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MARKER GENE

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(57) Claim

1. A chimeric selectable marker gene for transforming a plant to render it resistant to an aminoglycoside antibiotic (e.g., kanamycin) ; said marker gene comprising in sequence:

a) a plant-expressible promoter;

b) an aac(6') DNA; and

c) a 3' end formation and polyadenylation region active in plant cells.

CORRECTED VERSION*

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(57) Abstract

A method to select and identify transformed plant cells by expressing a chimeric gene encoding an aminoglycoside-6'-N-acetyltransferase in the plant cells in the presence of an aminoglycoside antibiotic.

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* (Referred to in PCT Gazette No. 33/1997, Section II)

MARKER GENE

This invention relates to a chimeric selectable marker gene comprising: a plant-expressible promoter, DNA encoding an aminoglycoside-6'-N-acetyltransferase (the "AAC(6')"), and a 3' end formation and polyadenylation region active in plant cells.

This invention further relates to a process for selecting or identifying transformed plant cells by expressing the chimeric marker gene, encoding the AAC(6'), in the plant cells. The chimeric marker gene confers, on the plant cells, resistance to normally lethal or growth-suppressive concentrations of an antibiotic which is efficiently detoxified by the AAC(6') in the cells.

This invention also relates to a plant cell, stably transformed with the chimeric marker gene, encoding the AAC(6'), and to a plant regenerated from this plant cell.

Background of the invention

Plant genetic engineering technology has made significant progress during the last decade. It has become possible to introduce stably foreign genes into plants. This has provided exciting opportunities for modern agriculture.

The use of chimeric selectable marker genes in plant cell transformation has considerably simplified the selection of transformed plant cells. For example, by the expression of such a marker gene, transformed plant cells can be made resistant to antibiotics that are cytotoxic or growth-suppressing to non-transformed cells. A commonly used chimeric marker gene contains the neomycin phosphotransferase-II or nptII coding region (Bevan et al (1983) Nature 304, 184-187; Fraley et al (1983) Proc. Natl. Acad. Sci USA 80, 4803-4807).

The nptII gene confers resistance to kanamycin, neomycin and G-418 antibiotics on plant cells expressing the gene (Reynaerts et al (1987) Plant Mol. Biol. Manual, Gelvin, S.B. & Schilperoort, R.A. (eds), Kluwer, Dordrecht, sect. A9, pp. 1-16).

Chimeric marker genes have typically contained: a plant-expressible promoter (with a 5' untranslated region); DNA (such as the npt II gene) encoding a selectable marker; and a 3' end formation and polyadenylation region active in plants. Although the versability of the nptII gene has been confirmed in chimeric marker genes in several plant systems over the years, there have been limitations on its use that have necessitated the development of alternative antibiotic-resistance genes for use in such chimeric selectable marker genes (Hayford et al (1988) Plant Physiol. 86, 1216). Furthermore, in many situations, a second complementary antibiotic-resistance gene has been needed for introduction into plants that have already been transformed with an antibiotic-resistance gene. Such alternative antibiotic-resistance genes already exist, but they often require the use of very toxic substrates and/or they do not allow efficient selection in all plant species. Certainly for species that are routinely vegetatively reproducible, like potato, antibiotic-resistance genes encoding different selectable markers, with different specific substrates, are required when different genes have to be engineered at different times into a plant.

Among the known antibiotic-resistance genes are those encoding aminoglycoside antibiotic-acetylating (AAC) enzymes, four types of which have been characterized (based on the position of the modified amino group of the 2-deoxystreptamine-derived aminoglycosides):AAC(1), AAC(2'), AAC(3) and AAC(6'). See Shaw et al (1989) Antimicrob. Agents & Chemotherapy

33, 2052-2062. High-pressure liquid chromatography (HPLC) analysis has demonstrated the differences among the acetylated products of these four types of enzymes, and aminoglycoside-resistance profiles can be used to identify the presence of each of these types of enzymes in a host strain (Shaw et al (1989) *supra*).

European patent publication ("EP") 0 289 478 (Rogers et al (1988), Hayford et al (1988) *supra*, and Carrer et al (1991) Plant Mol. biol. *17*, 301-303 describe the selection on gentamycin of plants transformed with an aminoglycoside-3-N-acetyltransferase-encoding gene (the "*aac*(3) gene"). The *aac*(3)-IV gene was found to confer resistance to kanamycin (in Petunia), but the level of resistance was, at most, only sufficient for marginal selection (Hayford et al (1988) *supra*). These publications also describe supertransformation of tobacco, previously transformed with the *npt*II gene, with the *aac*(3) gene by selection on gentamycin-containing medium. EP 0 289 478 also describes the use of gentamycin as a substrate in the transformation of petunia, soybean, oilseed rape and alfalfa transformed with the *aac*(3) gene. Carrer et al (1991) *supra* also describes the transformation of tobacco plants with an *aac*(3)-I gene, only conferring resistance to gentamycin, whereby the gentamycin-resistant plants retain their sensitivity to kanamycin. According to Carrer et al (1991) *supra*, it may be more advantageous to use a selectable marker gene with a narrow substrate specificity in some cases.

The AAC(6')-encoding genes (the "*aac*(6') genes") constitute a class of different but related genes acetylating the 6' amino group of several aminoglycoside antibiotics. Several bacterial *aac*(6') genes have been cloned and sequenced. According to Davis (1986) In *Antibiotics in Laboratory Medicine*, pp. 474-489, (ed.) Lorian V., Williams & Wilkins, Baltimore, Maryland and Phillips Shannon (1984) British Med. Bull. 40, 28-35, AAC(6') acetylates tobramycin, kanamycin, amikacyn, neomycin, gentamycin C_{IA} and C_2, sissomycin and netilmycin, although with varying efficiencies depending on the kind of AAC(6'). Two subtypes of *aac*(6') genes have been characterized by their aminoglycoside resistance profiles: *aac*(6')-I genes and *aac*(6')-II genes; the former subclass comprises the *aac*(6')-IV and -4 genes, and the latter subclass comprises the *aac*(6')-III gene (Shaw et al (1989) *supra*). However, other classifications of these genes have also been made.

Another acetyltransferase, phosphinotricin acetyltransferase, has also been found to be capable of conferring a selectable phenotype (i.e., a herbicide resistance) to plant calls (De Block at al (1987) EMBO J. 6, 2513-2518).

EP 0 248 207 (Wohllebon et al, 1987) describes a gentamycin-resistance gene that is active in *Streptomyces* and is obtainable from a strain of *S. ghanaensis* by total digestion with BglII.

French patent publication 2 601 965 (Courvalin, 1988) describes a bifunctional gene encoding AAC(6') and APH(2'') activities, the cloning and sequencing of this gene, and the use of parts of the gene as a DNA probe for detecting antibiotic-resistance development in bacterial cultures.

Summary of the invention

In accordance with this invention is provided a chimeric selectable marker gene (the "chimeric *aac*(6') gene"), for transforming a plant cell or a plant to render said plant cell or said plant resistant to an aminoglycoside antibiotic, said marker gene comprising the following operably linked elements:-

(a) a plant-expressible promoter;

(b) a DNA encoding an aminoglycoside-6'-N-acetyltransferase capable of modifying said aminoglycoside antibiotic; and

(c) a DNA region comprising suitable 3' transcription regulation signals active in plant cells.

Also in accordance with this invention is provided a method for selecting or identifying transformed plant cells by:transforming the cells with the chimeric *aac*(6') gene; and then contacting the cells with concentrations of an aminoglycoside antibiotic that are lethal or growth-suppressive to non-transformed plant cells.

Further in accordance with the invention is provided a plant cell, stably transformed with the chimeric *aac*(6') gene, a plant cell culture and plant regenerated from this plant cell, and a plant transformation vector, a plasmid and an *Agrobacterium* strain containing the chimeric *aac*(6') gene.

Detailed Description of the Invention

The term "*aac*(6') DNA" as used herein means a DNA coding sequence encoding a protein (an "AAC (6')") which catalyses the acetylation of the 6' amino group of aminoglycoside antibiotics. This term includes a partly or fully synthetic DNA sequence, as well as a naturally occurring DNA sequence encoding an AAC(6'). Preferred *aac*(6') DNAs according to this invention include the DNA of SEQ ID No. 1 and substantially similar DNAs, such as the *aac*(6') DNAS described by Nobuta et al (1988) J. Bacteriol. 170, 3769), Tolmaski (1990) Plasmid 24, 219-226 and Than van Nhieu and Collatz (1987) J. Bacteriol., 169, 5708. Other aac (6') DNAs of this invention include those described by Davies and Smith (1978) Ann. Rev. Microbiol. 32, 469-518, Morohoshi et al (1984) J. Antibiotics 37, 1687-1691, Tenover et al., (1988) J. Bacteriol. 170, 471, Ferretti et al (1986) J. Bacteriol. 167, 631, and Shaw et al (1989) Antimicrob. Agents and Chemotherapy 33, 2052.

The term "chimeric aac(6') gene" as used herein means a chimeric selectable marker gene comprising the aac(6') DNA, operably linked to a plant-expressible promoter (including a 5' untranslated region) and a 3' end formation and polyadenylation region active in plants. The aac(6') DNA can also be expressed as a fusion protein in a chimeric gene fusion with another transforming DNA, so as to enhance selection for the desired genotype. The construction of this chimeric gene fusion can be carried out according to techniques derived from methods currently used for constructing chimeric genes comprising known markers.

The term "selectable marker gene" as used herein means a DNA sequence, the expression of which in a plant cell confers a selectable phenotype (e.g., antibiotic resistance) to the plant cell.

The term "translationally neutral modifications" as used herein means modifications of a gene or DNA sequence that do not affect the amino acid sequence encoded by the gene or DNA sequence. Preferred examples of such translationally neutral modifications are changes, by means of nucleotide substitutions, of codons into other codons encoding the same amino acids.

The term "suitable substrate" or "suitable substrate antibiotic" as used herein is an aminoglycoside antibiotic (e.g., kanamycin) which is efficiently modified by AAC(6') so that expression of the aac(6') DNA in a plant cell confers resistance on the plant cell to the antibiotic. Hence, the term "substrate of an AAC(6')" as used herein means any aminoglycoside antibiotic which can be modified, i.e. acetylated, by the aac(6') gene product.

An aac(6') DNA of this invention can easily be isolated from bacteria by routine procedures after cultivation on a suitable substrate containing normally inhibitory levels of an aminoglycoside antibiotic, such as kanamycin, for example as described by Nobuta et al (1988) J. Bacteriol. 170, 3769. AAC(6') activity can be assayed by conventional methods (Davies (1986) supra; Shaw et al (1989) supra).

Preferably, an aac(6') DNA of this invention is inserted in a plant genome downstream (i.e., 3') of, and under the control of, a promoter which can direct the expression of the gene in plant cells. Preferred promoters include, but are not limited to, the strong constitutive 35S promoter (Odell et al (1985) Nature 313, 810) or duplicated 35S promoter (Kay et al (1987) Science 236, 1299) of cauliflower mosaic virus; 35S promoters have been obtained from different isolates (Hull & Howell (1987) Virology 86, 482-493). Other preferred promoters include the TR1' promoter and the TR2' promoter (Velten et al (1984) EMBO J. 3, 2723). Also preferred are monocot promoters such as the promoters described in EPO 0 342 926 or EP 0 459 643. Alternatively, a promoter can be utilized which is not constitutive but rather is specific for one or more plant tissues or organs. For example, the aac(6') DNA can be selectively expressed in the green tissues of a plant by placing the DNA under the control of a light-inducible promoter such as the promoter of the ribulose-1,5-bisphosphate-carboxylase small subunit gene as described in EP 0 193 259. Another alternative is to use a promoter whose expression is inducible by temperature or chemical factors or a promoter that is expressed preferentially or selectively in the time period or the developmental stage at which cells are selected, such as a callus-specific promoter, which have been used previously with other markers. In any event, it is evident that a promoter for use in this invention must at least allow sufficient expression of the aac(6') DNA in plant cells to confer antibiotic-resistance to the plant cells.

It is preferred that the aac(6') DNA be inserted upstream (i.e., 5') of suitable 3' transcription regulation signals (i.e., transcript 3' end formation and polyadenylation signals). Preferred 3' transcription regulation signals include those of the chalcone synthase gene (Sommer & Saedler (1986) Mol. Gen. Genet. 202, 429), the cauliflower mosaic virus (Mogen et al (1990) The Plant Cell 2, 1261), the octopine synthase gene (Gielen et al (1984) EMBO J. 3, 835) or the T-DNA gene 7 (Velten & Schell (1985) Nucl. Acids Res. 13, 6981).

In accordance with this invention, all or part of an aac(6') DNA of this invention can be stably inserted in a conventional manner into the nuclear genome of a plant cell, and the so-transformed plant cell can be used to produce a transgenic plant showing resistance to aminoglycoside antibiotics. In this regard, a disarmed Ti-plasmid containing the chimeric aac(6') gene in Agrobacterium (e.g., A. tumefaciens) can be used to transform a plant cell using the procedures described, for example, in EP 0 116 718 and EP 0 270 822, PCT publication WO 84/02913, EP 0 242 246, De Block (1988) Theor. Appl. Genet. 76 767-774, and Gould et al (1991) Plant Physiol. 95, 426 (which are incorporated herein by reference). Preferred Ti-plasmid vectors contain the chimeric aac(6') gene between the border sequences, or at least located to the left of the right border sequence, of the T-DNA of the Ti-plasmid. Of course, other types of vectors can be used to transform the plant cell, using procedures such as direct gene transfer (as described, for example, in EP 0 233 247), pollen mediated transformation (as described, for example, in EP 0 270 356, PCT publication WO 85/01856, and US patent 4,684,611, plant RNA virus-mediated transformation (as described, for example, in

EP 0 067 553 and US patent 4,407,956), liposome-mediated transformation (as described, for example, in US patent 4,536,475) and other methods such as the methods for transforming monocots (e.g., the major cereals including corn, rice, wheat, barley and rye) as described in PCT publication WO 92/09696. In case the plant to be transformed is corn, other recently developed methods can also be used such as, for example, the method described for certain lines of corn by Fromm et al (1990) Bio/Tech. 8, 833, Gordon-Kamm et al (1990) The Plant Cell 2, 603, and Gould et al (1991) supra. In case the plant to be transformed is rice, other recently developed methods can also be used such as, for example, the methods described by Shimamoto et al (1989) Nature 338, 274), Datta et al (1990) Bio/tech. 8, 736 and Hayashimoto et al (1990) Plant Physiol. 93, 857.

In order to improve the expression of the chimeric aac(6') gene of this invention in a plant, the aac(6') DNA can be modified, for example by changing its natural codon usage to form an equivalent artificial aac(6') DNA. Such modifications can include introducing functional introns and/or translationally neutral modifications into the aac(6') DNA sequence in order to eliminate deleterious DNA sequences present in this bacterial DNA, such as are described in PCT patent application PCT/EP92/02547. This can be done directly by modifying (in a translationally neutral manner) such deleterious DNA sequences, inhibiting expression in the plant cells, or indirectly by adapting the codon usage of the aac(6') DNA to that preferred by the plant, for example as described by Murray et al (1989) Nucleic Acids Res. 17, 477. Additionally for achieving sufficient expression in monocot plants such as corn, an efficiently spliced monocot intron, e.g. intron 1 from the corn adh gene, can be added to the chimeric aac(6') gene (Koziel et al (1993) Bio/Tech. 11, 194-200).

A transformed plant of this invention, regenerated from a plant cell transformed with the chimeric aac(6') gene, shows resistance against suitable substrate antibiotics by virtue of the production of AAC(6') activity in its plant cells. Such a plant can be used in a conventional breeding scheme to produce more transformed plants with the same aminoglycoside antibiotic-resistance characteristics or to introduce the chimeric aac(6') gene into other varieties of the same or related plant species by standard breeding techniques. Seeds, which are obtained from the transformed plants, contain the chimeric aac(6') gene as a stable genomic insert.

Since the spectrum of antibiotics which can be chemically modified by expression of an aac(6') DNA of this invention is different from that of the nptII coding region, the aac(6') DNA can be used with the nptII coding region in different chimeric selectable marker genes where two different foreign genes are to be introduced into a plant, with each foreign gene being associated with its own chimeric marker gene. When engineering multiple chimeric marker genes in a plant, it may be advantageous to use a chimeric aac(6') gene only conferring resistance to a limited group of aminoglycoside antibiotics (Carrer et al (1991) supra). However, if only one chimeric marker gene is to be used, it is preferred to use a chimeric aac(6') gene with kanamycin as the suitable substrate for plant cells. In this regard, the enzymatic assay for detecting acetyltransferase activity is often quicker and more convenient to use than the phosphotransferase assay used to detect nptII-activity.

To test for the successful transformation of plants with an aac(6') DNA, different methods are available. For example, antibiotic-resistance can be checked in a callus induction test or a dot application assay; the presence and the activity of AAC (6') can also be analyzed by an enzymatic assay; Western blotting can provide an easy immunological test but is less sensitive; and kanamycin resistance can be followed in the progeny of transgenic plants by the sowing of seeds on kanamycin-containing media.

The following Examples illustrate the invention. Unless otherwise stated in the Examples, all procedures for making and manipulating recombinant DNA are carried out by the standardized procedures described in Sambrook et al, *Molecular Cloning - A laboratory Manual, Second Ed.*, Cold Spring Harbor Laboratory Press, NY (1989) or in Ausubel et al., *Current Protocols in Molecular Biology*, vols. 1 and 2, Current Protocols, USA (1994).

Sequence Listing

SEQ ID No. 1 shows : i) the DNA sequence of an *aac*(6') DNA which comes from a *Shigella* plasmid, and ii) the corresponding amino acid sequence encoded by the *aac*(6') DNA.

SEQ ID No. 2 shows: the amino acid sequence of the AAC(6') protein.

SEQ ID No. 3 shows: the nucleotide sequence of PCR primer "RVA61".

SEQ ID No. 4 shows: the nucleotide sequence of PCR primer "OFD15".

SEQ ID No. 5 shows: the plasmid "pTRVA3" containing a chimeric *aac*(6') gene with a 35S-2 promoter, the *aac*(6') DNA of SEQ ID No. 1, and the 3' end formation

	and polyadenylation region from T-DNA gene 7.
SEG ID No. 6 shows:	the amino acid sequence of the AAC(6') protein.

Examples

Example 1. Cloning of an aac(6') DNA and construction of a chimeric aac(6') gene

The aac(6') DNA was obtained from the mini-Sa plasmid, pGV1106 (Leemans et al (1982) Gene 19, 361-364). The 1.5 Kb PvuII/HindIII fragment of pGV1106, containing the aac(6') DNA, was ligated to the ScaI-linearized plasmid pGSC1600 (Cornelissen & Vandewiele (1989) Nucl. Acids Res. 17, 19-29) after Klenow treatment. The resultant plasmid, pFD1002A, was used as a template for PCR, using the primers RVA61 and OFD15, of SEQ ID Nos. 3 and 4, respectively. RVA61 is complementary to the non-coding strand of the aac(6') DNA and its untranslated sequences in pFD1002A. The SalI/BamHI aac(6') PCR fragment of pFGD1002A was ligated to the 7.2 Kb SalI/BamHI fragment of the plasmid pGSJ290, containing the chimeric gene, P35S-nptII-3'g7, to yield the plasmid, PTRVA3, of SEQ ID No. 5.

pGSJ290 was derived from pGV825 (Deblaere et al (1985) Nucl. Acids Res. 13, 4777-4787), in which a chimeric P35S-nptII-3'g7 gene construct was cloned between T-DNA border repeats. The 3' untranslated end of the T-DNA gene 7 (i.e., 3'g7) was as described by Velten & Schell (1985) aac(6')supra, the nptII gene was from pKM109/90 (Reiss et al (1984) EMBO J. 3, 3317-3322), and the CaMV 35S promoter (i.e., P35S) was a cauliflower mosaic virus 35S-2 promoter as described in EP 0 193 259 and in Gardner et al (1981) Nucl. Acids

Res. 9, 2871-2888. The chimeric p35S-*npt*II-3'g7 gene construct was cloned between the HpaI and BglII sites of the T-DNA between the right and left border repeats of pGV825.

Due to the introduction of a *Bam*HI restriction site downstream of its translation initiation site, the *aac*(6') DNA sequence in pTRVA3, as shown in SEQ ID No. 5, contained the amino acids Asp and Pro at amino acid positions 2 and 3, respectively, and contained the chimeric *aac*(6') gene, P35S-*aac*(6') -3'g7, including the *aac*(6') DNA with the sequence of SEQ ID No. 1.

Example 2. Selection of *aac*(6')-transformed tobacco cells on kanamycin.

Plasmids pTRVA3 and pGSJ290 were mobilized from *E. coli* into *A. tumefaciens* strain C58C1-Rif^R (pGV2260; Deblaere et al (1985) *supra*) by means of a triparental cross as described by Deblaere et al (1985) *supra* and EP 0 193 259. The resultant *Agrobacteria* were selected on minimal A medium (Miller (1972) *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). Tobacco cv Petit Havana SR1 cells were cocultivated with these *Agrobacteria*, and transformed tobacco cells were selected using antibiotic resistance according to De Block et al (1984) EMBO J. 3, 1681-1689. *Agrobacterium* strains used for infection of tobacco protoplasts were: C58C1-Rif^R (pGV2260) as a negative control, C58C1-Rif^R (pGV2260::pTRVA3) which contains the *aac*(6') DNA, and C58C1-Rif^R (pGV2260::pGSJ290) which contains the *npt*II gene. One week after infection, the protoplasts were transferred to selective media containing one of the following different concentrations of kanamycin sulphate (Km):0-25-50-100,6200 µg/ml.

Seven weeks after infection, calli started growing from cells cocultivated with C58C1-Rif^R

(pGV2260::pTRVA3) and C58C1-Rif^R (pGV2260::pGSJ290) at all Km concentrations. Protoplasts infected with pGV2260 (negative control) only formed calli on medium without Km. After transfer of the calli to shoot-inducing medium containing 200 µg/ml Km, shoots were readily formed. Southern analysis of DNA extracted from kanamycin-resistant regenerated tobacco plants confirmed the stable integration of the aac(6') DNA.

Example 3. Selection of aac(6')-transformed potato cells on kanamycin.

Leaf discs of potato variety Yesmina were cocultivated with Agrobacterium tumefaciens strains C58C1-Rif^R (pGV2260::pTRVA3) and C58C1-Rif^R (pGV2260::pGSJ290), carrying the chimeric genes of Example 2 containing either the aac(6') DNA or nptII gene as described by De Block (1988) supra. The leaf discs were transferred to callus induction medium containing 50 µg/ml kanamycin sulphate. About 30% of the leaf discs that were cocultivated with Agrobacterium C58C1-Rif^R (pGV2260::pGSJ290) produced growing calli in the presence of kanamycin, while about 70% of the leaf discs that were cocultivated with Agrobacterium C58C1-Rif^R (pGV2260::pTRVA3) produced growing calli after culturing for 6 weeks on kanamycin medium. After transfer of the aac(6') -transformed calli to shoot-regenerating medium, shoots readily formed. The regenerated potato plants are found to retain the kanamycin-resistant phenotype. Southern analysis of DNA extracted from the kanamycin-resistant potato plants and their progeny confirms the integration of the aac(6') DNA.

Example 4. Assay for enzymatic activity in aac(6')-transformed plants.

The tobacco callus and leaf tissue of Example 2 containing the chimeric aac(6') gene, showed AAC(6') activity when tested in a Thin Layer Chromatography (TLC)-acetyltransferase assay for kanamycin. The acetyltransferase assay for determining phosphinotricin-acetyltransferase activity according to De Block et al (1987) EMBO J. 6, 2513-2518 was used, with the exception that kanamycin sulphate was used as a substrate instead of phosphinotricin in a concentration which was twice the concentration of phosphinotricin used by De Block et al (cf. supra), and 2 μl of ^{14}C-acetylcoenzyme A were added instead of a mixture containing both the radioactive and the non-radioactive form of the enzyme. Upon incubation for 30 minutes at 37°C, the reaction mixture was spotted onto TLC plates, and the reaction products were separated by chromatography in 1-propanol/NH_4OH (3/2). Extracts of the calli containing the chimeric aac(6') gene catalyzed the acetylation of kanamycin, while no reaction was observed with extracts of calli from non-transformed SR1 plants and from nptII-expressing calli. Also extracts from leaf tissue of regenerated aac(6') -transformed tobacco plants were found to efficiently acetylate kanamycin.

Needless to say, this invention is not limited to tobacco or potato plants transformed with the aac(6') DNA. It includes any plant, such as tomato, cotton, rapeseed, alfalfa, sunflower, corn, rice, soybean, brassica, sugar beet and other vegetables, transformed with an aac(6') DNA.

Nor is this invention limited to the use of the chimeric aac(6') gene of SEQ ID No. 5 or the aac(6') DNA of SEQ ID No. 1 for transforming plant cells so that they express an AAC(6'). Other natural and artificial chimeric genes and DNA can be used. In this regard, the aac(6') DNA sequences of SEQ ID Nos. 1 and 5 can be modified by: 1) replacing some codons with others that code either for the same or different, preferably the same, amino acids; and/or 2) deleting or adding some codons; provided that such modifications do not substantially alter the properties, especially the capacity to detoxify aminoglycoside antibiotics, of the encoded AAC(6').

All publications referred to in this application are hereby incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: PLANT GENETIC SYSTEMS N.V.
(B) STREET: Plateaustraat 22
(C) CITY: GENT
(E) COUNTRY: BELGIUM
(F) POSTAL CODE (ZIP): B-9000

(ii) TITLE OF INVENTION: MARKER GENE

(iii) NUMBER OF SEQUENCES: 6

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: EP 93401237.8

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 612 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..612
(D) OTHER INFORMATION: /codon_start= 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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  1               5                  10                  15

TAC AGC ATC GTG ACC AAC AGC ACC GAT TCC GTC ACA CTG CGC CTC ATG        96
Tyr Ser Ile Val Thr Asn Ser Thr Asp Ser Val Thr Leu Arg Leu Met
             20                  25                  30

ACT GAG CAT GAC CTT GCG ATG CTC TAT GAG TGG CTA AAT CGA TCT CAT       144
Thr Glu His Asp Leu Ala Met Leu Tyr Glu Trp Leu Asn Arg Ser His
         35                  40                  45

ATC GTC GAG TGG TGG GGC GGA GAA GAA GCA CGC CCG ACA CTT GCT GAC       192
Ile Val Glu Trp Trp Gly Gly Glu Glu Ala Arg Pro Thr Leu Ala Asp
```

```
    50                  55                  60

GTA CAG GAA CAG TAC TTG CCA AGC GTT TTA GCG CAA GAG TCC GTC ACT       240
Val Gln Glu Gln Tyr Leu Pro Ser Val Leu Ala Gln Glu Ser Val Thr
65                  70                  75                  80

CCA TAC ATT GCA ATG CTG AAT GGA GAG CCG ATT GGG TAT GCC CAG TCG       288
Pro Tyr Ile Ala Met Leu Asn Gly Glu Pro Ile Gly Tyr Ala Gln Ser
                85                  90                  95

TAC GTT GCT CTT GGA AGC GGG GAC GGA TGG TGG GAA GAA GAA ACC GAT       336
Tyr Val Ala Leu Gly Ser Gly Asp Gly Trp Trp Glu Glu Glu Thr Asp
            100                 105                 110

CCA GGA GTA CGC GGA ATA GAC CAG TCA CTG GCG AAT GCA TCA CAA CTG       384
Pro Gly Val Arg Gly Ile Asp Gln Ser Leu Ala Asn Ala Ser Gln Leu
        115                 120                 125

GGC AAA GGC TTG GGA ACC AAG CTG GTT CGA GCT CTG GTT GAG TTG CTG       432
Gly Lys Gly Leu Gly Thr Lys Leu Val Arg Ala Leu Val Glu Leu Leu
    130                 135                 140

TTC AAT GAT CCC GAG GTC ACC AAG ATC CAA ACG GAC CCG TCG CCG AGC       480
Phe Asn Asp Pro Glu Val Thr Lys Ile Gln Thr Asp Pro Ser Pro Ser
145                 150                 155                 160

AAC TTG CGA GCG ATC CGA TGC TAC GAG AAA GCG GGG TTT GAG AGG CAA       528
Asn Leu Arg Ala Ile Arg Cys Tyr Glu Lys Ala Gly Phe Glu Arg Gln
                165                 170                 175

GGT ACC GTA ACC ACC CCA GAT GGT CCA GCC GTG TAC ATG GTT CAA ACA       576
Gly Thr Val Thr Thr Pro Asp Gly Pro Ala Val Tyr Met Val Gln Thr
            180                 185                 190

CGC CAG GCA TTC GAG CGA ACA CGC AGT GAT GCC TA                        612
Arg Gln Ala Phe Glu Arg Thr Arg Ser Asp Ala
        195                 200
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 203 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Asp Pro Ser Ile Gln His Phe Gln Thr Lys Leu Gly Ile Thr Lys
1               5                   10                  15

Tyr Ser Ile Val Thr Asn Ser Thr Asp Ser Val Thr Leu Arg Leu Met
            20                  25                  30

Thr Glu His Asp Leu Ala Met Leu Tyr Glu Trp Leu Asn Arg Ser His
        35                  40                  45
```

```
Ile Val Glu Trp Trp Gly Gly Glu Glu Ala Arg Pro Thr Leu Ala Asp
     50                  55                  60

Val Gln Glu Gln Tyr Leu Pro Ser Val Leu Ala Gln Glu Ser Val Thr
 65                  70                  75                  80

Pro Tyr Ile Ala Met Leu Asn Gly Glu Pro Ile Gly Tyr Ala Gln Ser
                 85                  90                  95

Tyr Val Ala Leu Gly Ser Gly Asp Gly Trp Trp Glu Glu Glu Thr Asp
            100                 105                 110

Pro Gly Val Arg Gly Ile Asp Gln Ser Leu Ala Asn Ala Ser Gln Leu
        115                 120                 125

Gly Lys Gly Leu Gly Thr Lys Leu Val Arg Ala Leu Val Glu Leu Leu
    130                 135                 140

Phe Asn Asp Pro Glu Val Thr Lys Ile Gln Thr Asp Pro Ser Pro Ser
145                 150                 155                 160

Asn Leu Arg Ala Ile Arg Cys Tyr Glu Lys Ala Gly Phe Glu Arg Gln
                165                 170                 175

Gly Thr Val Thr Thr Pro Asp Gly Pro Ala Val Tyr Met Val Gln Thr
            180                 185                 190

Arg Gln Ala Phe Glu Arg Thr Arg Ser Asp Ala
        195                 200
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..54
(D) OTHER INFORMATION: /note= "upstream oligonucleotide primer, designated RVA61"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
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```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..31
(D) OTHER INFORMATION: /note= "oligonucleotide primer, designated OFD15"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
AATAATGTCG ACGTCCCCCT CGATGGAAGG G                                    31
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7811 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (synthetic)

(ix) FEATURE:
(A) NAME/KEY: misc_recomb
(B) LOCATION: 1..7811
(D) OTHER INFORMATION: /label= vector pTRVA3

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 194..218
(D) OTHER INFORMATION: /note= "T-DNA right border"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 484..684
(D) OTHER INFORMATION: /note= "the 3' end formation and polyadenylation region of T-DNA gene 7"

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: complement (729..1340)
(D) OTHER INFORMATION: /note= "the aac(6') coding sequence"

(ix) FEATURE:
(A) NAME/KEY: promoter
(B) LOCATION: 1341..1756
(D) OTHER INFORMATION: /label= 35S promoter

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 3001..3023

(D) OTHER INFORMATION: /note= "T-DNA left border sequences"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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TCCGGTGAAA TGGGCTGCAC TCCAACAGAA ACAATCAAAC AAACATACAC AGCGACTTAT     180
TCACACGAGC TCAAATTACA ACGGTATATA TCCTGCCAGT CAGCATCATC ACACCAAAAG     240
TTAGGCCCGA ATAGTTTGAA ATTAGAAAGC TCGCAATTGA GGTCTACAGG CCAAATTCGC     300
TCTTAGCCGT ACAATATTAC TCACGGGTGC GATGCCCCCC ATCGTAGGTG AAGGTGGAAA     360
TTAATGATCC ATCTTGAGAC CACAGGCCCA CAACAGCTAC CAGTTTCCTC AAGGGTCCAC     420
CAAAAACGTA AGCGCTTACG TACATGGTCG ATAAGAAAAG GCAATTTGTA GATGTTAATT     480
CCCATCTTGA AAGAAATATA GTTTAAATAT TTATTGATAA AATAACAAGT CAGGTATTAT     540
AGTCCAAGCA AAAACATAAA TTTATTGATG CAAGTTTAAA TTCAGAAATA TTTCAATAAC     600
TGATTATATC AGCTGGTACA TTGCCGTAGA TGAAAGACTG AGTGCGATAT TATGTGTAAT     660
ACATAAATTG ATGATATAGC TAGCTTAGCT CATCGGGGGA TCTGTCGACG TCCCCCTCGA     720
TGGAAGGGTT AGGCATCACT GCGTGTTCGC TCGAATGCCT GGCGTGTTTG AACCATGTAC     780
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CATCGGATCG CTCGCAAGTT GCTCGGCGAC GGGTCCGTTT GGATCTTGGT GACCTCGGGA     900
TCATTGAACA GCAACTCAAC CAGAGCTCGA ACCAGCTTGG TTCCCAAGCC TTTGCCCAGT     960
TGTGATGCAT TCGCCAGTGA CTGGTCTATT CCGCGTACTC CTGGATCGGT TTCTTCTTCC    1020
CACCATCCGT CCCCGCTTCC AAGAGCAACG TACGACTGGG CATACCCAAT CGGCTCTCCA    1080
TTCAGCATTG CAATGTATGG AGTGACGGAC TCTTGCGCTA AAACGCTTGG CAAGTACTGT    1140
TCCTGTACGT CAGCAAGTGT CGGGCGTGCT TCTTCTCCGC CCCACCACTC GACGATATGA    1200
GATCGATTTA GCCACTCATA GAGCATCGCA AGGTCATGCT CAGTCATGAG GCGCAGTGTG    1260
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TGTTGAATAC TCGGATCCAT CGATTTGTAG AGAGAGACTG GTGATTTCAG CGTGTCCTCT    1380
CCAAATGAAA TGAACTTCCT TATATAGAGG AAGGGTCTTG CGAAGGATAG TGGGATTGTG    1440
CGTCATCCCT TACGTCAGTG GAGATATCAC ATCAATCCAC TTGCTTTGAA GACGTGGTTG    1500
GAACGTCTTC TTTTTCCACG ATGCTCCTCG TGGGTGGGGG TCCATCTTTG GGACCACTGT    1560
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CTGTATCTTT GATATTCTTG GAGTAGACGA GAGTGTCGTG CTCCACCATG TTGACGAAGA 1800

TTTTCTTCTT GTCATTGAGT CGTAAAAGAC TCTGTATGAA CTGTTCGCCA GTCTTCACGG 1860

CGAGTTCTGT TAGATCCTCG ATCTGAATTT TTGACTCCAT GGCCTTTGAT TCAGTAGGAA 1920

CTACTTTCTT AGAGACTCCA ATCTCTATTA CTTGCCTTGG TTTATGAAGC AAGCCTTGAA 1980

TCGTCCATAC TGGAATAGTA CTTCTGATCT TGAGAAATAT ATCTTTCTCT GTGTTCTTGA 2040

TGCAGTTAGT CCTGAATCTT TTGACTGCAT CTTTAACCTT CTTGGGAAGG TATTTGATCT 2100

CCTGGAGATT ATTACTCGGG TAGATCGTCT TGATGAGACC TGCCGCGTAG GCCTCTCTAA 2160

CCATCTGTGG GTCAGCATTC TTTCTGAAAT TGAAGAGGCT AATCTTCTCA TTATCGGTGG 2220

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AGTCGTCCAT GGTGATCTCC GGGGCAAAGG AGATCTTATA ATTAAATGGC CTTCGCTGCC 2340

CATATTATTG GTAACTCAAC AGCATCAATC ACGGGATTTT TCTCGAATTA ATTGGGTCGA 2400

ATCTCAGCAT CGAAATATTC GCCTTTTTCG TCCATTAGAC TATCTATTGT GATGGTGGAT 2460

TTATCACAAA TGGGACCGGC CGCCGACAGA GGTGTGATGT TAGGCCAGGA CTTTGAAAAT 2520

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TATGTATATG AGTGTATATT TTCGCATAAT CTCAGACCAA TCTGAAGATG AAATGGGTAT 2700

CTGGGAATGG CGAAATCAAG GCATCGATCG TGAAGTTTCT CATCTAAGCC CCCATTTGGA 2760

CGTGAATGTA GACACGTCGA AATAAAGATT TCCGAATTAG AATAATTTGT TTATTGCTTT 2820

CGCCTATAAA TACGACGGAT CGTAATTTGT CGTTTTATCA AAATGTACTT TCATTTTATA 2880

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TTCTCCATAT TGACCATCAT ACTCATTGCT GATCCATGTA GATTTCCCGG ACATGAAGCC 3000

ATTTACAATT GAATATATCC TGCCGCCGCT GCCGCTTTGC ACCCGGTGGA GCTTGCATGT 3060

TGGTTTCTAC GCAGAACTGA GCCGGTTAGG CAGATAATTT CCATTGAGAA CTGAGCCATG 3120

TGCACCTTCC CCCCAACACG GTGAGCGACG GGGCAACGGA GTGATCCACA TGGGACTTTT 3180

AAACATCATC CGTCGGATGG CGTTGCGAGA GAAGCAGTCG ATCCGTGAGA TCAGCCGACG 3240

CACCGGGCAG GCGCGCAACA CGATCGCAAA GTATTTGAAC GCAGGTACAA TCGAGCCGAC 3300

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[illegible]TA CCCGAACGAC CAAGCAAGCT AGCTTAGTAA AGCCCTCGCT AGATTTTAAT    3360
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TGACCTGATA GTTTGGCTGT GAGCAATTAT GTGCTTAGTG CATCTAACGC TTGAGTTAAG    3540
CCGCGCCGCG AAGCGGCGTC GGCTTGAACG AATTGTTAGA CATTATTTGC CGACTACCTT    3600
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CACTGCGGAG CCGTACAAAT GTACGGCCAG CAACGTCGGT TCGAGATGGC GCTCGATGAC    4320
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TGGCGGGTGT CGGGGCGCAG CCATGACCCA GTCACGTAGC GATAGCGGAG TGTATACTGG 5280

CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC ACCATATGCG GTGTGAAATA 5340

CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCT CTTCCGCTTC CTCGCTCACT 5400

GACTCGCTGC GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT CAGCTCACTC AAAGGCGGTA 5460

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AGGACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT 6060

AGCTCTTGAT CCGGCAAACA AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG 6120

CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT 6180

GACGCTCAGT GGAACGAAAA CTCACGTTAA GGGATTTTGG TCATGAGATT ATCAAAAAGG 6240

ATCTTCACCT AGATCCTTTT AAATTAAAAA TGAAGTTTTA AATCAATCTA AAGTATATAT 6300

GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC 6360

TGTCTATTTC GTTCATCCAT AGTTGCCTGA CTCCCCGTCG TGTAGATAAC TACGATACGG 6420

GAGGGCTTAC CATCTGGCCC CAGTGCTGCA ATGATACCGC GAGACCCACG CTCACCGGCT 6480

CCAGATTTAT CAGCAATAAA CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA 6540

ACTTTATCCG CCTCCATCCA GTCTATTAAT TGTTGCCGGG AAGCTAGACT AAGTAGTTCG 6600

CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTGCAG GCATCGTGGT GTCACGCTCG 6660

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CCCATGTTGT GCAAAAAAGC GGTTAGCTCC TTCGGTCCTC CGATCGTTGT CAGAAGTAAG 6780

TTGGCCGCAG TGTTATCACT CATGGTTATG GCAGCACTGC ATAATTCTCT TACTGTCATG 6840

CCATCCGTAA GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG 6900

TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAACAC GGGATAATAC CGCGCCACAT 6960

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GCATCTTTTA CTTTCACCAG CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA 7140

AAAAAGGGAA TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT 7200

TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA ATGTATTTAG 7260

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GAAACCATTA TTATCATGAC ATTAACCTAT AAAAATAGGC GTATCACGAG GCCCTTTCGT 7380

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ATAAGCACAA GTTTTATCCG GCCTTTATTC ACATTCTTGC CCGCCTGATG AATGCTCATC 7800

CGGAATTAAT T 7811

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 203 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Asp Pro Ser Ile Gln His Phe Gln Thr Lys Leu Gly Ile Thr Lys
  1               5                  10                  15

Tyr Ser Ile Val Thr Asn Ser Thr Asp Ser Val Thr Leu Arg Leu Met
             20                  25                  30

Thr Glu His Asp Leu Ala Met Leu Tyr Glu Trp Leu Asn Arg Ser His
         35                  40                  45

Ile Val Glu Trp Trp Gly Gly Glu Glu Ala Arg Pro Thr Leu Ala Asp
```

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     50                  55                  60

Val Gln Glu Gln Tyr Leu Pro Ser Val Leu Ala Gln Glu Ser Val Thr
 65                  70                  75                  80

Pro Tyr Ile Ala Met Leu Asn Gly Glu Pro Ile Gly Tyr Ala Gln Ser
                 85                  90                  95

Tyr Val Ala Leu Gly Ser Gly Asp Gly Trp Trp Glu Glu Glu Thr Asp
            100                 105                 110

Pro Gly Val Arg Gly Ile Asp Gln Ser Leu Ala Asn Ala Ser Gln Leu
        115                 120                 125

Gly Lys Gly Leu Gly Thr Lys Leu Val Arg Ala Leu Val Glu Leu Leu
    130                 135                 140

Phe Asn Asp Pro Glu Val Thr Lys Ile Gln Thr Asp Pro Ser Pro Ser
145                 150                 155                 160

Asn Leu Arg Ala Ile Arg Cys Tyr Glu Lys Ala Gly Phe Glu Arg Gln
                165                 170                 175

Gly Thr Val Thr Thr Pro Asp Gly Pro Ala Val Tyr Met Val Gln Thr
            180                 185                 190

Arg Gln Ala Phe Glu Arg Thr Arg Ser Asp Ala  *
        195                 200
```

CLAIMS

1. A chimeric selectable marker gene for transforming a plant cell or a plant to render said plant cell or said plant resistant to an aminoglycoside antibiotic, said marker gene comprising in the following operably linked elements:

a) a plant-expressible promoter;

b) a DNA encoding an aminoglycoside-6'-N-acetyltransferase capable of modifying said aminoglycoside antibiotic; and

c) a DNA region comprising suitable 3' transcription regulation signals active in plant cells.

2. The chimeric selectable marker gene of Claim 1, wherein said aminoglycoside-6'-N-acetyltransferase is capable of acetylating at least kanamycin.

3. The chimeric selectable marker gene of Claim 2, wherein said aminoglycoside-6'-N-acetyltransferase is further capable of acetylating gentamycin.

4. The chimeric selectable marker gene of Claim 1, wherein said DNA encoding an aminoglycoside-6'-N-acetyltransferase has the DNA sequence of SEQ ID No. 1 or has a DNA sequence substantially similar to the DNA sequence of SEQ ID No. 1.

5. The chimeric selectable marker gene of Claim 1, wherein said DNA encoding an aminoglycoside-6'-N-acetyltransferase has the DNA sequence of SEQ ID No. 1.

6. The chimeric selectable marker gene of Claim 1, wherein said aminoglycoside-6'-N-acetyltransferase has the sequence of SEQ ID No. 2.

7. The chimeric selectable marker gene of

Claims to 6, which is integrated in the nuclear DNA of a plant cell.

8. A plant transformation vector, particularly a Ti plasmid, containing the marker gene of any one of Claims 1 to 6.

9. *Agrobacterium tumefaciens* containing the vector of Claim 8.

10. A plant cell, the nuclear DNA of which contains the marker gene of any one of Claims 1 to 6.

11. A plant cell culture, comprising a plurality of plant cells of Claim 10.

12. A seed comprising the chimeric selectable marker gene of any one of Claims 1 to 6.

13. A plant comprising the chimeric selectable marker gene of any one of Claims 1 to 6.

14. A method for selecting or identifying transformed plant cells using an aminoglycoside antibiotic, said method comprising the steps of:

transforming plant cells with a foreign DNA comprising the marker gene of any one of Claims 1 to 6; and then growing said transformed cells in concentrations of said aminoglycoside antibiotic that are lethal or growth-suppressive to non-transformed cells.

15. The method of Claim 14, wherein said aminoglycoside antibiotic is kanamycin.

16. A method for rendering a plant cell resistant to an aminoglycoside antibiotic, said method comprising the step of transforming the nuclear DNA of said cell with the marker gene of any one of Claims 1 to 6.

17. The method of Claim 16, wherein said aminoglycoside antibiotic is kanamycin.

18. A method for detoxifying an

aminoglycoside antibiotic in a plant cell the method comprising the step of providing the marker gene of any one of Claims 1 to 6 in the nuclear DNA of the cell.

19. The use of the marker gene of any one of Claims 1 to 6 to produce an aminoglycoside-6'-N-acetyltransferase in cells of a plant.

20. The use of the marker gene of any one of Claims 1 to 6 to produce an aminoglycoside-6'-N-acetyltransferase in cells of a plant.

21. The chimeric selectable marker gene as defined in Claim 1 and substantially as hereinbefore described with reference to Example 1.

22. A plant cell, the nuclear DNA of which contains the gene of Claim 1 and substantially as hereinbefore described with reference to Example 2 or Example 3.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 94/01560

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/82 C12N15/54 C12N1/21 C12N5/10 A01H5/00
C12N9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PLANT PHYSIOL., vol.86, 1988 pages 1216 - 1222 HAYFORD, M.B., ET AL. 'Development of a plant transformation selection system based on expression of genes encoding gentamicin acetyltransferases' cited in the application see the whole document	1-10
A	EP,A,0 289 478 (MONSANTO) 2 November 1988 cited in the application see the whole document	1-10

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search
25 October 1994

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Authorized officer
Maddox, A

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No

PCT/EP 94/01560

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0289478	02-11-88	NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)