial PAT enzyme are ca. 30% identical at the amino acid level (ca. 50% using conservative replacements).

Treatment of genetically modified plants carrying a *bar* gene with glufosinate or bialaphos provides a very efficient means of selection in genetic transformation protocols. Although this use of the *bar* gene was gaining favor in many laboratories, according to some, the patent owners have expressed legitimate concern about indiscriminate use of the gene in

fields. Such use could lead to cross-pollination and possible tolerance buildup in weeds, thereby undermining commercial applications of glufosinate. This has led to enforcement of restrictions to the use of the *bar* gene as a selectable marker gene (personal communication).

Commercial use so far has been restricted to a few major crops, and seed distribution is mainly in the hands of the patent owner, nowadays Bayer Crop Science, and very few other companies.

In the few countries with commercial transgenic crops, crops carrying the *bar* gene include sugar beet, canola, soybean, rice and maize. A few resistant hybrid maize lines are registered to Dekalb, Mycogen and Syngenta. A male sterile chicory variety carrying the gene is registered to Bejo Zaden, NL (data obtained from [www.agbios.com](http://www.agbios.com)).

## Patent issues surrounding the *bar* gene

When using the *bar* gene, besides the gene itself, several IP protected materials and processes may be involved, such as processes for plant transformation, use of genetic regulatory elements, use of antibiotic resistance genes as selectable markers, etc. These topics are discussed in the corresponding white papers, e.g. "*Agrobacterium*-mediated plant transformation", "Promoters", and "Antibiotic Resistance". In this whitepaper, we analyze patent issues around the *bar* gene as such.

In the case of the *bar* gene (not its uses) we are faced with a relatively simple intellectual property ownership situation. Essentially all key patents are held by Bayer Crop Science, although assignees listed on the patent documents include Plant Genetic Systems, Hoechst, AgrEvo and Aventis. To understand why the *bar* gene patent portfolio is now in the hands of Bayer Crop Science, a schematic overview of [the corporate consolidation history](#) which led to the creation of one of the major players in the agrichemicals business worldwide is included in the analysis.

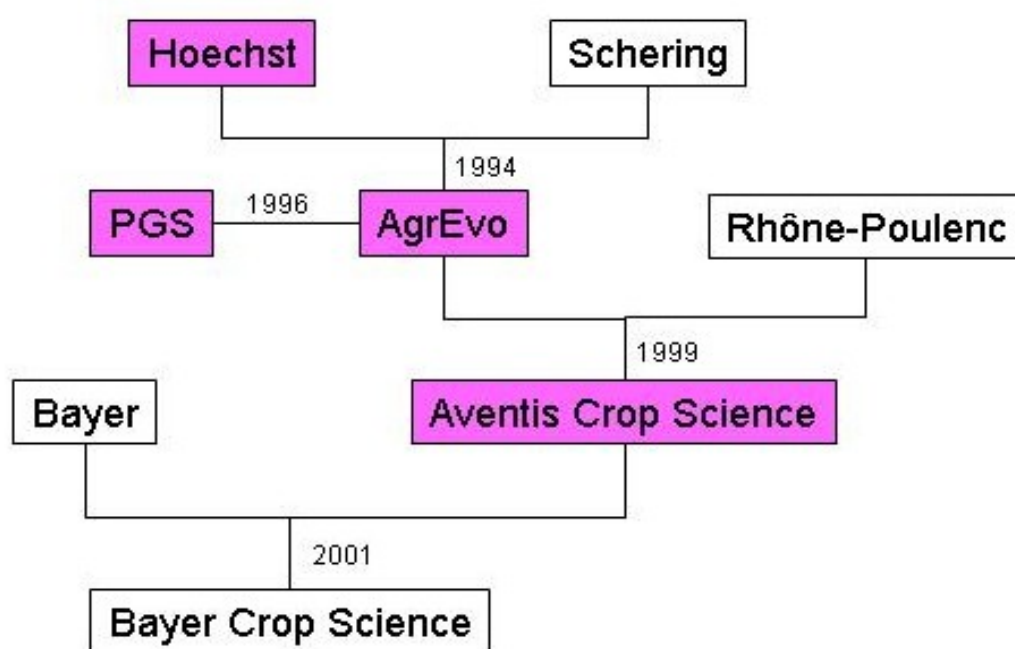
The *bar* gene patents owned by Bayer Crop Science are divided into three main families. The first patent family is the dominant family and was originally assigned to Plant Genetic Systems (PGS) and Biogen NV. It protects the use of the *bar* gene in plants and plant products. More specifically, this patent family protects the use of the gene in creating herbicide resistant crops and also its use as a selectable marker.

The other two patent families in the Bayer portfolio (assigned originally to Hoechst AG) strengthen the corporate position on the *bar* gene by claiming additional *bar* genes from other organisms and uses, e.g. isolating the gene from gram-negative bacteria, the gene itself, its use as a selectable marker in bacteria, codon-optimized versions for expression in plant cells, and treatment of sewage contaminated with phosphinothricin.



## Who owns the dominant patents on the *bar* gene?

Currently patents claiming the *bar* gene are mainly in the hands of Bayer Crop Science. The schematic representation below displays the corporate history of the *bar* gene patent portfolio. In all the *bar* gene patents discussed in this white paper the reader will find applicants (assignees) listed as Hoechst AG, Plant Genetic Systems NV, AgrEvo (Hoechst Schering GmbH), and Aventis (Crop Science GmbH); these company names appear in color in the graphic. For clarity, mergers, demergers, takeovers and spin-offs not related to the carryover of the portfolio relevant to this discussion have not been included in the graphic.



The acquisition of the Belgian company Plant Genetic Systems (PGS) by AgrEvo in 1996 was an important strategic move to gain access to a broad portfolio of traits and enabling technologies required to participate in the highly competitive market of genetically engineered crops. At the time AgrEvo had fallen behind Monsanto and Novartis (now Syngenta) in securing a competitive market position in the area of genetically modified insect and herbicide resistant crops. With the acquisition of PGS, AgrEvo took a serious step to enter the U.S. and the Canadian markets for transgenic crops, two of the largest markets in world. Companies are still preparing for a definitive opening of the European markets for genetically modified crops, a development stalled by public perception.

With the acquisition of PGS, AgrEvo gained access to various technologies of PGS in its patent portfolio, such as gene promoters, marker genes, techniques to insert specific genes into plant cells, and gene expression technology to optimise the efficacy of expression of foreign genes in plants. Additionally, PGS had engaged in research and development of

novel technologies, particularly in the area of functional genomics, but also in engineering disease tolerant plants and modifying certain quality traits. PGS's products included corn, oilseed rape (canola) and selected vegetables engineered for insect protection (based on the expression of Bt toxin), herbicide tolerance and pollination control.

PGS's herbicide tolerance technology was developed in collaboration with AgrEvo, based on tolerance to AgrEvo's herbicide Liberty™ (glufosinate) by virtue of the *bar* gene. PGS's SeedLink™ pollination control technology is also based on tolerance to Liberty™.

After the merger of AgrEvo and Rhône-Poulenc, which gave rise to Aventis, the agricultural section of this merger was called Aventis Crop Science. The Hoechst conglomerate, holder of Aventis and other companies, finally decided to shed its agrichemicals section by selling to Bayer AG, which recently gave rise to Bayer Crop Science, explaining thereby the migration of the *bar* gene portfolio over time.

## Dominant *bar* gene patents

The first and most dominant patent family has been divided into three individual key patents in the United States. The three key patents cover:

- a) the use of the *bar* gene in a plant cell (US 5,561,236);
- b) a process for the production of a plant cell tolerant or resistant to glufosinate (PPT) or any compound containing the PPT moiety, by nuclear integration of a compound-specific acetyl transferase gene (US 5,646,024); and
- c) a plant transformation vector carrying such a gene (US 5,648,477).

The other patent in this dominant family is European Patent 242 236. These patents have extremely broad claims, particularly European Patent 242 236 and the United States patent 5,561,236. A summary of the claims for these patents can be viewed below, and is followed by an analysis of the scope of the granted claims.

<b>Bayer Crop Science portfolio (original assignees Plant Genetic Systems NV and Biogen NV)</b>		
<b>PAT No</b>	<b>ISSUE DATE</b>	<b>SUMMARY OF PATENTS</b>
<a href="#">EP 242 236 B2</a>	21 Aug 1996	This patent has the broadest coverage with respect to the <i>bar</i> gene. Claims recite a process to inactivate a glutamine synthetase (GS) inhibitor by expression of a heterologous resistance gene in plants and plant cells. Other claims specify that the enzyme is a phosphinothricin acetyl transferase (PAT) from <i>Streptomyces</i> sp. The use of

		<p>this gene to produce herbicide resistant plants is also claimed. In another claim, resistance is exploited to protect plants from fungal infection by treating plants with phosphinothricin, which is not only a herbicide but an antibiotic. (see note below).</p> <p><a href="#">View Claims</a></p>
<a href="#">US 5,561,236</a>	1 Oct 1996	<p>The broadest claim covers a plant cell (and by extension a plant) transformed with an acetyl transferase gene capable of inactivating a GS inhibitor. The dependent claims then proceed to define the enzyme as PAT with a defined sequence (isolated from <i>Streptomyces</i> sp).</p> <p><a href="#">View Claims</a></p>
Note:	<p>In the U.S., three patents are related to EP 242 236 and to each other.</p>	

## EP 242 236

European Patent 242 236 was first filed in March 1986. However, it did not issue until 10 years later (21 Aug 1996). Bibliographic information is presented below.

<b>EP 242 236 B2</b>	
<b>Title</b>	Plant cells resistant to glutamine synthetase inhibitors, made by genetic engineering
<b>Issue date</b>	21 Aug 1996
<b>Assignees</b>	Plant Genetic Systems NV and Biogen NV
<b>Inventors</b>	Leemans J, Botterman J, de Block M, Thompson C, Mouva R
<b>Appl No and filing date</b>	EP 87/400141 21 Jan 1987
<b>Priority</b>	EP 86/400521 11 Mar 1986
<b>Abstract</b>	The invention relates to a DNA fragment containing a determined gene, the expression of which inhibits

	the antibiotic and herbicidal effects of Bialaphos and related products. It also relates to recombinant vectors, containing such DNA fragment, which enable this protective gene to be introduced and expressed into cells and plant cells.
--	---

As mentioned previously, the claims in this patent are extremely broad. The patent claims seek to cover transformation of plant cells with the *bar* gene. Independent Claim 1 covers the introduction of a foreign coding gene that inhibits glutamine synthetase (*bar* gene) in plant cells (transformation). Importantly, this claim does not indicate the source of the engineered foreign coding gene sequence and therefore the foreign gene to be inserted is literally not restricted to any particular enzyme or gene source. The foreign gene to be introduced is only described in terms of its function, i.e. a gene encoding a product capable of inactivating the inhibitor (see actual claims). Recent court decisions in the U.S. suggest that if this claim was included in a U.S. patent application it would be considered indefinite in its meaning and therefore fail the U.S. enablement requirement for patentability.

In dependent Claim 2 a preferred embodiment of the foreign gene sequence is revealed as an acetyl transferase. Claim 2 recites:

“...a process wherein an acetyl transferase capable of inactivating PPT or its derivatives is used.

Hence this claim restricts the foreign gene sequences to those encoding an acetyl transferase enzyme. Claim 2 is written broadly enough, however, to include acetyl transferases capable of inactivating a novel, not yet identified derivative of PPT. There is no restriction in the claim with respect to the source of the enzyme. The enzyme can be isolated from any microorganism.

Further dependent claims limit this process to situations where the foreign nucleotide sequence is derived from the genome of an antibiotic-producing *Streptomyces* strain or another nucleotide sequence that encodes the same antibiotic-producing activity (Claim 3). This broad claim also includes antibiotic-producing organisms that have not yet been identified.

Independent claims 7, 11, 14, 18 and 23 extend protection to the application of the process outlined in Claim 1 to developing herbicide resistant plants. The claims cover non-biologically transformed plant cells and plants displaying resistance to glutamine synthetase (Claim 14) as well as suitable DNA fragments and recombinants containing sequences encoding resistance to glutamine synthetase inhibitors (Claim 31).

A remarkable feature of this patent is that the method of inactivation of PPT is not identified. This gives the patent a broad, far reaching scope and potentially covers all processes and methods by which PPT is broken down and inactivated.

This patent has equivalents in numerous countries including Austria, Germany, Denmark, Hungary, Portugal, Spain, Israel, Australia, Brazil, Finland, Hong Kong, Greece, Japan and South Africa.

### **Opposition of EP 242 236**

The European patent EP 242 236 B1 was granted in 1990 by the European Patent Office (EPO). In 1991, Greenpeace formally opposed the issue of the patent. The EPO Board of Appeals (BOA) considered the opposition by Greenpeace in 1993 (decision T 356/93) and, as a result, an amended version of the patent (EP 242 236 B2) was granted in 1996.

A number of objections in relation to EP 242 236 B1 were raised by Greenpeace in the opposition procedure. Greenpeace argued that transgenic plants were outside the scope of patent protection. In making their decision the EPO board had to address the following questions.

### **Is genetic manipulation an essentially biological process and therefore not patentable?**

Essential biological processes are not patentable under the law. Greenpeace argued that some steps involved in obtaining a transgenic plant, such as the regeneration of plant callus tissue, were based on an "essentially biological process". Part of the Board's discussion surrounding this point focused on defining the amount of human intervention required to execute the "essentially biological process". In the present case, the Board concluded that without human intervention the transgenic plant wouldn't be obtainable, even though some steps, like regeneration of a plant from callus tissue, did not require human intervention. Human intervention was therefore required in carrying out the process and, as a result, genetically modified cells were deemed patentable.

### **Can plant cells be patented?**

The Board found that plant cells as such do not fall under the definition of a plant or of a plant variety, both of which are patentable under the European Patent Convention (EPC). Rather, plant cells are considered to be "microbiological products" in the broad sense and are therefore patentable.

### **Can a patent be allowed where the subject matter is contrary to public opinion?**

In this argument Greenpeace provided evidence based on public surveys in Sweden and in Switzerland. Public perception and speculative data about potentially serious damage to the environment were not deemed to support the argument made, therefore this point was rejected.

While the EPO quashed these grounds of rejection put forward by Greenpeace, Claim 21 of the patent in contention played a central role in the proceedings. Claim 21 sought to cover:

"A plant, modified by introducing a gene encoding an enzyme capable of inactivating or neutralizing an inhibitor of glutamine synthetase, a key enzyme in nitrogen assimilation."



In its decision the Board held that Claim 21 was directed to transgenic plants and, as a result, embraced plant varieties, even though no plant variety was individually claimed. In later years, the Board has revised their standing in this area, largely as a result of the European Directive 1998 and The Agreement on Trade

Related Aspects of Intellectual Property Rights (TRIPS) which requires that biotechnological process are patentable provided that no individual plant varieties are claimed.

To comply with the interpretation of the law at the time, some claims were eliminated from the original application after the opposition procedure ([abandoned claims](#)) and EP 242 236 B2 was granted three years later in 1996.

### US 5,561,236

The other dominant United States patent in the Bayer Crop Science portfolio originally assigned to Plant Genetic Systems NV and Biogen NV is US 5,561,236. This patent protects transgenic plant cells and plants containing a *bar* gene. Its bibliographic information is outlined below.

<b>US 5,561,236</b>	
<b>Title</b>	Genetically engineered plant cells and plants exhibiting resistance to glutamine synthetase inhibitors, DNA fragments and recombinants for use in the production of said cells and plants
<b>Issue date</b>	1 Oct 1996 (expires 1 Oct 2013)
<b>Assignees</b>	Plant Genetic Systems NV and Biogen NV (now Bayer Crop Science)
<b>Inventors</b>	Leemans J, Botterman J, de Block M, Thompson C, Mouva R
<b>Appl No and filing date</b>	US 07/525,300 17 May 1990
<b>Priority</b>	US 07/131,140 (WO 87/05629 11-03-1987)
<b>Abstract</b>	The invention relates to a DNA fragment containing a determined gene, the expression of which inhibits the antibiotic and herbicidal effects of Bialaphos and related products. It also relates to recombinant vectors, containing such DNA fragment, which

	enable this protective gene to be introduced and expressed into cells and plant cells.
--	--

This United States patent obtains priority from the same PCT application from which EP 242 236 is derived. The claims in this patent are similar to the European patent in their objective; however they are not as broad.

Claim 1 claims a plant cell in which inactivation of a glutamine synthetase inhibitor is achieved by acetyl transferase activity of an introduced heterologous gene product. This contrasts to EP 242 236, whereby the foreign gene to be inserted into the cell is only described in terms of its function. Claim 2 of US 5,561,236 further limits the introduced gene product referred to in Claim 1 as a polypeptide having an acetyl transferase activity with respect to phosphinothricin.

At this point we are not aware of other glutamine synthetase inhibitors that are inactivated by enzymatic acetylation. Hence, in practice, Claim 1 would likely refer to a *bar* gene product as specified in Claim 2. While Claim 1 is literally very broad, in reality the patentability requirements of enablement, written description and issues of interpretation would limit Claim 1 to that of Claim 2.

Dependent claims in the patent extend protection to whole plants, seeds of plants and any other plant tissue that contain the cells described in Claim 1.

Similarly to the European patent, the source of the gene product in the United States patent is not limited to a *Streptomyces* strain. Given this, the United States patent and the European patent likely protect the use of the *bar* gene, regardless of its source, in plants. Considering that both patents are part of the Bayer Crop Science portfolio, Bayer Crop Science have a controlling commercial position with respect to the use of the *bar* gene in both the U.S. and Europe.

**A *bar* gene from *Streptomyces* sp.**

The remaining patents in the dominant family now owned by Bayer Crop Science are discussed below. All of the remaining patents protect the *bar* gene specifically isolated from species of *Streptomyces*. The patents cover both a) a process for the production of a plant cell tolerant or resistant to glufosinate (PPT) or any compound containing the PPT moiety, by nuclear integration of a compound-specific acetyl transferase gene (US 5,646,024) and b) a plant transformation vector carrying such a gene (US 5,648,477). A summary of the claims of these patents and other related patents are found in the table below.

<b>Bayer Crop Science portfolio (original assignees Plant Genetic Systems NV and Biogen NV)</b>		
<b>PAT No</b>	<b>ISSUE DATE</b>	<b>SUMMARY OF PATENTS</b>

<a href="#">EP 242 246 B1</a>	11 Nov 1992	A DNA fragment coding for a PAT gene isolated from a defined <i>S. hygrosopicus</i> strain and used in processes as described in EP 242 236. <a href="#">View Claims</a>
<a href="#">US 5,646,024</a>	8 Jul 1997	The patent claims the transformation of a plant cell with the <i>bar</i> gene isolated from <i>Streptomyces</i> sp, the expression of which inhibits herbicidal effects of Bialaphos. Recombinant vectors containing such a fragment are also claimed. <a href="#">View Claims</a>
<a href="#">US 5,648,477</a>	15 Jul 1997	The patent claims a vector containing a foreign DNA fragment capable of inactivating a GS inhibitor. A dependent claim then specifies that the inhibitor is phosphinothricin. <a href="#">View Claims</a>
Related patents and applications	The following patents are related to the PGS assigned patent family (including the dominant patents). Granted patents in: Austria AT 57390, AT 82323, Germany DE 3765449, DE 3782526 T2, Spain ES 2052588 T3, Hungary HU 213580 B, Portugal PT 84448 B; Australia AU 612570 B2, Japan JP 3142848 B2, South Africa ZA 8701754. Applications: Brazil BR 8706204, Denmark DK 5898/87, DK 9099, Spain ES 20525/88 T, Finland FI 87/4883, Greece GR 3001220 T3, GR 3006936 T3, GR 3021380 T3, Hungary HU 46063 A2, Israel IL 81838, Hong Kong HK 1000519 A1, Japan JP 11262394 A2, JP 1503434 T2	

## EP 242 246 B1

This patent protects plant cells containing DNA that provides resistance to PPT and its products, while at the same time protecting the process that inactivates the GS inhibitor, namely Bialaphos or PPT.

<b>EP 242 246 B1</b>	
<b>Title</b>	Plant cells resistant to glutamine synthetase inhibitors, made by genetic engineering
<b>Issue date</b>	11 Nov 1992
<b>Assignees</b>	Plant Genetic Systems NV and Biogen NV



<b>Inventors</b>	Leemans J, Botterman J, de Block M, Thompson C, Mouva R
<b>Appl No and filing date</b>	EP 87/400544 11 Mar 1987
<b>Priority</b>	EP 86/400521 11-03-1986; EP 87/400141 21-01-1987
<b>Abstract</b>	The invention relates to a DNA fragment containing a determined gene, the expression of which inhibits the antibiotic and herbicidal effects of Bialaphos and related products. It also relates to recombinant vectors, containing such DNA fragment, which enable this protective gene to be introduced and expressed into cells and plant cells.

Independent claim 1 protects a *bar* gene, either partially or in its entirety, that is isolated from *Streptomyces hygrosopicus* and encodes an active enzyme. It is inferred by the construction of the following dependent claims that such a nucleotide sequence would include various codon combinations yielding the same peptide sequence. The claims therefore encompass codon degeneracy. The peptide sequence corresponds to a *bar* gene isolated from a specific *S. hygrosopicus* strain.

Claims 4 and 5 recite a process to produce a transgenic plant resistant to a glutamine synthetase inhibitor by introducing a gene encoding a phosphinothricin acetyl transferase. The breadth of the claims is restricted by their dependence on Claims 1 and 2. Thus, the processes claimed in Claims 4 and 5 are limited to the transformation of plant tissue with the specific nucleotide sequence in Claim 1 (i.e. the *bar* gene) that codes for a protein having phosphinothricinacetyl transferase activity from *S. hygrosopicus*.

Similarly to EP 242 236, Claims 19 and 23 recite the processes of using the gene construct described above for weed and fungal control.

## **US 5,646,024**

The following patent (US 5,646,024) complements the dominant *bar* gene patents by claiming a process involved in producing transgenic plants resistant to a glutamine synthetase inhibitor.

<b>US 5,646,024</b>	
<b>Title</b>	Genetically engineered plant cells and plants exhibiting resistance to glutamine synthetase inhibitors, DNA fragments and recombinants for use in the production of said cells and plants

<b>Issue date</b>	8 Jul 1997 (17 year term)
<b>Assignees</b>	Bayer Crop Science
<b>Inventors</b>	Leemans J, Botterman J, de Block M, Thompson C, Mouva R
<b>Appl No and filing date</b>	US 08/463,241 5 June 1995
<b>Priority</b>	US 07/131,140 05-11-1987 (WO 87/05629); divisional application of US 07/525,300 17-05-1990; US 08/463,241 05-06-1995; EP 86/400521 11-03-1986; EP 87/400141 21-01-1987
<b>Abstract</b>	The invention relates to a DNA fragment containing a determined gene, the expression of which inhibits the antibiotic and herbicidal effects of Bialaphos and related products. It also relates to recombinant vectors, containing such DNA fragment, which enable this protective gene to be introduced and expressed into cells and plant cells.

As recited in Claim 1, resistance is achieved by introducing a gene obtained from a microorganism that produces the glutamine synthetase inhibitor encoding an acetyl transferase capable of inactivating phosphinothricin or another compound. In additional claims the introduced gene is the *bar* gene isolated from either *Streptomyces hygroscopicus* or *Streptomyces viridochromogenes* (Claims 2-4).

Other claims recite processes of weed and fungal control, and plants transformed with the *bar* gene that are cultivated in a field which is subsequently treated with herbicide containing glutamine synthetase inhibitor.

Claim 38 recites a process for the production of a pure culture of transformed plant cells using the glutamine synthetase inhibitor as a means of selection.

### **US 5,648,477**

The following patent claims only methods involved in producing a plant cell resistant to glufosinate.

<b>US 5,648,477</b>	
<b>Title</b>	Genetically engineered plant cells and plants exhibiting resistance to glutamine synthetase inhibitors, DNA fragments and recombinants for use in the production of said cells and plants

<b>Issue date</b>	15 Jul 1997
<b>Assignees</b>	Bayer Crop Science
<b>Inventors</b>	Leemans J, Botterman J, Thompson C, Mouva R
<b>Appl No and filing date</b>	US 08/477,320 7 June 1995
<b>Priority</b>	US 07/131,140 05-11-1987 (WO 87/05629); US 08/477,320 07-06-1995; EP 86/400521 11-03-1986; EP 87/400141 21-01-1987; division of US 07/525,300 17-05-1990
<b>Abstract</b>	The invention relates to a DNA fragment containing a determined gene, the expression of which inhibits the antibiotic and herbicidal effects of Bialaphos and related products. It also relates to recombinant vectors, containing such DNA fragment, which enable this protective gene to be introduced and expressed into cells and plant cells.

The claims of this patent are directed to vectors carrying a gene encoding an enzymatic activity capable of inactivating a glutamine synthetase inhibitor by acetylation. Such genes would comprise a *bar* gene (as specified in Claim 2) as well as an acetyl transferase capable of inactivating an unspecified inhibitor, not necessarily phosphinothricin.

Independent Claim 1 recites a vector which contains not only a gene that inactivates a glutamine synthetase inhibitor but also includes a plant promoter. Claim 25 extends the protection of the patent to any vector that contains the coding DNA fragment, regardless of the promoter that is used.

### **A *bar* gene from *Streptomyces viridochromogenes***

The patent family discussed here is assigned to Hoechst AG. The patents in this family have restricted scope: the main independent claims are limited to a *bar* gene isolated from one specific strain of *Streptomyces viridochromogenes* (Actinobacteria: Actinomycetales), best exemplified by European patent EP 257 542 B1, and the same gene, codon-optimized for expression in plant cells, as in patent EP 275 975 B1. These patents, which fall within the literal scope of the claims of the assigned patent family discussed previously, are also now part of the consolidated patent portfolio of Bayer Crop Science.



The Hoechst family consists of related patents and applications in many jurisdictions, including several in Europe, as well as in Australia, Canada, China, Israel, Japan, New Zealand, South Africa and the U.S. Unusually, no PCT application was filed for conversion into the numerous jurisdictions.

A summary of the claims in this patent family can be seen below.

<b>Assigned to Hoechst AG</b>		
<b>PATENT No</b>	<b>ISSUE DATE</b>	<b>SUMMARY</b>
<a href="#">EP 257 542 B1</a>	2 Mar 1988	A <i>bar</i> gene obtained from a <i>Streptomyces viridochromogenes</i> strain* and plant cells transformed with that gene. Also a process to acetylate PPT using bacteria expressing the gene and the enzyme as such. <a href="#">View Claims</a>
<a href="#">EP 275 957 B1</a>	27 Aug 1988	A plant codon-optimized <i>bar</i> gene isolated from the same bacterial strain as above*. The modifications consist of an altered G/C ratio and elimination of potentially deleterious palindromic sequences. <a href="#">View Claims</a>
<a href="#">US 5,273,894</a>	28 Dec 1993	A <i>bar</i> gene obtained from same bacterial strain as above* and plant cells transformed with that gene. Also a process to acetylate PPT using bacteria expressing the gene and the enzyme as such. <a href="#">View Claims</a>
<a href="#">US 5,637,489</a>	10 Jun 1997	A process to produce plant cells resistant to PPT by introducing the same <i>bar</i> gene described above and the resulting resistant plants. <a href="#">View Claims</a>
<a href="#">US 5,879,903</a>	9 Mar 1999	A method to select bacterial or plant cells using the <i>bar</i> gene described above as a selectable marker. <a href="#">View Claims</a>
<a href="#">US 5,276,268</a>	4 Jan 1994	A plant codon-optimized <i>bar</i> gene isolated from same bacterium as above*. Modifications consist of an

		altered G/C ratio and elimination of potentially deleterious palindromic sequences. <a href="#">View Claims</a>
--	--	--

Related patents: EP in force in Germany DE 3778792 C0, Austria AT 75776, Belgium, Switzerland, France, Great Britain, Greece GR 3005200, Spain ES 2038631 T3, Italy, Sweden; other related patents: Australia AU 604743, Canada CA 1337597, CA 1321364, Finland FI 100251 B1, Hungary HU 217208 B, HU 215079 B, Japan JP 2993964 B2, JP 2749424 B2, JP 2815847 B2, JP 3093686 B2, JP 7097994 B4.  
Related applications: China CN 1040772 B, CN 1225393, Denmark DK 4378/87, Finland FI 88/0216, Greece GR 3007859 T3, Israel IL 83604 A0, IL 85143 A0, Japan JP 7097994 B4, JP 3103193 A2, JP 63071183 A2, JP 63273479 A2, JP 7147985 A2, JP 9107981 A2, JP 10080278 A2, JP 10080289 A2, New Zealand NZ 221526 A, South Africa ZA 87/06210 A, ZA 88/00368 A

\* *Streptomyces viridochromogenes* strain DSM 40736 (catalog #DSM 4112, deposit under the Budapest Convention) obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ).

## EP 257 542

The European patent EP 257 542 protects the use of a specific strain of *Streptomyces viridochromogenes*.

Widely protected in many jurisdictions, the independent claim of this patent recites a *bar* gene from a specific *Streptomyces viridochromogenes* strain, DSM 4112. Dependent claims recite the use of the *bar* gene from strain DSM 4112 as a selectable marker for genetically modified bacterial and plant cells. The use of the *bar* gene as a selectable marker in bacteria (not mentioned in the claims of the "Dominant *bar* gene patents" belonging to Plant Genetic Systems (PGS)) is restricted here to the *bar* gene as described in Claim 1.

Any phosphinothricin-resistant plants that contain the described *bar* gene are also protected (Claim 8).

Claim 7 recites another use of the *bar* gene isolated from *S. viridochromogenes* not present in the claims of the dominant patents, namely to acetylate L-phosphinothricin. This acetylated compound is of commercial interest in another field of application, the selective destruction of tissues expressing the deacetylase gene, e.g. for the production of male sterile plants when the gene is expressed in the pollen sac.

## EP 275 957

European patent 275 957 protects the same gene as the previous patent. However it is codon-optimized for expression in plant cells.

Due to differences in codon usage, bacterial genes are not always expressed well in eukaryotic cells. These differences are a consequence of divergent evolution of the protein-synthesizing machinery. As we learn more from genomic sequence analysis of many species, we can infer which codons work best for different plant families. Most variation is found in the last base of the triplet that codes for an amino acid (wobble hypothesis). The wobble can also account for differences between the average GC content of plant versus bacterial genes.

Claim 1 recites in general terms an adaptation of the genetic code to that of plants. This exploits the flexibility provided by the redundancy of the genetic code (wobble) and it also allows consideration of other relevant issues in gene design, e.g. the elimination of repetitive or palindromic sequences that might interfere with gene expression.

An example of an optimized sequence is provided in dependent Claim 2. Plant cells harboring the transgene are protected (Claim 8) but so too are host cells, typically bacteria, used to manipulate the intermediate and used during the transformation process.

The remaining patents in the Hoechst AG assigned family are derived from the same German patent applications that gave rise to European patent EP 257 542 B1. The earliest priority date is 23 Aug 1986, which corresponds to German application DE 3628747. The following four U.S. patents are all related to each other by continuation and division procedures. Divisional applications arise from when the claimed subject matter covers multiple inventions according to U.S. Patent Office Standards.

### **US 5,273,894**

Claims in this patent are essentially the same as those described in European patent EP 257 542 B1, namely a *bar* gene isolated from *Streptomyces viridochromogenes* strain DSM 4112. Other claims recite plant and bacterial cells transformed with the gene.

Claim 10 recites the use of the *bar* gene as a tool to acetylate L-phosphinothricin, similar to Claim 7 in European patent EP 257 542 B1.

### **US 5,276,268**

Claims in this patent are similar to those in European patent EP 275 957 B1. Claim 1 refers to a *bar* gene modified to reflect plant codon usage to enhance gene expression. Modifications are based on the flexibility conferred by the redundancy of the genetic code. Other allowed claims recite plant and bacterial cells expressing the introduced gene (Claims 5-11) as well as a process to generate plants resistant to the herbicide phosphinothricin (Claims 12-14).

### **US 5,637,489**

Claim 1 recites a process to obtain a phosphinothricin-resistant plant by incorporating a *bar* gene isolated from *Streptomyces viridochromogenes*

strain DSM 4112 into the plant genome. Claim 4 recites a plant containing such a gene.

Claim 3 states that the sequence should contain at least nucleotides 258-806 of the given sequence, which corresponds exactly to the structural gene.

### **US 5,879,903**

United States patent 5,879,903 recites methods of using the *bar* gene isolated from *Streptomyces viridochromogenes* strain DSM 4112 to select for transgenic bacterial and plant cells respectively.

Again, the patent does not protect *bar* genes isolated from other bacterial species or genera. Use of the *bar* gene as a selectable marker in bacteria is not mentioned in the claims of the dominant *bar* gene patent family — issued to PGS - where the gene is mainly used to confer herbicide resistance to transgenic plants in the field. The present patent falls within the scope of the dominant patent family because the granted main claims in those patents are written in broad terms.

### **A *bar* gene from *Alcaligenes* sp and other gram-negative bacteria**

The third patent family making up the Bayer Crop Science portfolio was originally assigned to Hoechst AG. The patent family is directed to a *bar* gene isolated from gram-negative, non-spore forming, (facultative) aerobic bacteria. In the U.S., the claims recite an isolated gene and enzyme restricted to *Alcaligenes* sp. The use of this gene(s) to confer resistance to glutamine synthetase inhibitors in plants is dominated by the broad claims of the earlier PGS patent family, now part of the same IP portfolio.

A summary of the claims of the patents based on the *bar* gene from the *Alcaligenes* sp patent family are presented below.

PAT No	ISSUE DATE	SUMMARY OF PATENTS
<a href="#">EP 290 986 B1</a>	23 Jun 1993	This patent covers <i>bar</i> genes isolated from basically any Gram-negative, non-spore forming, (facultative) aerobic bacterium, e.g. <i>Alcaligenes</i> , <i>Agrobacterium</i> , <i>Pseudomonas</i> , and various enterobacteria, as opposed to gram-positive <i>Streptomyces</i> spp from which the gene was first isolated. <a href="#">View Claims</a>
<a href="#">US 5,077,399</a>	31 Dec 1991	A phosphinothricin resistance gene isolated from <i>Alcaligenes</i> sp (Proteobacteria: Beta subdivision). The gene product exhibits PAT activity in dependent claims. <a href="#">View Claims</a>

Related patents	<p>Granted patents in: Australia AU 613367 B2, Germany DE 3881959 C0, Spain ES 2058172 T3.</p> <p>Applications in: China CN 88/102798 A, Germany DE 3716309 A1, Denmark DK 2642/88 A, Finland FI 88/2227 A, Hungary HU 46943 A2, Israel IL 86378 A0, Japan JP 1005493 A2, New Zealand NZ 224602 A, South Africa ZA 88/03390 A</p>
Commentary	<p>From the latest patent status search it appears that the assignee has dropped its patents in all major jurisdictions. It is possible that all patents and applications in this family might have been dropped.</p> <p>Having consolidated dominant patents in the area, economically it is no longer necessary to maintain this group of patents. The subject matter disclosed here constitutes prior art, thus making sure that nobody else can block sub-sets of possible applications of the technology.</p>

## EP 290 986 B1

European patent 290 986 is the most recently issued patent in this family. Claim 1 recites a phosphinothricin resistance gene obtained from bacteria that are not fungus-like. This description discloses a resistance gene from bacteria other than those specified in the dominant PGS patent. Although the broadest independent claim in the PGS patent covers, in principle, any microorganism, the specification teaches only so-called fungus-like bacteria — *Streptomyces* sp to be more precise — which belong to the gram-positive Actinomycetales family. Although there are many genera that are not fungus-like within the gram-positive group, the patent family discussed here lists only gram-negative bacteria in its dependent claims. Furthermore, only the genus *Alcaligenes* was granted in the claims of the corresponding U.S. patent (US 5,077,399; see below). Plants and propagules harboring the gene are also covered in the claims (see Claims 8 and 9).

Claim 10 recites a novel application of the gene expressed in microorganisms, namely for degradation of sewage contaminated with phosphinothricin, which could be a useful way to clean up contamination.

The corresponding granted claims for Spain have also been included in the Appendix. These provide an example of how claims must be sometimes reformulated to comply with national legislation. Patenting of genes and plants is not allowed in Spain, but processes to generate or to select genes and transgenic plants are allowed.

## US 5,077,399

As mentioned earlier, the U.S. patent in this family has a very narrow scope given the broad claims of the PGS patents. Claims are limited to a phosphinothricin resistance gene isolated from the genus *Alcaligenes* (Proteobacteria: beta subdivision). The peptide sequence of this enzyme is



33% identical to the homolog isolated from *Streptomyces*, and 53% similar in terms of conservative amino acid exchanges.

Transgenic plants containing the gene or other applications of the gene expressed in microorganisms (e.g. the treatment of phosphinothricin-contaminated sewage) are not included in the granted claims of this patent, although these possibilities are mentioned in the specifications.

The dominant claim in this patent has the form of a "product by process" claim, i.e. it follows the basic form "a product X obtained by carrying out process Y". The question here is whether obtaining the same product



using a different process would constitute infringement. In the United States this issue is yet to be considered at the Federal Circuit level. Meanwhile, some district courts are treating the sale or use of product X not produced by process Y as infringement. In the ongoing discussion by the courts, two issues are separated, i.e. determination of patentability and assessment of infringement. Patentability is dependent on novelty of the product. Inclusion of the process in the claims is allowed to facilitate the definition of the product (also contemplated by the European Patent Convention). Existing case law supports the notion that the scope was limited by the

process stated. If this becomes the guiding principle, then, taking the above patent as an example, performing PCR on non-selected bacteria would not constitute an infringement of the patent.

### **A *bar* gene in combination with a virus resistance gene**

This group of patents covers the combination of virus resistance genes and herbicide resistance genes in one single transgenic plant. Having one or the other is already a valuable asset in agriculture. Having both has the potential of adding significant value to crops, as weed control and viruses are two major problems in crop management. Use of this invention, however, by the assignees or licensees might still require separate negotiations with owners of patents on virus resistance genes or methods to obtain transgenic virus resistant plants.

<b>Assigned to Hoechst Schering AgrEvo GmbH (now part of the Bayer Crop Science)</b>		
<b>PATENT No</b>	<b>ISSUE DATE</b>	<b>SUMMARY</b>
<a href="#">EP 513 054 B1</a>	21 Apr 1999	Plant cells containing a combination of a phosphinothricin and a virus resistance gene. A process to generate plants with improved agronomic characteristics from these cells. <a href="#">View Claims</a>

<a href="#">EP 899 340 A2</a>	3 Mar 1999 (publ. date)	Plants, plant cells or parts, and seed containing a gene construct combining virus and herbicide resistance. A preferred embodiment contains a phosphinothricin resistance gene from <i>Streptomyces</i> sp. <a href="#">View Claims</a>
<a href="#">EP 714 237 B1</a>	4 Nov 1998	A method to increase yield (not necessarily through weed control) by treating plants resistant to glutamine synthetase inhibitors —transgenic or not— with inhibitors such as glufosinate. <a href="#">View Claims</a>
<a href="#">US 5,633,434</a>	27 May 1997	A DNA construct combining a bar gene and a virus resistance gene. Plant cells expressing the construct. A method to improve growth by treating plants expressing the construct with phosphinothricin. <a href="#">View Claims</a>
<a href="#">US 5,792,926</a>	11 Aug 1998	Plants and plant cells transformed with a construct comprising a phosphinothricin resistance gene and a viral coat protein capable of conferring virus resistance to the plant. <a href="#">View Claims</a>
<a href="#">US 5,908,810</a>	1 Jun 1999	A method to improve crop yield by treating transgenic plants resistant to a glutamine synthetase inhibitor with an inhibitor such as phosphinothricin —in case the resistance is conferred by the <i>bar</i> gene. <a href="#">View Claims</a>
<a href="#">US 5,739,082</a>	14 Apr 1998	A method to improve growth of crops by treating plants resistant to glutamine synthetase inhibitors with a stimulating amount of an inhibitor such as phosphinothricin —in case the resistance is conferred by the <i>bar</i> gene. <a href="#">View Claims</a>
Related patents and applications	Granted patents in : Austria AT 172847 E; Australia AU 654662 B2; AU 700325 B2; China CN 1089555 B; Czech Rep CZ 286623 B6; Germany DE 59109123 C0; DE 59407241; Hungary HU 219059 B; HU 220775 B1; OAPI OA 9664 A; Poland PL 180005 B1; Romania RU 2166850 C2. Applications in : Canada CA 2,075,209 AA, CA 2,169,282 AA, Germany DE 4003045 A1, DE 4327056 A1, Denmark DK 714237 T3, Spain ES 2131050 T3, ES	

2124906 T3, Hungary HU 64395 A2, JP 5506568 T2, Japan JP 9501179 T2, New Zealand NZ 236979 A, NZ 271372 A, South Africa ZA 9406038 A
--

The number of related patents suggests that this invention represents a high value to the assignee. The combination of two agronomically important genes in one invention — a virus resistance gene and a herbicide resistance gene — increases the commercial options of a transgenic crop.

Beside the obvious use of a herbicide to eliminate weeds the inventors claim improved growth of crops after application. Dependent claims in this patent family mention phosphinothricin as the herbicide of choice.

### **EP 513 054**

Claim 1 recites a plant cell that comprises both a phosphinothricin resistance gene and a gene coding for a virus resistance. This is a broad claim which is limited by the embodiments cited in the following claims.

The phosphinothricin gene must be obtained from a *Streptomyces* species, and hence includes the *bar* gene (Claim 4). Furthermore, the virus resistance gene must code for a virus coat protein and be obtained from RNA of either cucumber mosaic virus, alfalfa mosaic virus or of brom mosaic virus (Claims 2-3). Dependent Claim 5 recites a process for producing plants with the transgenes, as described in Claim 1, that display improved growth after treatment with phosphinothricin. The use of the plant cell for the regeneration of plants, with or without improved herbicide or virus resistance, is also claimed (Claims 6-7)

### **EP 899 340 A2**

This patent application is still active (office actions have taken place in 2002). Hence the application seems to be still alive and it must be noted that the claims have not yet been granted and are therefore subject to alteration.

Independent Claim 1 of this patent application is written in more general terms than European patent EP 513 014 B1. It recites "a gene coding for virus resistance" in combination with "a herbicide resistance gene". In the mentioned patent, the herbicide was phosphinothricin. The object of this patent application seems to be to ensure wider coverage for the gene.

### **EP 714 237**

This member of the family deals exclusively with the growth-promoting effects of herbicide treatment and not with virus resistance. The patent claims the application of glutamine synthetase inhibitors to crop plants that are resistant to such inhibitors (Claim 1). The application of such herbicides has commercial implications in that crop yield is expected to increase after application as a result of decreased competition from weeds and other non-resistant plants.

The patent specifically claims the application of herbicides to plants (including transgenic plants) that express an N-acetyl-transferase gene (Claim 2).

### **US 5,633,434**

Claim 1 recites the combination of two DNA molecules, one encoding a phosphinothricin resistance gene and the other a virus coat protein capable of conferring virus resistance. Dependent Claim 3 recites *Streptomyces* as the source of the phosphinothricin gene. The patent extends to cover plants, plant cells or seeds of plants that express the two resistance genes (Claim 6). The patent also covers a method to improve the growth of plants involving the transformation of plant cells with the above genes.

### **US 5,792,926**

This patent is a division of U.S. patent application No 08/279,706, Patent No 5,633,434. The emphasis here is on a transformed, transgenic plant cell expressing both a phosphinothricin (PPT) resistance gene and a viral coat protein (Claim 1). Utilizing the herbicide resistance to improve growth is the subject of Claim 2. Claim 2 recites the transformation, selection and regeneration of a plant from a plant cell containing the genes for herbicide and virus resistance as in Claim 1. The treatment of such regenerated plants with phosphinothricin is also claimed. The phosphinothricin resistance gene must be from a *Streptomyces* species (Claim 6). However in the specifications the resistance gene from *Streptomyces viridochromogenes* is preferred. This patent also claims the isolated DNA sequence used to transform the plant cells (Claim 3).

### **US 5,739,082**

This patent is a continuation-in-part of U.S. patent application No 08/279,706, Patent No 5,633,434, i.e. it contains additional disclosure. Claims deal solely with the growth-enhancing effects when treating transgenic plants containing a glutamine synthetase inhibitor resistance gene with the corresponding herbicide. Claims are also directed to field treatment details to achieve the desired results.

### **US 5,908,810**

This patent covers the application of a glutamine synthetase inhibitor to plants that, through transformation, have been modified to be resistant to glutamine synthetase inhibitors (Claim 1). The transformation of the plant cells with a DNA fragment coding for n-acetyltransferase is also claimed (Claim 6).

## APPENDIX

[1. Dominant \*bar\* gene patents](#)

[2. \*Bar\* genes from \*Streptomyces\* sp.](#)

[3. \*Bar\* genes from \*Streptomyces viridochromogenes\*](#)

[4. \*Bar\* genes from \*Alliccaligenes\* sp and other gram-negative bacteria](#)

[5. \*Bar\* genes in combination with a virus resistance gene](#)

## 1. Dominant *bar* gene patents

Patents originally assigned to Plant Genetic Systems NV and Biogen NV. These patents are now part of the dominant patent family in the Bayer Crop Science portfolio.

Granted actual claims.

### EP 242 236 B2

#### **Claim 1**

Process for controlling the action in plant cells and plants comprising such cells of a glutamine synthetase inhibitor when the former are contacted with the latter, which comprises causing the stable integration in the genomic DNA of said plant cells of a heterologous DNA including a promoter recognized by polymerases of said plant cells and a foreign nucleotide sequence capable of being expressed in the form of a protein in said plant cells and plants, under the control of said promoter, and wherein said protein has an enzymatic activity capable of causing inactivation or neutralization of said glutamine synthetase inhibitor.

#### **Claim 2**

Process according to claim 1, wherein the heterologous DNA fragment comprises a foreign nucleotide sequence coding for a polypeptide having an acetyl transferase activity particularly PPT acetyltransferase activity with respect to said glutamine synthetase inhibitor.

#### **Claim 3**

Process according to claims 1 or 2, wherein the foreign nucleotide sequence is derived from the genome of an antibiotic-producing *Streptomyces* strain or is a nucleotide sequence encoding the same activity.

#### **Claim 7**

A process for producing a plant or reproduction material of said plant including a heterologous genetic material stably integrated therein and capable of being expressed in said plants or reproduction material in the form of a protein capable of inactivating or neutralizing the activity of a glutamine synthetase inhibitor, which process comprises transforming cells or tissue of said plants with a DNA recombinant containing a heterologous DNA including a foreign nucleotide sequence encoding said protein as well as the regulatory elements selected among those which are capable of causing the stable integration of said heterologous DNA in said plant cells or tissue and of enabling the expression of said foreign nucleotide sequence in said plant cells or plant tissue, regenerating plants or reproduction material of said plants or both from the plants cells or tissue transformed with said heterologous DNA and, optionally biologically replicating said last mentioned plants or reproduction material or both.

#### **Claim 18**

Process for selectively protecting the culture of a plant species and

selectively destroying weeds which comprises the steps of treating the field with a herbicide consisting of a glutamine synthetase inhibitor, wherein the cells of the plant species contain in their genome a foreign nucleotide sequence encoding a protein having an enzymatic activity capable of neutralizing or inactivating said glutamine synthetase inhibitor under the control of a promoter recognized by the polymerases of the cells of said plant.

**Claim 23**

Process for selectively protecting a plant species in a field against fungal diseases comprising the steps of treating said field with a herbicide consisting of a glutamine synthetase inhibitor, wherein the plant species contains in the genome of its cells a heterologous DNA including a promoter recognized by the polymerases of said cell and a foreign nucleotide sequence encoding a protein having an enzymatic activity capable of neutralizing or inactivating said glutamine synthetase inhibitor under the control of said promoter.

**Abandoned Claims**

**Claim 18**

Seeds, non biologically transformed which possess, stably integrated in the genome of their cells, a heterologous DNA containing a promoter recognized by the polymerases of said seeds and a foreign nucleotide sequence encoding a protein having a non-variety-specific enzymatic activity capable of inactivating or neutralizing a glutamine synthetase inhibitor under the control of said promoter.

**Claim 19**

Seeds according to claim 18, which are capable of germinating into a plant capable of producing seeds having a non-variety-specific enzymatic activity capable of inactivating or neutralizing glutamine synthetase inhibitor.

**Claim 20**

Seeds according to claim 18 or 19, which are transformed by the process of any one of claims 7 to 13.

**Claim 21**

Plant, non biologically transformed, which possesses, stably integrated in the genome of its cells, a foreign DNA nucleotide sequence encoding a protein having a non-variety-specific enzymatic activity capable of neutralizing or inactivating a glutamine synthetase inhibitor under the control of a promoter recognized by the polymerases of said cells.

**Claim 22**

Plant according to claim 21, which is capable of producing seeds having a non-variety-specific enzymatic activity capable of inactivating or neutralizing a glutamine synthetase inhibitor.

**Claim 23**

Plants according to claim 21 or 22, which are transformed by the process if any of claims 7 to 13.

## US 5,561,236

**Claim 1**

A plant cell having a heterologous DNA stably integrated into its genome; said DNA comprising a heterologous DNA fragment encoding a protein having an acetyl transferase activity which inactivates a glutamine synthetase inhibitor in said cell.

**Claim 2**

The cell of claim 1 wherein said DNA fragment encodes a polypeptide having a phosphinothricin acetyl transferase activity with respect to Bialaphos or phosphinothricin.

**Claim 8**

A plant which consists of the cells of claim 1 and which is susceptible to infection and transformation by *Agrobacterium* and capable of regeneration thereafter.

## 2. *Bar* genes from *Streptomyces* sp.

These patents make up the remaining part of the dominant family now owned by Bayer Crop Science and originally assigned to Plant Genetic Systems NV and Biogen NV.

Actual granted claims.

## EP 242 246 B1

**Claim 1**

A DNA fragment, for the subsequent transformation of plant cells, coding for a polypeptide having phosphinothricin-acetyl-transferase activity, which consists of a nucleotide sequence coding for the following amino acid sequence

V S P E R R P V E I R P A T A A D M A A V C D I V N H Y I E T S T V N P R  
T E P Q T P Q E W I D D L E R L Q D R Y P W L V A E V E G V V A G I A Y  
A G P W K A R N A Y D W T V E S T V Y V S H R H Q R L G L G S T L Y T  
H L L K S M E A Q G F K S V V A V I G L P N D P S V R L H S A L G Y T A  
R G T L R A A G Y K H G G W H D V G F W Q R D F E L P A P P R P V R P  
V T Q I \*

or of a part of said nucleotide sequence of sufficient length to code for a polypeptide still having phosphinothricin-acetyl-transferase activity.



**Claim 2**

The DNA fragment of claim 1, which comprises the following nucleotide sequence

```
TAAAGAGGTGCCCCACCCGCTTTCCOCAGXACAC
CGAAGGAGACCACACGTGAGCCCAGAACGACGCC
GGTCGAGATCCGTCCCGCCACCGCCGCCGACATCG
CGGCGGTCTGCGACATCGTCAATCACTACATCGAG
ACGAGCACGGTCAACTTCCGTACGGAGCCGCAGAC
TCCGCAGGAGTGGATCGACGACCTGGAGCGCCTCC
AGGACCGCTACCCCTGGCTCGTCGCCGAGGTGGAG
GGCGTCGTCCGCCGATCGCCTACGCCGGCCCTG
GAAGGCCCGCAACGCCTACGACTGGACCGTCGAGT
CGACGGTGTACGTCTCCACCGGCACCAGCGGCTC
GGACTGGGCTCCACCCTCTACACCCACCTGCTCAA
GTCCATGGAGGCCAGGGCTTCAAGAGCGTGGTCG
CCGTCATCGGACTGCCCAACGACCCGAGCGTGC GC
CTGCACGAGGCGCTCGGATACACCGCGCGCGGAC
GCTGCGGGCAGCCGGCTACAAGCACGGGGGCTGG
CACGACOTGGGGTTCTGGCAGCGCGACTTCGAGCT
GCCGGCCCCGCCCCGCCCGTCCGGCCCGTCAAC
AGATCTGAGCGGGAGAGCGCATGGC
```

or of a part thereof expressing a polypeptide having phosphinothricin-acetyl-transferase activity.

**Claim 4**

Process for controlling the action in plant cells and plants comprising such cells of a glutamine synthetase inhibitor when the former are contacted with the latter, which comprises causing the stable integration in the genomic DNA of said plant cells of a heterologous DNA including a promoter recognized by polymerases of said plant cells and a foreign nucleotide sequence capable of being expressed in the form of a protein in said plant cells, and wherein said protein has an enzymatic activity capable of causing inactivation or neutralization of said glutamine synthetase inhibitor, characterized in that said foreign nucleotide sequence is the nucleotide sequence or the DNA fragment of any of claims 1 to 3.

**Claim 5**

Process for producing a plant or reproduction material of said plant including a heterologous genetic material stably integrated therein and capable of being expressed in said plants or reproduction material in the form of a protein capable of inactivating or neutralizing the activity of a glutamine synthetase inhibitor, which process comprises transforming cells or tissue of said plants with a DNA recombinant containing a heterologous DNA, as well as the regulatory elements selected among those which are capable of causing the stable integration of said heterologous DNA in said plant cells or tissue and of enabling the expression of said foreign nucleotide sequence in said plant cells or plant tissue, regenerating plants or reproduction material of said plants or both from the plants cells or tissue transformed with said heterologous DNA and, optionally, biologically replicating said last mentioned plants or

reproduction material or both, characterized in that said heterologous DNA has the nucleotide sequence of the DNA fragment of any of claims 1 to 3 or of said part that codes for a protein having phosphinothricin acetyl transferase activity.

**Claim 19**

Process for selectively protecting a plant species and selectively destroying weeds in a field which comprises the steps of treating a field with a herbicide, wherein the plant species contain in their genome a heterologous DNA as defined in any of claims 1 to 3, and wherein the used herbicide is a glutamine synthetase inhibitor.

**Claim 23**

Process for selectively protecting a plant species in a field against fungal diseases comprising the steps of treating a field with a herbicide consisting of a glutamine synthetase inhibitor, wherein the plant species contain in the genome of its cells a heterologous DNA as defined in any of claims 1 to 3 and wherein the used herbicide is a glutamine synthetase inhibitor.

**US 5,646,024**

**Claim 1**

A process for the production of a plant cell that is tolerant or resistant to the herbicidal activity of a glutamine synthetase inhibitor including phosphinothricin or a compound with a phosphinothricin moiety, which comprises the step of incorporating into the nuclear genome of a starting plant cell a recombinant DNA comprising:

- a) a promoter recognized by the polymerases of said starting plant cell, and
- b) a coding region comprising a DNA fragment from a microorganism which produces said glutamine synthetase inhibitor, wherein said DNA fragment encodes a protein with acetyl transferase activity to said glutamine synthetase inhibitor.

**Claim 2**

The process of claim 1, in which said DNA fragment is from a *Streptomyces* species and said DNA fragment encodes a protein with phosphinothricin acetyl transferase activity.

**Claim 17**

A process for the protection of a group of cultivated plants of a plant species in the field by destroying weeds and/or fungi wherein said plants have incorporated into the genome of their cells a recombinant DNA comprising

- a) a promoter recognized by the polymerases of cells of said plants, and
- b) a coding region comprising a DNA fragment from a microorganism which produces a glutamine synthetase inhibitor including

phosphinothricin or a compound having a phosphinothricin moiety, wherein said DNA fragment encodes a protein with acetyl transferase activity to said glutamine synthetase inhibitor, and wherein said weeds and/or fungi are destroyed by application of a herbicide comprising said glutamine synthetase inhibitor as an active ingredient.

**Claim 38**

A process for the production of a pure culture of transformed plant cells that have a foreign DNA incorporated into the nuclear genome of their cells which comprises the steps of:

- i) transforming starting plant cells in a plant cell culture with a foreign DNA which comprises:
  - a) a promoter recognized by the polymerases of said starting plant cell, and
  - b) a coding region comprising a DNA fragment from a microorganism which produces a glutamine synthetase inhibitor, including phosphinothricin or a compound with a phosphinothricin moiety wherein said DNA fragment encodes a protein with acetyl transferase activity to said glutamine synthetase inhibitor; and
- ii) selecting the transformed plant cells by applying to the plant cell culture said glutamine synthetase inhibitor at a sufficient concentration to kill the untransformed plant cells.

**US 5,648,477**

**Claim 1**

A vector comprising a chimeric gene comprising in sequence:  
(a) a promoter recognized by polymerases of a plant cell; and  
(b) a DNA fragment encoding a protein with acetyl transferase activity on a glutamine synthetase inhibitor, wherein said protein is capable of inactivating said glutamine synthetase inhibitor in a plant cell.

**Claim 2**

The vector of claim 1, wherein said DNA fragment encodes a protein with phosphinothricin acetyl transferase activity.

**Claim 25**

A vector comprising a DNA fragment encoding a protein with acetyl transferase activity on a glutamine synthetase inhibitor, wherein said protein is capable of inactivating said glutamine synthetase inhibitor in a plant cell.

### 3. *Bar* genes from *Streptomyces viridochromogenes*

The following patents were originally assigned to Hoechst AG and are now part of the Bayer Crop Science portfolio.

Actual granted independent claims.

#### EP 257 542 B1

**Claims for the following contracting states: BE, CH, DE, FR, GB, GR, IT, LI, LU, NL, SE**

**Claim 1**

A phosphinothricin (PTC) resistance gene which can be obtained by cutting, with BamHI, the total DNA from *Streptomyces viridochromogenes* DSM 4112 which has been selected for phosphinothricyl-alanyl-alanine (M) resistance, by cloning fragment 4.0 kb in size, and by selection for PTT resistance.

**Claims for the following contracting states: AT, ES**

**Claim 1**

A process for obtaining a phosphinothricin (PTC) resistance gene which comprises isolation of a fragment 4.0 kb in size by cutting, with BamHI, from the total DNA from *Streptomyces viridochromogenes* DSM 4112 which has been selected for phosphinothricyl-alanyl-alanine (M) resistance, cloning the whole of this fragment or a part thereof containing the resistance gene, and selecting for PTT resistance.

#### EP 275 957 B1

**Claims for the following contracting States: AT, BE, CH, DE, FR, GB, GR, IT, LI, LU, NL, SE**

**Claim 1**

A resistance gene coding for the protein of amino acid sequence 1 (annex), in that ATG is used as start codon and TGA is used as stop codon, and the GC content of the gene is adapted to that in plants.

**Claim 7**

A host cell containing a vector as claimed in claim 4, 5 or 6.

**Claim 8**

A plant cell containing a gene as claimed in claim 1, 2 or 3.

**Claims for the following contracting state: ES**

**Claim 1**

A process for the preparation of a phosphinothricin (PTC)-resistance

gene, which comprises synthesis of a gene which codes for the protein of amino acid sequence 1 (annex), wherein ATG is used as start codon and TGA is used as stop codon, and the GC content of the gene is adapted to that in plants.

**Claim 3**

A process for the generation of PTC-resistant plant cells, plants, parts of plants or seeds, which comprises coupling a gene which has been obtained as claimed in claim 1 or 2 to regulation and expression signals active in plants, introducing the resulting gene structure into plant cells, and bringing about its expression therein.

**US 5,273,894**

**Claim 1**

Phosphinothricin (PTC)-resistance gene obtainable by selecting *Streptomyces viridochromogenes* DSM 4112 for resistance to phosphinothricyl-alanyl-alanine (PTT), cutting with BamHI the total DNA from the resistant strains, cloning a fragment 4.0 kb in size, and selecting for PTT resistance.

**Claim 4**

A plant cell transformed with the gene of claim 2.

**Claim 5**

The gene as claimed in claim 1, comprising at least the positions 258-806 of the DNA sequence I: [sequence omitted]

**Claim 8**

A bacterium transformed with the gene of claim 1.

**Claim 9**

A plant cell transformed with the gene of claim 1.

**Claim 10**

A process for the selective acetylation of the NH<sub>2</sub>-group of the L-form of racemic PTC which comprises contacting racemic PTC with (a) a cell expressing a phosphinothricin (PTC)-resistance gene, wherein said gene is obtainable by selecting *Streptomyces viridochromogenes* DSM 4112 for resistance to phosphinothricyl-alanyl-alanine (PTT), cutting with BamHI the total DNA from the resistant strains, cloning a fragment 4.0 kb in size, and selecting for PTT resistance, or (b) the enzyme encoded by said gene and fractionating the D-form and the acylated L-form of PTC.

**US 5,276,268**

**Claim 1**

An isolated resistance gene coding for the protein of amino acid sequence III, which gene is adapted to codon usage in plants so that it

is expressed in plant cells at a level sufficient to confer resistance to phosphinothricin in said plant cells.

**Claim 3**

A gene structure having DNA sequence III operatively linked to regulation and expression signals active in plants so that it is expressed in plant cells at a level sufficient to confer resistance to phosphinothricin in said plant cells.

**Claim 6**

A plant cell containing a gene as claimed in claim 1.

**Claim 9**

Plants and their propagules containing a gene as claimed in claim 1.

**Claim 12**

A process for generating phosphinothricin resistant plant cells, plants, and their propagules which comprises transforming plant cells with the gene as claimed in claim 1, and regenerating the transformed plant cells to plants which produce propagules.

**US 5,637,489**

**Claim 1**

A process for the production of a PTC-resistant plant which comprises incorporating into the genome of the plant a phosphinothricin (PTC)-resistance gene obtainable by selecting *Streptomyces viridochromogenes* DSM 4112 for resistance to phosphinothricyl-alanyl-alanine (PTT), cutting with BamHI the total DNA from the resistant strains, cloning a fragment 4.0 Kb in size, and selecting for PTT resistance.

**Claim 3**

A process for the production of a PTC-resistant plant which comprises incorporating into the genome of the plant a phosphinothricin (PTC)-resistance gene obtainable by selecting *Streptomyces viridochromogenes* DSM 4112 for resistance to phosphinothricyl-alanyl-alanine (PTT), cutting with BamHI the total DNA from the resistant strains, cloning a fragment 4.0 Kb in size, and selecting for PTT resistance, wherein the PTC-resistance gene so-obtained contains at least positions 258-806 of the DNA sequence I [sequence omitted here].

**Claim 4**

A plant which contains the PTC-resistance gene said gene is obtainable by selecting *Streptomyces viridochromogenes* DSM 4112 for resistance to phosphinothricyl-alanyl-alanine (PTT), cutting with BamHI the total DNA from the resistant strains, cloning a fragment 4.0 Kb in size, and selecting for PTC resistance.

## US 5,879,903

### Claim 1

A method of using a phosphinothricin (PTC)-resistance gene as a resistance marker in bacteria comprising the steps of transforming the bacteria with the phosphinothricin (PTC)-resistance gene, culturing the bacteria in a medium, exposing the bacteria to phosphinothricin, and determining whether the bacteria is resistant to the phosphinothricin, wherein the phosphinothricin (PTC)-resistance gene is obtainable by selecting *Streptomyces viridochromogenes* DSM 4112 for resistance to phosphinothricyl-alanyl-alanine (PTT), cutting with BamHI the total DNA from the resistant strains, cloning a fragment 4.0 kb in size, and selecting for PTT resistance.

### Claim 4

A method of using a phosphinothricin (PTC)-resistance gene as a resistance marker in plant cells comprising the steps of transforming the plant cells with the phosphinothricin (PTC)-resistance gene, culturing the plant cells in a medium, exposing the plant cells to phosphinothricin, and determining whether the plant cells are resistant to the phosphinothricin, wherein the phosphinothricin (PTC)-resistance gene is obtainable by selecting *Streptomyces viridochromogenes* DSM 4112 for resistance to phosphinothricyl-alanyl-alanine (PTT), cutting with BamHI the total DNA from the resistant strains, cloning a fragment 4.0 kb in size, and selecting for PTT resistance.

## 4. Bar genes from *Alcaligenes* sp and other gram-negative bacteria

The third patent family making up the Bayer Crop Science portfolio. These patents were originally assigned to Hoechst AG.

Granted actual claims.

## EP 290 986 B1

**Claims for the following contracting states : CH, DE, FR, GB, IT, LI, NL**

### Claim 1

A phosphinothricin(PTC)-resistance gene, obtainable by selection of bacteria, which are not fungus-like, for PTC resistance, extraction of the DNA, construction of a gene bank, isolation of PTC-resistant clones, and extraction of the PTC-resistance gene from these clones.

### Claim 2

A gene as claimed in claim 1, wherein bacteria of the genus

*Pseudomonas, Alcaligenes, Agrobacterium, Enterobacter, Serratia* or *Cedecea* bacteria of the species *Pseudomonas paucimobilis, Alcaligenes faecalis* or *eutrophus, Agrobacterium tumefaciens, Enterobacter agglomerans, Serratia plymuthica* or *Cedecea* Gr. V are used.

**Claim 8**

Useful plants and parts thereof, which harbor an expressible resistance gene as claimed in one or more of claims 1 to 5.

**Claim 9**

Propagation material from useful plants, which harbors an expressible resistance gene as claimed in one or more of claims 1 to 5.

**Claim 10**

The use of microorganism populations which express the resistance gene as claimed in one or more of claims 1 to 5 in sewage treatment plants or on areas under agricultural use

**Claims for the following contracting state : ES**

**Claim 1**

A process for obtaining a phosphinothricin(PTC)-resistance gene, which comprises selecting bacteria which are not fungus-like for PTC resistance, isolating the DNA from the selectants, constructing a gene bank therefrom, isolating PTC-resistant clones, and obtaining the resistance gene from the DNA thereof.

**Claim 2**

The process as claimed in claim 1, wherein bacteria of the genus *Pseudomonas, Alcaligenes, Agrobacterium, Enterobacter, Serratia* or *Cedecea* are used.

**Claim 6**

A process for the production of PTC-resistant plant cells, plants, parts of plant and propagation material, which comprises inserting into the plant cells an expressible gene which has been obtained as claimed in claim 1 to 5.

**Claim 7**

A process for the selection of plants, plant cells or bacteria, which comprises inserting into the cells an expressible gene which has been obtained as claimed in claim 1 to 5, and selection for PTC resistance.

**Claim 8**

A process for the biological degradation of PTC in PTC-containing residues, areas under agricultural use, and sewage, which comprises contacting the residues or sewage with microorganism populations which express a gene obtained as claimed in claim 1 to 5.

**US 5,077,399**

**Claim 1**

A phosphinothricin (PTC)-resistance gene obtained by selecting bacteria



from the genus *Alcaligenes* for PTC resistance, extracting the DNA, constructing a gene bank, isolating PTC-resistant clones, and obtaining the PTC-resistance gene from these clones.

**Claim 2**

A gene as claimed in claim 1, wherein bacteria of the species *Alcaligenes faecalis* or *eutropus* are selected.

## 5. *Bar* genes in combination with a virus resistance gene

These patents were all originally assigned to Hoeschst Schering AgrEvo GmbH and are now part of the Bayer Crop Science portfolio.

Actual granted claims.

### EP 513 054 B1

**Claim 1**

Plant cells comprising a gene coding for a phosphinothricin resistance and a gene coding for a virus resistance.

**Claim 5**

A process for producing plants with improved properties, which comprises plants being regenerated from plant cells as claimed in claim 1, and these plants being treated with phosphinothricin.

### EP 899 340 A2

**Claim 1**

Plants, plant cells and parts of plants or seed of those plants comprising a gene coding for virus resistance in combination with a herbicide resistance gene.

### EP 714 237 B1

**Claim 1**

A method of increasing the yield of crop plants which are resistant to glutamine synthetase inhibitors, which comprises treating the plants with glutamine synthetase inhibitors at application rates which are not harmful to the plants.

## US 5,633,434

### **Claim 1**

An isolated DNA molecule consisting of a nucleotide sequence coding region for a phosphinothricin acetyl transferase protein which confers phosphinothricin resistance and a nucleotide sequence coding region for a virus coat protein which confers virus resistance.

### **Claim 10**

A transformed plant cell containing and expressing an isolated DNA molecule consisting of a nucleotide sequence coding region for a phosphinothricin acetyl transferase protein which confers phosphinothricin resistance and, an isolated DNA molecule consisting of a nucleotide sequence coding region for a virus coat protein which confers virus resistance.

### **Claim 11**

A method for improving growth of a plant comprising: transforming plant cells so that the cells contain an isolated DNA molecule consisting of a nucleotide sequence coding region for a phosphinothricin acetyl transferase protein which confers phosphinothricin resistance and, an isolated DNA molecule consisting of a nucleotide sequence coding region for a virus coat protein which confers virus resistance; selecting transformed cells; regenerating plants from the cells; and treating the regenerated plants with a phosphinothricin herbicide.

## US 5,792,926

### **Claim 1**

A transformed plant cell containing and expressing an isolated DNA sequence comprising a first DNA sequence coding for a protein which confers phosphinothricin resistance and, a second DNA sequence coding for a virus coat protein which confers virus resistance.

### **Claim 2**

A method for improving growth of a plant comprising: transforming a plant cell so that the cell contains an isolated DNA sequence comprising a first DNA sequence coding for a protein which confers phosphinothricin resistance and, a second DNA sequence coding for a virus coat protein which confers virus resistance; selecting transferred cells; regenerating the plant from the cell; and treating the regenerated plant with phosphinothricin.

### **Claim 3**

An isolated DNA sequence comprising a first DNA sequence coding for a protein which confers phosphinothricin resistance and, second DNA sequence coding for a virus coat protein which confers virus resistance.

## **US 5,908,810**

### **Claim 1**

A method of improving the yield of crop plants which are transformed so as to be resistant to glutamine synthetase inhibitors, which comprises treating the plants with a glutamine synthetase inhibitor.

## **US 5,739,082**

### **Claim 1**

A method of improving the growth of crop plants which are transformed so as to be resistant to glutamine synthetase inhibitors, which comprises treating the plants with a growth stimulating amount of a glutamine synthetase inhibitor.