OPIC
Office de la propriété intellectuelle du Canada

CIPO
Canadian Intellectual Property Office

(12) (19) (CA) **Demande-Application**

(21) (A1) **2,224,736**
(86) 1996/07/11
(87) 1997/02/06

(72) GREENLAND, Andrew James, GB
(72) BRIGHT, Simon William Jonathan, GB
(72) DRAYTON, Paul Richard, GB
(72) BELL, Philip John, GB
(71) ZENECA LIMITED, GB

(51) Int.Cl.^{6} A01H 5/00, C12N 15/82
(30) 1995/07/24 (9515161.9) GB

(54) **INHIBITION DE LA RESPIRATION CELLULAIRE ET PRODUCTION DE PLANTES MALES STERILES**

(54) **INHIBITION OF CELL RESPIRATION AND PRODUCTION OF MALE STERILE PLANTS**

(57) Procédé d'inhibition de l'expression génique dans un tissu végétal cible, lequel procédé consiste à transformer de manière stable une cellule végétale d'un type à partir duquel un végétal entier peut être régénéré à l'aide d'une construction génique porteuse, d'une part, d'un promoteur spécifique d'un tissu ou spécifique du développement, agissant dans les cellules du tissu végétal cible, et d'autre part, d'un gène de rupture codant une protéine, laquelle peut, lorsqu'elle est exprimée, inhiber la respiration dans les cellules dudit tissu cible, avec pour conséquence la mort de ces cellules, ce gène de rupture étant choisi dans le groupe comprenant le gène T-urf13, des gènes codant une .alpha.- ou une .beta.-tubuline, une co-suppression sens court de deux gènes essentiels du cycle cellulaire du maïs, à savoir le cdc25 et un activateur d'origine de réplication, ainsi qu'une construction sens court du translocateur nucléotidique de l'adénine de la membrane mitochondriale interne.

(57) A method of inhibiting gene expression in a target plant tissue which comprises stably transforming a plant cell of a type from which a whole plant may be regenerated with a gene construct carrying a tissue-specific or a development-specific promoter which operates in the cells of the target plant tissue and a disrupter gene encoding a protein which is capable, when expressed, of inhibiting respiration in the cells of the said target tissue resulting in death of the cells, the said disrupter gene is selected from the group consisting of the T-urf13 gene, genes encoding an .alpha.- or .beta.-tubulin, short sense co-suppression of two essential maize cell cycle genes, cdc25 and replication origin activator (ROA) and a short sense construct to the adenine nucleotide translocator (ANT) of the inner mitochondrial membrane.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/82, 5/10 — A1

(11) International Publication Number: WO 97/04116

(43) International Publication Date: 6 February 1997 (06.02.97)

(21) International Application Number: PCT/GB96/01675

(22) International Filing Date: 11 July 1996 (11.07.96)

(30) Priority Data:
9515161.9 24 July 1995 (24.07.95) GB

(71) Applicant *(for all designated States except US)*: ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: GREENLAND, Andrew, James [GB/GB]; Tree Tops, Kingswood Court, Braywick Road, Maidenhead, Berkshire SL6 1DA (GB). BRIGHT, Simon, William, Jonathan [GB/GB]; 24 Pound Lane, Marlow, Buckinghamshire SL7 2AY (GB). DRAYTON, Paul, Richard [GB/GB]; 42 Notton Way, Lower Earley, Reading, Berkshire RG6 4AJ (GB). BELL, Philip, John [GB/GB]; 344 Rochford Gardens, Slough, Berkshire SL2 5XN (GB).

(74) Agents: ROBERTS, Alison, Christine et al.; Zeneca Agrochemicals, Intellectual Property Dept., Jealott's Hill Research Station, P.O. Box 3538, Bracknell, Berkshire RG42 6YA (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: INHIBITION OF CELL RESPIRATION AND PRODUCTION OF MALE STERILE PLANTS

(57) Abstract

A method of inhibiting gene expression in a target plant tissue which comprises stably transforming a plant cell of a type from which a whole plant may be regenerated with a gene construct carrying a tissue-specific or a development-specific promoter which operates in the cells of the target plant tissue and a disrupter gene encoding a protein which is capable, when expressed, of inhibiting respiration in the cells of the said target tissue resulting in death of the cells, the said disrupter gene is selected from the group consisting of the T-urfl3 gene, genes encoding an α- or β-tubulin, short sense co-suppression of two essential maize cell cycle genes, cdc25 and replication origin activator (ROA) and a short sense construct to the adenine nucleotide translocator (ANT) of the inner mitochondrial membrane.

INHIBITION OF CELL RESPIRATION AND PRODUCTION OF MALE STERILE PLANTS

The present invention relates to a method of producing male sterile plants by use of a gene, which is expressible in plants and inhibits an essential cell function, hence disrupting full expression of a selected plant characteristic.

Our International Patent Application No. WO 90/08831 describes and claims the disruption of respiration using a variety of disrupter genes which we refer to also as "pollen-inactivating genes".

The ability of such inactivating genes to function in this way varies and, therefore, there is a need for further improved gene sequences so that appropriate selection for specific applications may be made.

An object of this invention is to provide genes for use in inhibiting gene expression.

According to the present invention there is provided a method of inhibiting gene expression in a target plant tissue comprising stably transforming a plant cell of a type from which a whole plant may be regenerated with a gene construct carrying a tissue-specific or a development-specific promoter which operates in the cells of the target plant tissue and a disrupter gene encoding a protein which is capable, when expressed, of inhibiting respiration in the cells of the said target tissue resulting in death of the cells characterised in that the said disrupter gene is selected from the group consisting of the T-urf13 gene, genes encoding an α - or β-tubulin, short sense co-suppression of two essential maize cell cycle genes, cdc25 and replication origin activator (ROA) and a short sense construct to the adenine nucleotide translocator (ANT) of the inner mitochondrial membrane.

Down regulation of gene activity due to short sense co-suppression is described in our International Patent Application No.WO 90/08299.

The α- or β-tubulin genes act as disrupters by de-stabilizing microtubule arrays in plant cells, hence inhibiting essential microtubule function in the said target tissue resulting in death of the cells.

The use of short sense co-suppression of two essential maize cell cycle genes, cdc25 and replication origin activator (ROA) disrupts cell division and hence provide a growth defect in the targeted organ or tissue.

Preferably the promoter is an anther- and/or tapetum-specific promoter or a pollen-specific promoter, so that on expression of the said disrupter protein therein the regenerated plant is in male sterile. More preferably the said anther and/or tapetum-specific

promoter was isolated using the cDNA sequences shown in Figure 1 or 2 or 3 of the accompanying drawings and using the techniques described in our International Patent Application No. WO 90/08826.

Plasmids containing the DNA sequences shown in Figures 1, 2 and 3 have been deposited under the terms of the Budapest Treaty, details being as follows:

Plasmid pMS10 in an *Escherichia coli* strain RR1 host, containing the gene sequence shown in Figure 1 herewith, and deposited with the National Collection of Industrial & Marine Bacteria on 9th January 1989 under the Accession Number NCIB 40090.

Plasmid pMS14 in an *Escherichia coli* strain DH5α host, containing the gene sequence shown in Figure 2 herewith, and deposited with the National Collection of Industrial & Marine Bacteria on 9th January 1989 under the Accession Number NCIB 40099.

Plasmid pMS18 in an *Escherichia coli* strain RR1 host, containing the gene sequence shown in Figure 3 herewith, and deposited with the National Collection of Industrial & Marine Bacteria on 9th January 1989 under the Accession Number NCIB 40100.

The isolation and characterisation of these gene sequences of this invention are described in full in WO 93/01294.

Other promoters may also be used, for example a promoter such as the tapetum specific MFS14 promoter.

The present invention also provides a plant having stably incorporated in its genome by transformation a gene construct carrying a gene construct carrying a tissue-specific or a development-specific promoter which operates in the cells of the target plant tissue and a disrupter gene encoding a protein which is capable, when expressed, of inhibiting an essential cell function such as respiration, microtubule arrays or cell division in the cells of the said target tissue resulting in death of the cells.

The invention also provides a plant, particularly a monocotyledonous plant, and more particularly a corn plant, having stably incorporated within its genome a gene construct carrying a tissue-specific promoter which operates in the cells of the said target tissue and a disrupter gene encoding a protein which is capable of inhibiting an essential cell function such as respiration or microtubules in the said cells of the said target tissue resulting in death of the cells characterised in that the said disrupter gene is selected from the T-urf13 gene, a short sense construct of the adenine nucleotide translocator, genes encoding an α- or β-tubulin and short sense down-regulation of the essential cell cycle genes, cdc25 and ROA.

These gene constructs may be used as a means of inhibiting cell growth in a range of organisms from simple unicells to complex multicellular organisms such as plants and animals. By the use of tissue- or cell-specific promoters, particular cells or tissue may be targeted and destroyed within complex organisms. One particular application intended for this invention is in the destruction of cells essential for male flower development, leading to male sterility.

The invention therefore provides a method of preventing or inhibiting growth and development of plant cells based on gene constructs which inhibit an essential cell function such as respiration or microtubules. The technique has wide application in a number of crops where inhibition of particular cells or tissue is required.

Of particular interest is the inhibition of male fertility in maize for the production of F1 hybrids *in situ*. The concept of inhibition of mitochondrial function as a mechanism for male sterility arises from some previous research on T-type cytoplasmic male sterility in maize (cms-T) which has shown an association between the male sterile phenotype and mitochondrial dysfuction. Although a direct causal relationship has yet to be established between mitochondrial dysfunction and cms-T, an increasing body of evidence suggests that fully functional mitochondria, particularly in the tapetal cells, are essential. This is particularly critical during microsporogenesis since the metabolic demands placed on the tapetal cells results in a 40-fold increase in mitochondrial number.

Thus we provide a number of negative mutations which act upon mitochondria to inhibit functional respiration. When specifically expressed in maize anther tissue these mutations will result in a male sterile phenotype.

We also use expression of α- or β-tubulin genes to disrupt cell function. During normal cell life, expression of tubulin genes is closely regulated by their endogenous promoters and closely matches the requirements of cells for these proteins which are polymerised and assembled into microtubules during growth and development of the plant. By expressing tubulin genes in an unregulated fashion using non-tubulin promoters in a particular tissue or stage of development, the equilibrium between free tubulin monomers and those polmerised in microtubules is disrupted resulting in instability of the microtubule complex and cellular dysfuntion. When expressed in the tapetum or other cells of the anther this latter effect will cause the plants to be sterile.

We also propose the use of short sense down-regulation of essential cell cycle genes, eg cdc25 and ROA. When expressed in the tapetum or other cells of the anther this latter effect will cause the plants to be sterile.

The method employed for transformation of the plant cells is not especially germane to this invention and any method suitable for the target plant may be employed. Transgenic plants are obtained by regeneration from the transformed cells. Numerous transformation procedures are known from the literature such as agroinfection using *Agrobacterium tumefaciens* or its Ti plasmid, electroporation, microinjection of plant cells and protoplasts, microprojectile transformation and pollen tube transformation, to mention but a few. Reference may be made to the literature for full details of the known methods.

The development and testing of these gene constructs as disrupters of mitochondrial function in the unicellular organism, yeast, will now be described. A mechanism by which these gene constructs may be used to inhibit plant cell growth and differentiation in transformed plants will also be described. The object of these procedures is to use yeast as a model system for the identification and optimisation of gene constructs for expressing proteins which disrupt mitochondrial function. Plant cells will then be transformed with the selected constructs and whole plants regenerated therefrom.

The accompanying drawings are as follows:

Figure 1 shows the DNA sequence of an anther-specific cDNA, carried by plasmid pMS10;

Figure 2 shows the DNA sequence of a tapetum-specific cDNA, carried by plasmid pMS14;

Figure 3 shows the DNA sequence of an anther-specific cDNA, carried by plasmid pMS18;

Figure 4 shows the sequence of the T-urf13 gene (SEQ ID NO 1) with the primers Turf-1 (SEQ ID NO 2) and Turf-2R (SEQ ID NO 3) underlined;

Figure 5 shows DNA encoding the 59 amino acid region from the ATP-2 gene of *Nicotinia plumbaginifolia* ((SEQ ID NOS 4 and 5) with primers PREB-IB (SEQ ID NO 6) and PREB-R (SEQ ID NO 7) shown;

Figure 6 shows the cleavage site of the pre-β sequence;

Figure 7 is a map of vector pCaMVI$_1$N;

Figure 8 is a map of vector RMS17;

Figure 9 is a map of vector pIE109;

Figure 10 shows the MFS14 promoter sequence (SEQ ID NO 8) with the following features:

position 2198 transcription start CCT"A"CAA (concensus CTC"A"TCA)
position 2167 ATCCATT (possible TATA box motif)
position 2141 CCAT (possible CAAT box motif)
position 2233 cdna start CAC"A"CAG
position 2295 translation start GCAACAATGGCG (concensus TAAACAATGGCT);

Figure 11 is a map of vector RMS11;

Figure 12 is a map of vector pMANT3

Figure 13 illustrates the contruction of vectors for maize cell line transformation; and

Figure 14 is a plot showing numbers of transformants produced in the various different experiments.

The invention will now be illustrated by the following Examples.

Example 1

Construction of the maize transformation vector, RMS17.

We used the PCR to amplify the T-urf13 gene from cms-T maize (line RW33:TMS) and the mitochondrial targeting sequence, pre-β, from *Nicotiana plumbaginolia*. DNA samples from plant material were prepared using the method described by Edwards *et al* (Nucliec Acids Research 1991, 19, 1349).

The complete T-urf13 gene was amplified in the PCR using primers turf-1 (5'ATCGGATCCATGATCACTACTTTCTTAAACCTTCCT-3' , SEQ ID NO 2) and turf-2R (5'TAGTCTAGATCACGGTACTTGTACGCTATCGGT-3', SEQ ID NO 3) designed from sequence information provided by Dewey *et al* (1986, Cell, 44, 429-449). The PCR conditions were 35 cycles of denaturing at 94°C for 0.8 min, annealing at 65°C for 1 min and extension at 72°C for 2.5 mins. To aid subsequent cloning the PCR primers were designed such that they introduce unique *Bam*HI and *Xba*I restriction sites at the 5' and 3' ends of the gene respectively. The position of these primers relative to the T-urf13 gene sequence is shown in Figure 4.

Similarly the 59 amino region from the ATP2 gene of *Nicotiana plumbaginifolia* which encodes the functional pre-β mitrochondrial targeting sequence was amplified in the PCR using the primers PREB-IB (5'ATCGGTACCGCCATGGCTTCTCGGAGGCTTCTCGCCT-3', SEQ ID NO 6) and PREB-R (5'ATCGGATCCCGCTGCGGAGGTAGCGTA-3', SEQ ID NO 7) designed using sequence information provided from Boutry *et al* (1987, Nature, 328, 341). The PCR

conditions were as described above except that the annealing temperature was reduced to 60°C. To aid subsequent cloning PBEB-IB and PREB-2R were designed such that they introduce unique *Kpn*I and *Bam*HI restriction sites at the 5' and 3' ends of the amplified fragment respectively. The position of these primers relative to the ATP2 gene is shown in Figure 5.

Following amplification the pre-B PCR fragment was digested with *Kpn*I and *Bam*HI to generate cohesive ends and cloned into the corresponding sites of the vector pUC18 to give plasmid pPB1. The TURF-13 PCR product was then digested with *Bam*HI and *Xba*I and cloned into the corresponding sites in pPB1 to give plasmid pPB2. In pPB2 the pre-β sequence is fused in frame with the T-urf13 gene so that following expression in a plant cell the full product will be transported to mitochondria. Cleavage of the pre-β sequence at the predicted site between residues 55-56 will release the T-urf13 protein which includes at its NH_2-terminus an additional 4 residues from the pre-β sequence (Figure 6).

The pre-β/T-urf13 gene fusion in pBB2 removed by digestion with the enzymes *Kpn* I and Sal I, blunted-ended and cloned into the plasmid pCAMVI$_1$N (Figure 7) which was digested with *Bam*HI and blunt-ended to give pPB3. This cloning step places the pre-β/T-urf13 gene fusion under transcriptional control of the CAMV 35S promoter. The *Adh*I intron is present in this construct to boost expression levels in corn cells (Mascarenhas *et al.*, 1990. Plant Mol. Biol., 15, 913-920) and the nos 3' sequence provides a polyA addition site. To produce the final vector, RMS17 (Figure 8) the PAT selection cassette from p1E109 (Figure 9) which allows in vitro selection of transformed corn cells on bialaphos was introduced as an *Eco*RI fragment into the unique *Eco*RI site of pPB3.

Example 2

Transformation of BMS corn cells with RMS17.

The objective of this experiment was to show that expression of the preβ/TURF-13 gene construct in cultured BMS corn cells results in a reduction in cell viability as measured by the establishment of transgenic calli following transformation. The RMS17 vector (Figure 8) was introduced into cultured BMS cells using a silcon carbide fibre-mediated transformation technique as follows:

Preparation of silicon carbide whiskers

Dry whiskers were always handled in a fume cabinet, to prevent inhalation and possible lung damage. These whiskers may be carcinogenic as they have similar properties to

asbestos. The Silar SC-9 whiskers were provided by the Advanced Composite Material Corporation Greer, South Carolina,USA. The sterile whisker suspensions were prepared in advance as follows. Approximately 50mg of whiskers were deposited into a pre-weighed 1.5 ml Eppendorf tube, which was capped and reweighed to determine the weight of the whiskers. The cap of the tube was perforated with a syringe needle and covered with a double layer of aluminium foil. The tube was autoclaved (121°C, 15psi, for 20 minutes) and dried. Fresh whisker suspensions were prepared for each experiment, as it had been reported that the level of DNA transformation when using fresh suspensions was higher than that of older suspensions. A 5% (weight/volume) whisker suspension was prepared using sterile deionised water. This was vortexed for a few seconds to suspend the whiskers immediately before use.

DNA transformation into cells

All procedures were carried out in a laminar air flow cabinet under aseptic conditions. The DNA was transformed into the cells using the following approach. Specific modifications to this method are indicated in the text.

Cell and whisker suspensions were pipetted using cut down Gilson pipette tips. 100µl of fresh BMS medium (see appendix 1) was measured into a sterile Eppendorf tube. To this was added 40µl of the 5% (w/v) whisker suspension and 25µl (1mg/ml) of the plasmid DNA, which was vortexed at top speed for 60 seconds using a desktop vortex unit (Vortex Genie 2 Scientific industries, Inc). Immediately after this period of vortexing, 500µl of the cell suspension was added ie 250µl of packed cells. The Eppendorf tube was then capped and vortexed at top speed for 60 seconds in an upright position. The same procedure was used to transform the other cell lines.

Three controls were included in this experiment. Two positive control vectors were pPG3 which contains the PAT selection cassette alone and RMS15, which is identical to RMS17 except that the T-urf13 gene is replaced by the mitochondrial uncoupling protein gene, UCP, which jhas no effect on cultured BMS cells. The pre-β targeting sequence is present in both constructs. A negative control, which should completely prevent establishment of transgenic calli, was provided by RMS13, which is identical to RMS17 except that the preβ/T-urf13 gene fusion is replaced by the cytotoxic ribonuclease gene, barnase.

The mean numbers of transgenic calli established in this experiment are shown in Table 1.

TABLE 1

		No. of transgenic calli per replicate		
Vector	Mean	1	2	3
pPG3	-	44	33	39
RMS13	0	0	0	0
RMS15	40	30	43	38
RMS17	12	13	23	16

These data show that relative to the two positive controls, pPG3 and RMS15, expression of the preB/T-urf13 gene fusion results in a signficant decrease ($p<5\%$ or better) in the establishment of transgenic calli. This suggests that targeting T-urf13 protein to mitochondria has a deleterious effect on these cells, presumably due impartment of mitochondrial function. Expression of the cytotoxic ribonuclease, barnase, completely abolishes the establishment of transformed calli.

Example 3

Construction of the maize transformation vector, RMS11

RMS11 is a transformation vector in which expression of the pre-β/T-urf13 gene fusion is controlled by the maize tapteum promoter, MFS14. The sequence of the MFS14 promoter and untranslated leader region from position -2198 to +97 is shown in Figure 10. In this way, expression of the T-urf13 protein is limited to the cells producing pollen and not throughout the whole plant.

To construct RMS11, the *Kpn* I - *Sal*I fragment from pPB2 containing the pre-β/T-urf13 gene fusion was blunt-ended and ligated into the blunt ended *Bam*HI site of plasmid pSC9 to yield pPB4. In pPB4 the pre-β/T-urf13 gene fusion is now positioned between the -152 to +97 MFS14 promoter fragment and the nos 3' polyadenylation sequence. This complete cassette was removed from pPB4 by digestion with *Sac*I and *Eco*RI and cloned into the corresponding sites of pSC7 to give plasmid pB5. pSC7 contains the -153 to -5800 region of the MFS14 promoter so that the introduction of the *Sac*I - *Eco*RI fragment from pPB4 recreates the full 5.8 kb MFS14 promoter.

RMS11 (Figure 11) was completed by introduction of the PAT in vitro selection cassette from p1E109 into the unique *Eco*RI site of pPB5.

Example 4

Transformation of maize cells with RMS11 by particle bombardment to provide stably transformed, male sterile plants.

The maize transformation vector, RMS11 was used to transform regenerable maize cell cultures by particle bombardment.

Culture Material

Friable embryogenic Type II callus was initiated from immature zygotic embryos excised from either greenhouse or filed grown A188 plants 10-12 days after pollination with pollen from the inbred B73. The medium used for callus initiateion and maintenance was based onN6 medium as modified by Armstrong and Green. Specifically, the medium contained 6mM L-proline, 2% (w/v) sucrose, 2 mg/l 2,4-dichlorophenoxy- acetic acid (2,4-D) and 3% (w/v) Gelrite (Trade Mark, Caroline Biological Supply) at pH 6.0. Callus was grown for 4-4 weeks prior to suspension culture initiation. Suspension cultures were initiated in a MS-based liquid medium containing 100 mg/l myo-inositol, 2 mg/l 2,4-D, 2 mg/l 1-naphthaleneacetic acid (NAA), 6mM proline, 200 mg/l casein hydrolysate (Difco Laboratories), 35 (w/v sucrose and 5% (v/v) coconut water (Difco Laboratories) at pH 6.0. Cell suspensions were maintained in theis medium in 125 ml Erlenmeyer flasks at 28°C in the dark on a gyrating shaker at 125 rpm. Suspension were subcultured every 3.5 days by addition of 3ml packed volume of cells and 10 ml culture medium to 20 ml of fresh culture medium. The suspension cultures were typically 6 months to one year old at the time of bombardment. Suspension cultures recovered from cryopreservation were used in some transformations.

Microprojectile Bombardment

Cell suspensions were sieved through a 1.0 mm and then a 0.5mm screen. A packed volume of 0.2 ml of the cells which passed through the sieves was then suspended in 5 ml of suspension medium and evenly distributed on to a Whatman No.4 filter paper disc *via* vacuum filtration using a 4.7 cm microanalysis holder. Precipitation of supercoiled plasmid DNA on to tungsten particles and bombardment using the DuPont PDS-1000 Biolistics (Trade Mark) apparatus were essentialy as described by the manufacturers. Target plates were bombarded once.

Transformant selection and plant regeneration

Following bombardment, each filter disc (with cells) was transferred to N6 based medium containing 100 mg/l myo-inositol, 2 mg/l 2,4-D, 3% (w/v) sucrose, and 0.3% (w/v) Gelrite at pH 6.0. For selection using the NPTII gene, this medium was supplemented with 200 mg/l kanamycin sulphate. The filter discs were transferred to fresh medium containing the selection agent after seven and again after 14 days. The suspension was divided into two equal aliquots and each was evenly plated over 20 ml of solidified media containing the selective agent and 3% (w/v) Gelrite in 100 x 20 mm Petri dishes. After 2-5 weeks, rapidly growing, putatively transformed calli were removed and transferred to the surface of fresh selection medium. Plants were renerated by transferring tissue to MS based medium containing 1 g/l myo-inositol, 1 mg/l NAA, 6% (w/v) sucrose, and 3% (w/v) Gelrite at pH 6.0. After 2-3 weeks, the tissue was transferred to MS media containing 0.25 mg/l NAA, and 3% (w/v) sucrose and placed in the light, where embryo germination occurred. Plants were then grown in half-strength MS based medium containing 500 mg/l myo-inositol, 3% (w/v) sucrose and 0.3% Gelrite at pH 6.0 for approximately 1-2 weeks priro to transfer to the greenhouse.

After transfer to the glasshouse, plants within each independently transformed clone were tested for the presence of the MFS14/pre-B/T-urf13 gene construct using the PCR. DNA was extracted from small leaf samples using the technique described by Edwards *et al* (1991, Nucleic Acids Research, 19, 1349) and used in the PCR with the primers, 14-SA (5'-AGACGCTGAGCTCAAGGACGTGA-3' SEQ ID NO 9)and turf-2R (see Example 1 for the sequence of this primer).

At flowering the plants within each independently transformed clone were assessed in the glasshouse using a visual scale developed for CMS lines and described in Table 2. Plants scoring 4 and below are functionally sterile. The accumulated sterility scores for each of the independent PCR positive clones is shown in Table 3 and compared to a maize line which was generated by bombardment with RMS11 but which is PCR negative for the MFS14/pre-B/T-urf13 gene construct.

Sterile plants were backcrossed with pollen from fertile, non-transgenic BE70 plants. the progeny seeds arising from one of these crosses (clone YK23, plant 5 x BE70) were planted in the glasshouse and allowed to flower. The presence of the MFS14/pre-B/T-urf13 gene construct as assessed by PCR and PAT test (the latter determines whether the selectable

marker used inthe transformation process is present) and the fertility scores of the plants are shown in Table 4. Plants which were PCR negative were rogued from the glasshouse prior to flowering. Plants 1 and 2 had an inconclusive PCR test and were kept until flowering. Plant 12 was kept as a control. As can be seen in Table 4, 6 of the progeny plants were sterile and this sterility correlated with the presence of the transgene as assessed either by PCR or PAT testing. This is consistent the the presence of a single transgenic locus imparting sterility.

TABLE 2

Anther Classification (After L.M. Josephson)

Class 0	No anthers exerted
Class 1	Less than half of the anthers exerted and all were small, dry and hard with no pollen shed.
Class 2	Most of anthers exerted but all were small, dary and hard with no pollen shed.
Class 3	Partially fertile anthers exerted with some pollen shed, proportion of anthers exerted was highly variable.
Class 4	Slightly abnormal anthers with approximately 75 to 100 percent exertion.
Class 5	Normal anthers and fully fertile.

* Class 4 through 5 tassels were considered fertile.

TABLE 3

CLONE	NO. OF PLANTS	FERTILITY SCORE	P.C.R.
WK 23	2	0	+
	3	3	+
WK 23	2	0	+
YK 23	3	0	+
	1	4	+
YE 23	4	5	-

TABLE 4

PLANT	PCR TEST	PAT TEST	FERTILITY SCORE
YK23/5/1	+/-	-	0
YK23/5/2	+/-	+	0
YK23/5/3	-	ROGUED	
YK23/5/4	-	ROGUED	
YK23/5/5		NO GERMINATION	
YK23/5/6	+	+	1
YK23/5/7	+	+	0
YK23/5/8	-	ROGUED	
YK23/5/9	+	+	0
YK23/5/10	-	ROGUED	
YK23/5/11	+	+	0
YK23/5/12	-	-	5

Example 5

Construction of the maize transformation vector pRMS-23

We have tested whether expression of a short-sense construct from the maize adenine nucleotide translocator (ANT) gene will give rise to a defect in the growth of maize cells. A fragment of the maize ANT was isolated using the PCR and primers designed from the sequence of the maize gene published by Bathgate *et al* (1989, Eur. J. Biochem., 83, 303-310).

The fragment of theANT gene was amplified in the PCR using primers MANT-1(5'-ATGCCCGGGCTTGCAATGTCTGTTAGCGGTGGCATCA-3', SEQ ID NO 10) and MANT-2RB (5-ATGCCCGGGCGATGGGGTAAGATGCAAGACCA-3', SEQ ID NO 11). The PCR conditions were 35 cycles of denaturing at 94°C for 0.8 min, annealing at 65°C for 1 min and extension at 72°C for 2.5 mins. To aid subsequent cloning the PCR primers were designed such that they introduce unique *Sma*I restriction sites at the 5' and 3' ends of the gene. The sequence of the maize ANT gene was published in *Eur. J. Biochem.* (1989) **183**, 303-310. The MANT-1 primer sequence appears at the beginning of the coding sequence of the gene and the MANT-2R primer sequence is near the end of the gene.

Following PCR which produced a DNA fragment of the predicted size of 1050 bp, the DNA was digested with *Sma*I and subcloned into the *Sma*I site of pUC18 to give pMANT1. Subsequently the nos 3' polyadenylation signal sequence was introduced 3' to the ANT gene as a *Sac*I- *Eco*RI fragment into the corresponding sites in pMANT1 to yield pMANT2. A *Hin*dIII - *Bam*HI fragment carrying the CaMV 35s promoter and ADH 1intron from pCaMVI$_1$N (Figure 5) was introduced into the corresponding sites of pMANT2 to yield pMANT 3. pRMS-23 (Figure 12) was completed by introduction of the PAT in vitro selection cassette from pIE109 (Figure 7) into the unique *Eco*RI site of pMANT3.

Example 6

Transformation of BMS corn cells with pRMS-23

The objective of this experiment was to show that expression of pRMS-23 in cultured BMS corn cells results in a reduction in cell viability as measured by the establishment of transgenic calli following transformation in two separate experiments. The vector DNAs were introduced into cultured BMS cells using the silcon carbide fibre transformation techniqiue as described in Example 2.

Following transformation with pRMS23 the mean numbers of transgenic calli established were determined relative a positive control pPG3 wihich contains the *in vitro* selection cassette alone (Table 5). These data show that expression of a short sense adeneine nucleotide translocator gene results in a significant decrease in the establishment of transgenic calli.

TABLE 5

	No of transgenic calli per replicate			
	1	2	3	Mean
Experiment 1				
pPG3	16	20	22	19
pRMS-23	3	4	3	3
Experiment 2				
pPG3	49	48	40	46
pRMS-23	10	15	23	16

Example 7

Construction of the maize transformation vectors, pTBR and pTBS

We tested whether un-regulated expression of α-tubulin genes will give rise to a defect in the growth of maize cells. Two constructs containing the coding sequence from α-tubulin cDNAs isolated from two biotypes of *Eleusine indica* were prepared. pTBR (Figure 13) contains the α-tubulin cDNA from a dinitroaniline resistant biotype of *Eleusine indica* cloned as a blunt-ended *Hinf* I fragment into the blunt-ended *Bam*HI site of pCaMVI$_1$N (Figure 7). pTBS (Figure 13) contains the α-tubulin cDNA from a dinitroaniline sensitive biotype cloned exactly as described for pTBR.

Example 8

Transformation of BMS corn cells with pTBR and pTBS.

The objective of this experiment was to show that expression of pTBR and pTBS in cultured BMS corn cells results in a reduction in cell viability as measured by the establishment of transgenic calli following transformation. The vector DNAs were introduced into cultured BMS cells using the silcon carbide fibre transformation techniqiue as described in Example 2.

Following transformation with pTBR and pTBS the mean numbers of transgenic calli established were determined relative a positive control pPG3 wihich contains the *in vitro* selection cassette alone (Figure 14). These data show that non-regulated expression of an α-tubulin gene from either biotype of *Eleusine indica* results in a significant decrease in the establishment of transgenic calli.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: ZENECA LIMITED
(B) STREET: 15 Stanhope Gate
(C) CITY: London
(E) COUNTRY: UK
(F) POSTAL CODE (ZIP): W1Y 6LN

(ii) TITLE OF INVENTION: Production of Male Sterile Plants

(iii) NUMBER OF SEQUENCES: 11

(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 #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 357 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: T-urf13 gene

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

ATCGGATCCA TGATCACTAC TTTCTTAAAC CTTCCTCCCT TTGATCAAGG TTTGGTATTT 60

TTCGGTTCTA TTTTTATTTT TTTTTTGTGC ATATTATTGA TAAAGGGATA TCTCCGTAAA 120

ATGGATGATT CCTATTTGGC TCAACTCTCC GAGTTAGCCA ACCACAATAG AGTGGAAGCG 180

GCAAAAGCGG GCCACGTGGC CCTGCATGAG CTATCCTTCT CGTGGTTGAG GGGGGTTCAA 240

ATTAGGGTGA GGACCTTACC TATACAACGG AATGAAGGAG GGGGTCGAAG CAACGACCAA 300

TCCACTCTCT CTAAGCCTAA GTATTCCTCA ATGACCGATA GCGTACAAGT ACCGTGA 357

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Turf-1 primer

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

ATCGGATCCA TGATCACTAC TTTCTTAAAC CTTCCT 36

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Turf-2R primer

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

TAGTCTAGAT CACGGTACTT GTACGCTATC GGT 33

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 269 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: ATP-2 gene of Nicotinia plumbaginifolia

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..267
(D) OTHER INFORMATION:/codon_start= 1

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

```
ATG GCT TCT CGG AGG CTT CTC GCC TCT CTC CTC CGT CAA TCG GCT CAA        48
Met Ala Ser Arg Arg Leu Leu Ala Ser Leu Leu Arg Gln Ser Ala Gln
  1               5                  10                  15

CGT GGC GGC GGT CTA ATT TCC CGA TCG TCA GGA AAC TCC ATC CCT AAA        96
Arg Gly Gly Gly Leu Ile Ser Arg Ser Ser Gly Asn Ser Ile Pro Lys
            20                  25                  30

TCC GCT TCA CGC GCC TCT TCA CGC GCA TCC CCT AAG GGA TTC CTC TTA       144
Ser Ala Ser Arg Ala Ser Ser Arg Ala Ser Pro Lys Gly Phe Leu Leu
        35                  40                  45

AAC CGC GCC GTA CAG TAC GCT ACC TCC GCA GCG GCA CCG GCA TCT CAG       192
Asn Arg Ala Val Gln Tyr Ala Thr Ser Ala Ala Ala Pro Ala Ser Gln
    50                  55                  60

CCA TCA ACA CCA CCA AAG TCC GCC AGT GAA CCG TCC GGA AAA ATT ACC       240
```

Pro Ser Thr Pro Pro Lys Ser Ala Ser Glu Pro Ser Gly Lys Ile Thr
65 70 75 80

GAT GAG TTC ACC GGC GCT GGT TCG ATC GG 269
Asp Glu Phe Thr Gly Ala Gly Ser Ile
85

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 89 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Ala Ser Arg Arg Leu Leu Ala Ser Leu Leu Arg Gln Ser Ala Gln
1 5 10 15

Arg Gly Gly Gly Leu Ile Ser Arg Ser Ser Gly Asn Ser Ile Pro Lys
20 25 30

Ser Ala Ser Arg Ala Ser Ser Arg Ala Ser Pro Lys Gly Phe Leu Leu
35 40 45

Asn Arg Ala Val Gln Tyr Ala Thr Ser Ala Ala Ala Pro Ala Ser Gln
50 55 60

Pro Ser Thr Pro Pro Lys Ser Ala Ser Glu Pro Ser Gly Lys Ile Thr
65 70 75 80

Asp Glu Phe Thr Gly Ala Gly Ser Ile
85

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: PREB-IB primer

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

ATCGGTACCG CCATGGCTTC TCGGAGGCTT CTCGCCT 37

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: PREB-2R primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

ATCGGATCCC GCTGCGGAGG TAGCGTA 27

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2285 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:
(A) ORGANISM: MFS14 Promoter

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AAAAGCGTAC CAGTAAGGGA TAAAGAAAAT AAACAAACAC GAAATGCTTC CCCATCGGCC 60

AATTCGCCTA GGTGTGCTAG GAACTGGCCT ATATGTTCGT GTGTGCTTCT CCTATTTTCA 120

CCAGAAAACT TAGAAAACTC TGGTATCCTT GCCCCTTGTG GATATGGGAC AATGTCAAAC 180

CGGTGATCAT ATGGCTTCTG ATATGATTGC CCCACTCTAT CCACACCAAC TCCGAGTCTA 240

TCTCAAAATA GTTTAGCCAT CTCTTCCCTA ATTTTCTACA TTGCACTCGG TGGCAGACCA 300

CCGGACCCTA GGCTGTGGGG TTCATTCGGT CGGGCATTGT TATGCCGACC TTCTTGCCAT 360

GACCGATTGA TAATGTTGAT CGGCCTGTGA TCATATGGCG TGTTGTGGGT TAAATATGTA 420

GGGGGCAGAA CATACTGCCG TTGTGGTATG TAATAATTTG GTGCATAGTG TGCGACAGTA 480

GGTTCTGTGT ATGTGTATCC GATATGTCCG GTGGTACATC TGAACTGGCC GGTTGTGTTA 540

GCTATTATTG GGGCGCCACG CGTAGCCCTG GTGCGGCCCG GACTATCCGG CAGAGAAAGC 600

CGACGGTCTG TGTAAGGGCC GAACTATCCA GACAAAAGCT CGGACGGTCC GACCGTGTAG 660

AGGGCCGTCG ATCTGCCAAG CAAGGACGAT GGTGATGGTA TTTGCCCTGG ATATGAGTTC 720

ATCAACATAC CATATAATGG ATGGGGCTGC AAATCCCCAT TTGTCGCCGA TGTATTAGAC 780

ATAAATATCA TGTTACTAGT TTCATATGAT GGAAAACTAG GAGCAACAGA CTTCTCCAAC 840

ATACACGTTA ATTTTCTAAT TGGTTCTTCT AACCCTCTAA TCTAATGCTT CATTTGATTA 900

TGCAAATGGT CTACATACTG TTTAATAGAT TGGATGTCGT CGGGTTTACT TACGTTAGGG 960

ACTTGAAGCG AAGATAGAAG AGATGTGACG TCGGTATCGC ATGTTTGACA ACTTTCTGGT 1020

GACGATCCAC CATGTATTGT GACAAGAATT TCTCCTTCGT TTGACACATG TAGTCCTCGT 1080

ATTGTTGTTG CTCATCGGTC GTCGGACTCT TAATAGCCGG CTTTAGGATA TTGTCCGGGG 1140

AGATATCGGT GTGATCTTTA GAACCGCCAT TTGATGGCCT GAGTTTTAGT AGATCTAGAC 1200

ACATTTCCCC AACGGAGTCG CCAAAAAGTG TGTTGGCGCC GATCCAGGCG CGAAACACTG 1260

GAGATGGACC GTTTGGCGGT GTTCTCCGGG TGAGGACGGT CCGCGACCTG GGTCCAGCAG 1320

CGACTCTCCT CTACGTGTGT CCGGACGGTC CGTCGTCTGG GGCTCGGACG GTCGCGATGG 1380

CGCAGAGGGT CTTCTTCTTC GCAGCCGACC TAGATCTCGC CTCCCGGGAG GGACCGTCGG 1440

GGAGGAGAGA TTGTAGGGTG TGTCTTGGCG TCGACAGGCC ACACAATACG CCTCTAGTCG 1500

ACGTAGAGCC GAAGAGAGGT GAAGGATTGA GGTAGAAGGA GGCTAAACTT GGGCTAAACT 1560

AGAACTACTG CTAATGCATA AGGTAAAAAC GAGAAGTGGA CTTCATTTGA TCGATTGTGG 1620

AAGTAATCTG ACTGTAGCCC TTTATCTATA TAAAGGGGAG GTATGGACCC GTTACAAGCC 1680

GTTTTCCGAG CTAATCTCAC GGTTTTAGTT AATAAATCCT GCGAGAAACT CGGAACTCTA 1740

ACTGATTCTA CTCATGCGCG AACCATTCGT GCGCCACCGC TGCCCGTCCC GCGATCGCTC 1800

AGTTAACCCT GTGTTGTGCG CTGTGATTTG GTGGCATATA AAACCACATT TGCAATAAAA 1860

ATTTGTAGGG ATTTAACATA CCAAGTGCTG CGAAAGGAAT CGTTTTCGGA GGACCCAAAA 1920

TTAAAGAGGC AGATGCTAGA GCTCGTCCAG CTCAGCGCTG AGCACCTGTG TTGTCTTCCT 1980

CGTCCACGCC GGCGGAGATG AACGGCAACA AAGGCGGAAA GGCCGAGACG CTGAGCTCAA 2040

GGACGTGACA CCGCGCGTAC CTCGCGTTCA GTTGGCTCAC ACAACAGCAG CTCGCTCGCC 2100

CCAAGCTCCC GCGTCCTGAT CCGTAGGTGA GCCATGCAAA GGTCGCCGCG CGCCCTGATC 2160

CATTGCACCC TTCAAAGCTC GAACCTACAA ATAGCGTGCA CCAGGCATCC TGGCCACACC 2220

CACACAGCAA GCCAGCAGAG CAGAAAGCAG CCGCAGCCCC AGCCCCCACA AAGACGAAGG 2280

CAACA 2285

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: 14-SA Primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AGACGCTGAG CTCAAGGACG TGA 23

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: MANT-1 Primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

ATGCCCGGGC TTGCAATGTC TGTTAGCGGT GGCATCA 37

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: MANT-2RB Primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ATGCCCGGGC GATGGGGTAA GATGCAAGAC CA 32

CLAIMS

1. A method of inhibiting gene expression in a target plant tissue which comprises stably transforming a plant cell of a type from which a whole plant may be regenerated with a gene construct carrying a tissue-specific or a development-specific promoter which operates in the cells of the target plant tissue and a disrupter gene encoding a protein which is capable, when expressed, of inhibiting respiration in the cells of the said target tissue resulting in death of the cells, the said disrupter gene is selected from the group consisting of the T-urf13 gene, genes encoding an α- or β -tubulin, short sense co-suppression of two essential maize cell cycle genes, cdc25 and replication origin activator (ROA) and a short sense construct to the adenine nucleotide translocator (ANT) of the inner mitochondrial membrane.

2. A plant having stably incorporated in its genome by transformation a gene construct carrying a gene construct carrying a tissue-specific or a development-specific promoter which operates in the cells of the target plant tissue and a disrupter gene encoding a protein which is capable, when expressed, of inhibiting an essential cell function such as respiration, microtubule arrays or cell division in the cells of the said target tissue resulting in death of the cells wherein the disrupter gene is selected from the group consisting of the T-urf13 gene, genes encoding an α- or β-tubulin, short sense co-suppression of two essential maize cell cycle genes, cdc25 and replication origin activator (ROA) and a short sense construct to the adenine nucleotide translocator (ANT) of the inner mitochondrial membrane.

3. A plant having stably incorporated within its genome a gene construct carrying a tissue-specific promoter which operates in the cells of the said target tissue and a disrupter gene encoding a protein which is capable of inhibiting an essential cell function such as respiration or microtubules in the said cells of the said target tissue resulting in death of the cells characterised in that the said disrupter gene is selected from the T-urf13 gene, a short sense construct of the adenine nucleotide translocator, genes encoding an α- or β-tubulin and short sense down-regulation of the essential cell cycle genes, cdc25 and ROA.

4. A plant as claimed in claim 2 or claim 3 which is a monocotyledonous plant.

5. A plant as claimed in claim 4 which is a corn plant.

6. A method as claimed in claim 1 or a plant as claimed in any one of claims 2 to 5, wherein the promoter is an anther- and/or tapetum-specific promoter.

7. A method or a plant as claimed in claim 6 wherein the promoter may be isolated using the cDNA sequences of any of Figures 1 to 3.

8. A method or a plant as claimed in claim 6, wherein the promoter is the promoter from the MFS14 gene (SEQ ID NO 8)

9. A male sterile corn plant having stably incorporated within its genome a gene construct carrying a tapetum-specific promoter which operates in the cells of tapetum and a disrupter gene encoding a protein which is capable of inhibiting an essential cell function such as respiration or microtubules in the cells of the tapetum resulting in death of the cells characterised in that the said disrupter gene is selected from the T-urf13 gene, a short sense construct of the adenine nucleotide translocator, genes encoding an α- or β-tubulin and short sense down-regulation of the essential cell cycle genes, cdc25 and ROA.

FIG. 1.

Nucleotide and deduced amino acid sequence for male flower specific cDNA clone, pMS10.

```
                10                  20                  30                  40
GGC CTT GCC CGC TCG TTC CCC TCG CCT CCC CGG TCG CGC CGC TCC

    50                  60                  70                  80                  90
CGC TGC CGC CGT GGC GAT TCC TGC CCG GCG GCG GCG CCG GGT TCA

            100                 110                 120                 130
GGT CCA CGG CGG CGG CGG CTG CGC GGG GCG GGA CCG ACT ATG GGA
                                                        Met Gly

    140                 150                 160                 170                 180
CGG ACG ACA GCA AGA TCT CCC CCG ACG AGG AAT TCC TTC GAG GGC
Arg Thr Thr Ala Arg Ser Pro Pro Thr Arg Asn Ser Phe Glu Gly

            190                 200                 210                 220
TGC GAC TAC AAC CAC TGG CTC ATC ACC ATG GAC TTC CCG GAC CCC
Cys Asp Tyr Asn His Trp Leu Ile Thr Met Asp Phe Pro Asp Pro

    230                 240                 250                 260                 270
AAG CCG TCG CGC GAA GAG ATG ATC GAG ACA TAC CTC CAG ACT CTC
Lys Pro Ser Arg Glu Glu Met Ile Glu Thr Tyr Leu Gln Thr Leu

            280                 290                 300                 310
GCC AAG GTC GTC GGG AGT TAT GAG GAG GCC AAG AAG AGG ATG TAT
Ala Lys Val Val Gly Ser Tyr Glu Glu Ala Lys Lys Arg Met Tyr

    320                 330                 340                 350                 360
GCT TTT AGT ACG ACG ACT TAT GTT GGT TTT CAG GCT GTA ATG ACC
Ala Phe Ser Thr Thr Thr Tyr Val Gly Phe Gln Ala Val Met Thr

            370                 380                 390                 400
GAG GAA ATG TCA GAA AAA TTT CGC GGT TTG CCT GGA GTA GTT TTC
Glu Glu Met Ser Glu Lys Phe Arg Gly Leu Pro Gly Val Val Phe

    410                 420                 430                 440                 450
ATT TTG CCT GAT TCA TAT CTA TAT CCA GAA ACA AAG GAG TAC GGA
Ile Leu Pro Asp Ser Tyr Leu Tyr Pro Glu Thr Lys Glu Tyr Gly

            460                 470                 480                 490
GGA GAC AAA TAT GAC AAT GGT GTC ATC ACT CCA AGA CCA CCA CCT
Gly Asp Lys Tyr Asp Asn Gly Val Ile Thr Pro Arg Pro Pro Pro
```

FIG. 1.
(cont.)

```
    500         510         520         530         540
GTT CAT TAT AGC AGA CCA TCA AGA ACT GAC AGG AAC CGT AAC TAC
Val His Tyr Ser Arg Pro Ser Arg Thr Asp Arg Asn Arg Asn Tyr

          550         560         570         580
CGA GGA AAC TAC CAG GAT GGC CCT CCA CAG CAA GGA AAT TAC CAG
Arg Gly Asn Tyr Gln Asp Gly Pro Pro Gln Gln Gly Asn Tyr Gln

    590         600         610         620         630
AAC AAC CGT CCT CCA CCA GAA GGT GGT TAC CAG AAC AAC CCG CCG
Asn Asn Arg Pro Pro Pro Glu Gly Gly Tyr Gln Asn Asn Pro Pro

          640         650         660         670
CAG CAA GGA AAC TAC CAG ACA TAC CGC TCG CAG CAA GAT GGA AGA
Gln Gln Gly Asn Tyr Gln Thr Tyr Arg Ser Gln Gln Asp Gly Arg

    680         690         700         710         720
GGC TAT GCC CCA CAG CAG AAT TAT GCA CAA GGT GGT CAG GAT GGT
Gly Tyr Ala Pro Gln Gln Asn Tyr Ala Gln Gly Gly Gln Asp Gly

          730         740         750         760
AGA GGT TTT GGA AGG AAT GAT TAC ACA GAC CGT TCA GGC TAC AAT
Arg Gly Phe Gly Arg Asn Asp Tyr Thr Asp Arg Ser Gly Tyr Asn

    770         780         790         800         810
GGA CCC ACT GAT TTT CGA AGT CAA ACT CAG TAC CAA GGG CAT GTA
Gly Pro Thr Asp Phe Arg Ser Gln Thr Gln Tyr Gln Gly His Val

          820         830         840         850
AAT CCA GCT GGG CAA GGT CAA GGT TAC AAC AAC CCC CAA GAG CGT
Asn Pro Ala Gly Gln Gly Gln Gly Tyr Asn Asn Pro Gln Glu Arg

    860         870         880         890         900
ACG AAC TTC TCG CAA GGG CAG GGA GGA GGT TTT AGG CCT GGT GGT
Thr Asn Phe Ser Gln Gly Gln Gly Gly Gly Phe Arg Pro Gly Gly

          910         920         930         940
CCT TCA GCA CCT GGG TCT TAT GGC CAA CCA TCA GCA CCT GGA TCT
Pro Ser Ala Pro Gly Ser Tyr Gly Gln Pro Ser Ala Pro Gly Ser

    950         960         970         980         990
TAT GGT CAA CCT AAT ACA CTT GGT AAC TAT GGG CAG GTA CCT CCA
Tyr Gly Gln Pro Asn Thr Leu Gly Asn Tyr Gly Gln Val Pro Pro
```

FIG. 1.

(cont.)

```
          1000         1010         1020         1030
TCA GTG AAT CCT GGT GGT AAC AGA GTT CCT GGT GTG AAT CCT AGT
Ser Val Asn Pro Gly Gly Asn Arg Val Pro Gly Val Asn Pro Ser

  1040         1050         1060         1070         1080
TAT GGT GGG GAT GGC AGA CAG GGG GCT GGA CCA GCA TAT GGT GGA
Tyr Gly Gly Asp Gly Arg Gln Gly Ala Gly Pro Ala Tyr Gly Gly

          1090         1100         1110         1120
GAT AAC TGG CAA AGA GGT TCT GGT CAG TAT CCT AGC CCA GGT GAA
Asp Asn Trp Gln Arg Gly Ser Gly Gln Tyr Pro Ser Pro Gly Glu

  1130         1140         1150         1160         1170
GGA CAA GGA AAC TGG CAG GGA AGG CAG TAA GAG CTG ACG TGT TCC
Gly Gln Gly Asn Trp Gln Gly Arg Gln

          1180         1190         1200         1210
ACT GAA GAC AAG AAT GGC ACT TGA GAT TTA GAA ATC TCC ATC TGT

  1220         1230         1240         1250         1260
AAA ATA AAC GAC TGT GAT GCA TTA CTC TTT TTT TTT TTC TTG CAT

          1270         1280         1290         1300
TTG AAC TCT AAA CTT ATG GGC ATG CGT TAT TAC CAA ACT ACG GAT

  1310         1320         1330         1340         1350
GCA AAT TCA TTT TAG TTT TTT GGG CCA AAT GTT GGC ATT TTT AAA

AAA
```

FIG.2.

Nucleotide and deduced amino acid sequence for the male flower specific cDNA clone, pMS14.

```
          10          20          30          40
GCA GGG GGG GGG GCA CAG CAA GCC AGC AGA GCA GAA AGC AGC CGC
Ala Gly Gly Gly Ala Gln Gln Ala Ser Arg Ala Glu Ser Ser Arg

     50          60          70          80          90
AGC CCC AGC CCC CAC AAA GAC GAA GGC AAC AAT GGC GCT AGA AGC
Ser Pro Ser Pro His Lys Asp Glu Gly Asn Asn Gly Ala Arg Ser

          100         110         120         130
AGC CAC GCC CCC CGC GCA CTC CTC GCG CGT GCC TCG TCC TGC TGG
Ser His Ala Pro Arg Ala Leu Leu Ala Arg Ala Ser Ser Cys Trp

    140         150         160         170         180
TCC TCG GCG GCG GCA CCG GCC CGT CGT CGG TGC TCA GCG CGC CGG
Ser Ser Ala Ala Ala Pro Ala Arg Arg Arg Cys Ser Ala Arg Arg

          190         200         210         220
GGC GCA GGA CCG GCG GCA GTG CCT GCC GCA GCT GAA CGC CTC CTG
Gly Ala Gly Pro Ala Ala Val Pro Ala Ala Ala Glu Arg Leu Leu

    230         240         250         260         270
CGG TGC CGC GCG TAC CTG GTG CCG GCG CGC CGG ACC CCA GCG CGG
Arg Cys Arg Ala Tyr Leu Val Pro Ala Arg Arg Thr Pro Ala Arg

          280         290         300         310
ACT GCT GCA GCG CTG ACG CGC CGT GTG CAC GAG TGC GCC TGC AGC
Thr Ala Ala Ala Leu Thr Arg Arg Val His Glu Cys Ala Cys Ser

    320         330         340         350         360
ACC ATG GGC ATC ATC AAC AGC CTG CCC GGC CGG TGC CAC CTC GCC
Thr Met Gly Ile Ile Asn Ser Leu Pro Gly Arg Cys His Leu Ala

          370         380         390         400
CAA GCC AAC TGC TCC GCT TGA AGC AGG GAC CTG GCA CGC GTG CTG
Gln Ala Asn Cys Ser Ala

    410         420         430         440         450
CAA TGG ATG GCA GGA GGG GAG AGG AAT AAG AAG TGT TTC CAT TTC

          460         470         480         490
ACA GTG AGA GCA GTC GAG CTC CAA CGT TGT CGT CGT CGT CGT CTT
```

FIG.2.

(cont.)

```
    500                 510                 520                 530                 540
     |                   |                   |                   |                   |
CTT CTT TTG ATA TTC AGA CTC TGT CTT GCG GTC TAT ATC ATC AGC

              550                 560                 570                 580
               |                   |                   |                   |
ATA ATA ATA ATA AAA TAA GTA AAA CCA AAA AAA AAA AAA AA
```

FIG. 3.

Nucleotide and deduced amino acid sequence for the male flower specific cDNA clone, pMS18.

```
                10                  20                  30                  40
ACA GCA GTA GCA AGA GGG ATA GAG CAA GGC CAC ACA CAC ACA CAC

     50                  60                  70                  80                  90
ACC ACT AGG CTA GGT TAG CCT TTT AAT CGT CGT CGA GAA GCA AGA

           100                 110                 120                 130
AGG GCG CTG CAC CAA GCA GGC AAG CAA GAA GAG AGC CGA TCG ACC

    140                 150                 160                 170                 180
GAG AGC TAG CAC GCG ATG GCG AGG TCT TGC CAA GAT GAT GGT GGC
                      Met Ala Arg Ser Cys Gln Asp Asp Gly Gly

           190                 200                 210                 220
GCA CGT CTG CTG GCC TTG CGC TGG CGT GTC GAC CGC CGA GGC AGG
Ala Arg Leu Leu Ala Leu Arg Trp Arg Val Asp Arg Arg Gly Arg

    230                 240                 250                 260                 270
AAC ATC AAG ACC ACG ACG ACG GAG AAG AAG GAC GAC GCG GTG GTG
Asn Ile Lys Thr Thr Thr Thr Glu Lys Lys Asp Asp Ala Val Val

           280                 290                 300                 310
CAG CCG CAG AGG TTC CGC CCT TCG ACC GCC TCG GCG CGG CGC GTC
Gln Pro Gln Arg Phe Arg Pro Ser Thr Ala Ser Ala Arg Arg Val

    320                 330                 340                 350                 360
CCC GGC GTT CGG CGG CCT CCC CGG CGG CAC GAT TCC TGG CAG CAG
Pro Gly Val Arg Arg Pro Pro Arg Arg His Asp Ser Trp Gln Gln

           370                 380                 390                 400
CAT TCC CGG GTT CAG CAT GCC CGG CAG CGG CAG CAG CCT ACC CGG
His Ser Arg Val Gln His Ala Arg Gln Arg Gln Gln Pro Thr Arg

    410                 420                 430                 440                 450
GTT CAG CTT GCC CGG CAG CGG CAC GAT GCC CCT CTT CGG CGG CGG
Val Gln Leu Ala Arg Gln Arg His Asp Ala Pro Leu Arg Arg Arg

           460                 470                 480                 490
CTC CCC GGG CTT CAG CGG CTT CGG CGG CAT GCC CGG GTC GCC CAC
Leu Pro Gly Leu Gln Arg Leu Arg Arg His Ala Arg Val Ala His
```

FIG. 3.

(cont.)

```
    500           510           520           530           540
CGC CGG CTC CGT CCC CGA GCA CGC CAA CAA GCC CTG AAC GCC AAC
Arg Arg Leu Arg Pro Arg Ala Arg Gln Gln Ala Leu Asn Ala Asn

          550           560           570           580
AAG CGT GGT AGT AGA GGT GCT ACT GTT ACT GTA GTA CGT CGT CGT
Lys Arg Gly Ser Arg Gly Ala Thr Val Thr Val Val Arg Arg Arg

    590           600           610           620           630
CTT CAT GCA TGC GTG GTT CGT GGT TTC CCT AGC TCC ATA CGA GCA
Leu His Ala Cys Val Val Arg Gly Phe Pro Ser Ser Ile Arg Ala

          640           650           660           670
GTA GTT GGG CTT GCA CGT ACC GTA CGT CTA GCT AGC TAT ATA TAT
Val Val Gly Leu Ala Arg Thr Val Arg Leu Ala Ser Tyr Ile Tyr

    680           690           700           710           720
GCT TGT GTT CTA CTG CTT TTT AGT TTA ATT ACC TGC CTG CAT TGG
Ala Cys Val Leu Leu Leu Phe Ser Leu Ile Thr Cys Leu His Trp

          730           740           750           760
AGA GTT GGA TCT GTT TCA TTT GGT GGT GTT TGC TTT ACT ATT AGG
Arg Val Gly Ser Val Ser Phe Gly Gly Val Cys Phe Thr Ile Arg

    770           780           790           800           810
TCA GTA TCT GTT TGT GGA GAC TTG GTG TTT AAT TTA TTT AGC CGT
Ser Val Ser Val Cys Gly Asp Leu Val Phe Asn Leu Phe Ser Arg

          820           830           840           850
TTG TGA CTG GTT GTA GCT AGC GGT GGT GCG GTG GTG ATG TTC TTG
Leu

    860           870           880           890           900
AGG CAT GAA TAA TGC TAC ATG CAT GTG ATG TAT CCA TGT TTT GTG

          910           920           930
TGT GGT AAA CCT GTT GTT TGT ATA AGC TGT CCC
```

FIGURE 4

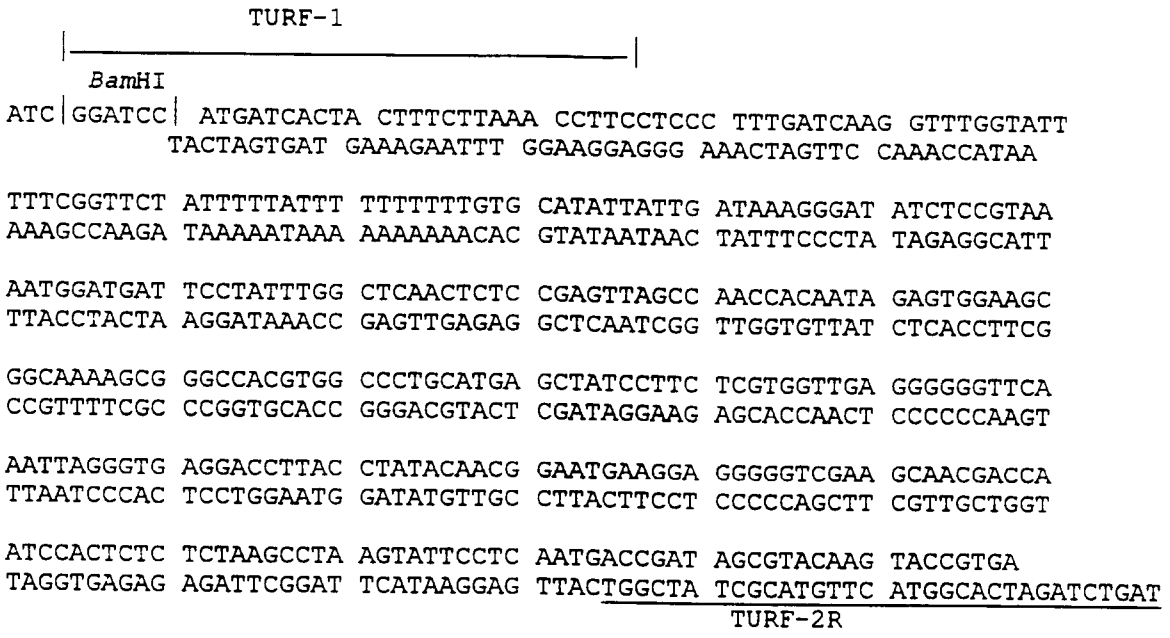


FIGURE 5

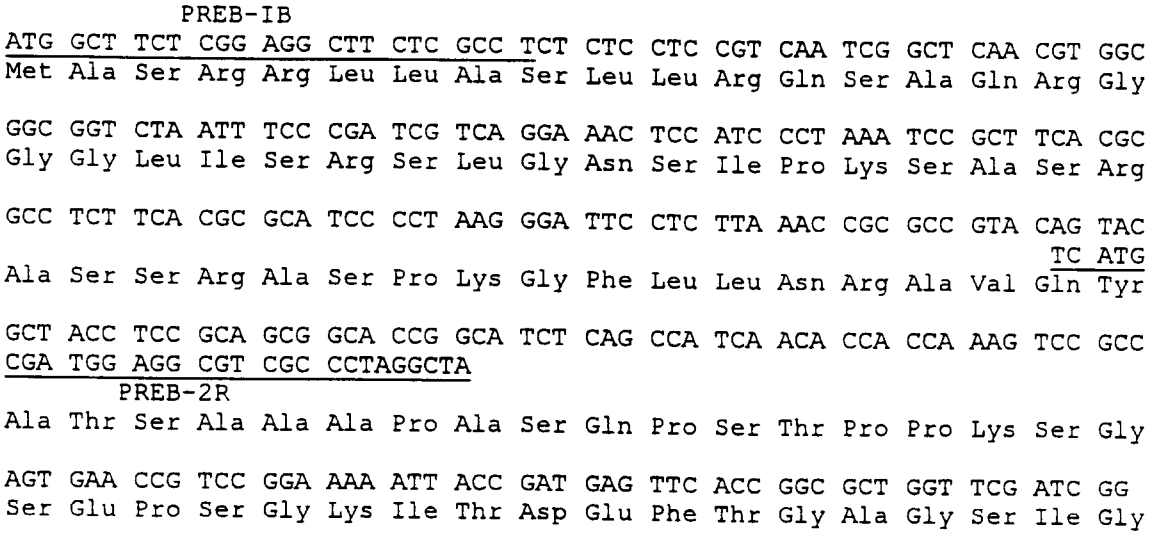


FIGURE 6

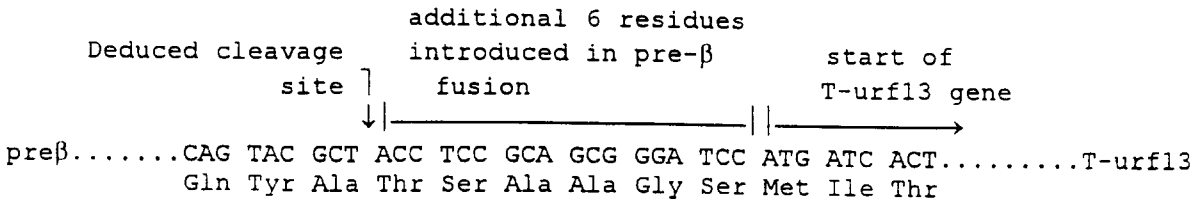
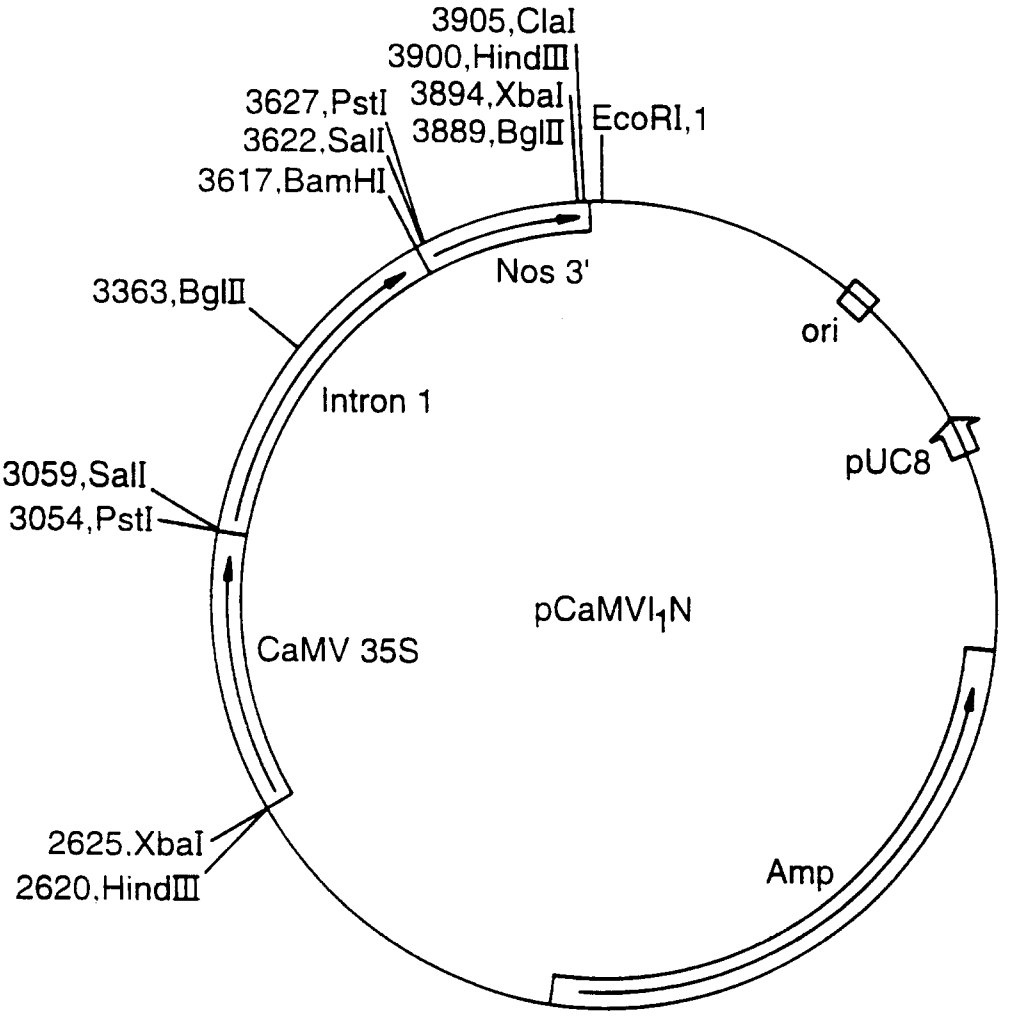


Fig.7.


3905,ClaI
3900,HindIII
3894,XbaI
3889,BglII
EcoRI,1
3627,PstI
3622,SalI
3617,BamHI
Nos 3'
3363,BglII
ori
Intron 1
pUC8
3059,SalI
3054,PstI
pCaMVI1N
CaMV 35S
2625,XbaI
2620,HindIII
Amp

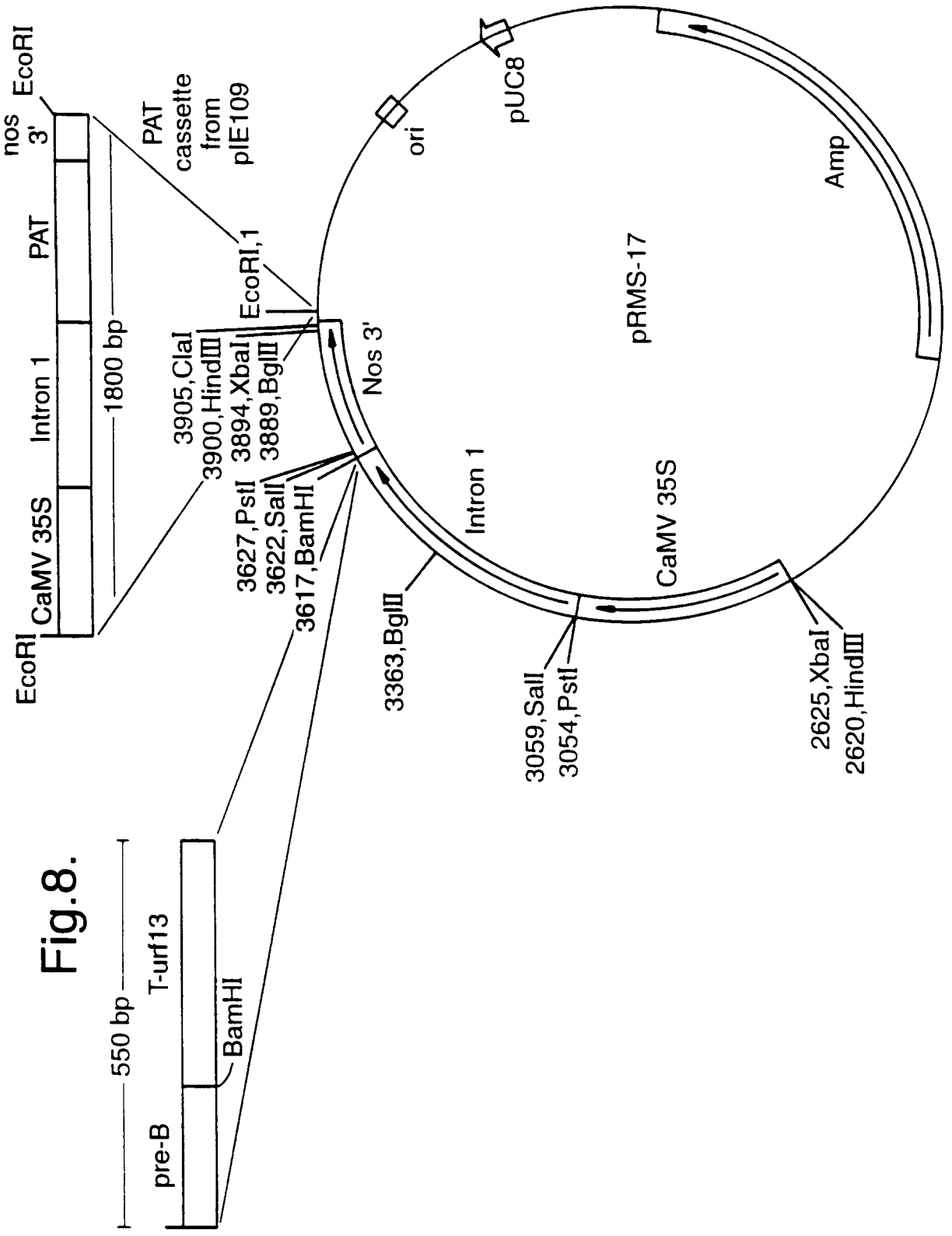
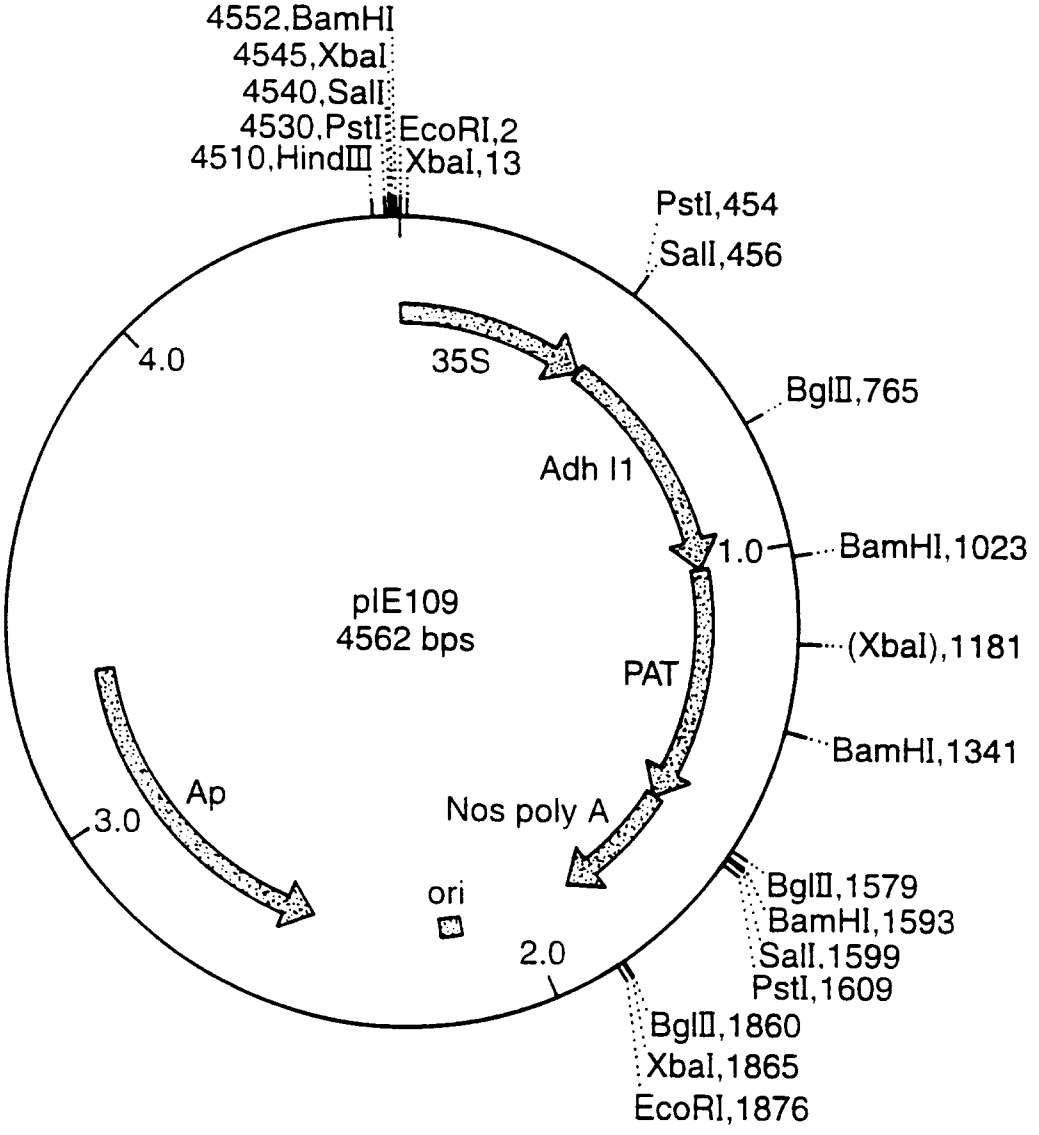

nos 3'
EcoRI
PAT
Intron 1
CaMV 35S
EcoRI
1800 bp
PAT cassette from pIE109
EcoRI,1
3905,ClaI
3900,HindIII
3894,XbaI
3889,BglII
3627,PstI
3622,SalI
3617,BamHI
3363,BglII
3059,SalI
3054,PstI
2625,XbaI
2620,HindIII
ori
pUC8
Amp
pRMS-17
Nos 3'
Intron 1
CaMV 35S
550 bp
T-urf13
BamHI
pre-B

Fig.8.

Fig.9.



35S=35S promoter from Cauliflower Mosaic Virus
AdhI1=maize Alcohol Dehydrogenase intron 1
PAT=phosphinothricin acetyl tranferase gene
nos polyA=nopaline synthase polyA region
Ap=bacterial ampicillin resistance
ori=ColE1n origin of replication (from pUC)

FIGURE 10

Promoter sequence from MFS14 Gene

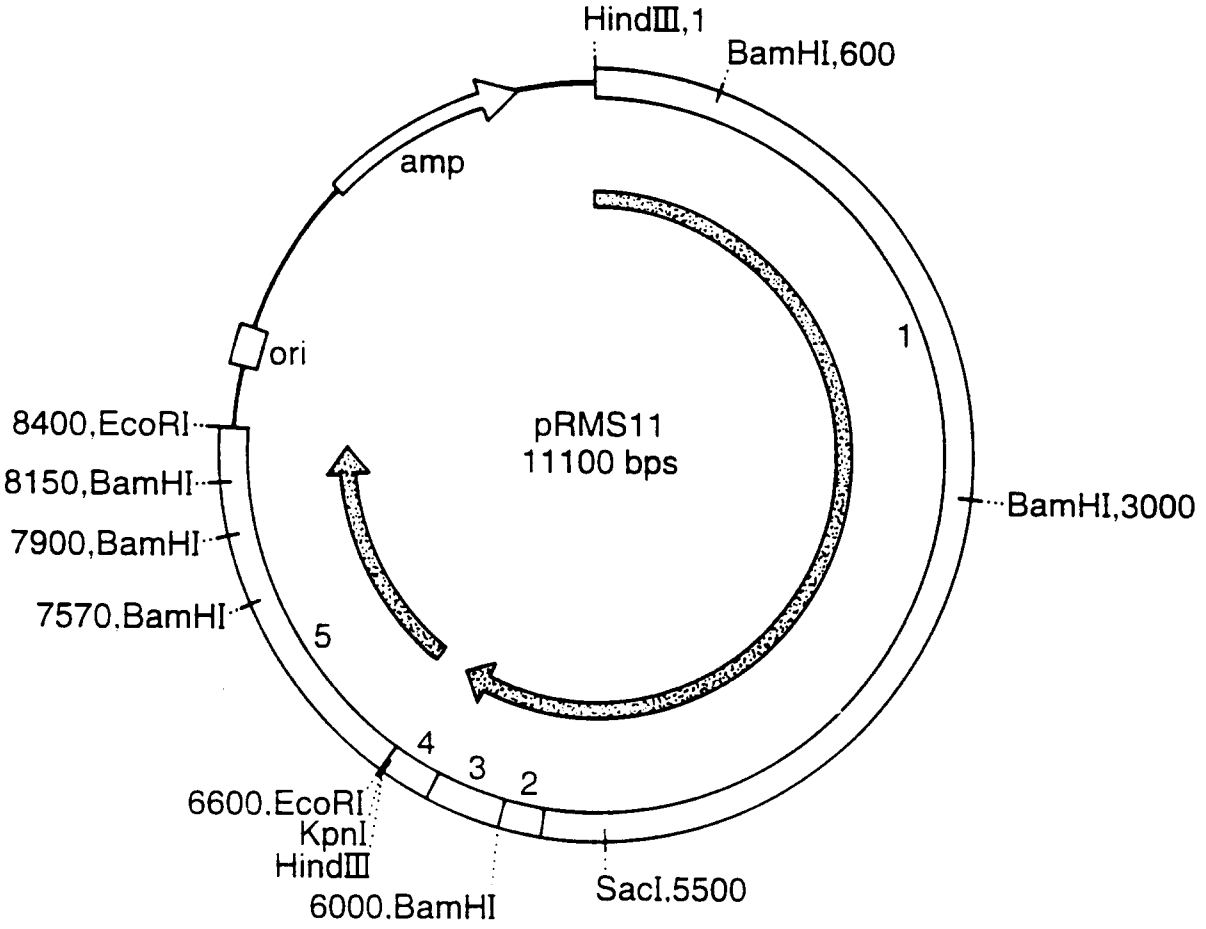
```
      3        9       15       21       27       33       39       45
      |        |        |        |        |        |        |        |
  1 AAA AGC GTA CCA GTA AGG GAT AAA GAA AAT AAA CAA ACA CGA AAT
    TTT TCG CAT GGT CAT TCC CTA TTT CTT TTA TTT GTT TGT GCT TTA

 46 GCT TCC CCA TCG GCC AAT TCG CCT AGG TGT GCT AGG AAC TGG CCT
    CGA AGG GGT AGC CGG TTA AGC GGA TCC ACA CGA TCC TTG ACC GGA

 91 ATA TGT TCG TGT GTG CTT CTC CTA TTT TCA CCA GAA AAC TTA GAA
    TAT ACA AGC ACA CAC GAA GAG GAT AAA AGT GGT CTT TTG AAT CTT

136 AAC TCT GGT ATC CTT GCC CCT TGT GGA TAT GGG ACA ATG TCA AAC
    TTG AGA CCA TAG GAA CGG GGA ACA CCT ATA CCC TGT TAC AGT TTG

181 CGG TGA TCA TAT GGC TTC TGA TAT GAT TGC CCC ACT CTA TCC ACA
    GCC ACT AGT ATA CCG AAG ACT ATA CTA ACG GGG TGA GAT AGG TGT

226 CCA ACT CCG AGT CTA TCT CAA AAT AGT TTA GCC ATC TCT TCC CTA
    GGT TGA GGC TCA GAT AGA GTT TTA TCA AAT CGG TAG AGA AGG GAT

271 ATT TTC TAC ATT GCA CTC GGT GGC AGA CCA CCG GAC CCT AGG CTG
    TAA AAG ATG TAA CGT GAG CCA CCG TCT GGT GGC CTG GGA TCC GAC

316 TGG GGT TCA TTC GGT CGG GCA TTG TTA TGC CGA CCT TCT TGC CAT
    ACC CCA AGT AAG CCA GCC CGT AAC AAT ACG GCT GGA AGA ACG GTA

361 GAC CGA TTG ATA ATG TTG ATC GGC CTG TGA TCA TAT GGC GTG TTG
    CTG GCT AAC TAT TAC AAC TAG CCG GAC ACT AGT ATA CCG CAC AAC

406 TGG GTT AAA TAT GTA GGG GGC AGA ACA TAC TGC CGT TGT GGT ATG
    ACC CAA TTT ATA CAT CCC CCG TCT TGT ATG ACG GCA ACA CCA TAC

451 TAA TAA TTT GGT GCA TAG TGT GCG ACA GTA GGT TCT GTG TAT GTG
    ATT ATT AAA CCA CGT ATC ACA CGC TGT CAT CCA AGA CAC ATA CAC

496 TAT CCG ATA TGT CCG GTG GTA CAT CTG AAC TGG CCG GTT GTG TTA
    ATA GGC TAT ACA GGC CAC CAT GTA GAC TTG ACC GGC CAA CAC AAT

541 GCT ATT ATT GGG GCG CCA CGC GTA GCC CTG GTG CGG CCC GGA CTA
    CGA TAA TAA CCC CGC GGT GCG CAT CGG GAC CAC GCC GGG CCT GAT

586 TCC GGC AGA GAA AGC CGA CGG TCT GTG TAA GGG CCG AAC TAT CCA
    AGG CCG TCT CTT TCG GCT GCC AGA CAC ATT CCC GGC TTG ATA GGT

631 GAC AAA AGC TCG GAC GGT CCG ACC GTG TAG AGG GCC GTC GAT CTG
    CTG TTT TCG AGC CTG CCA GGC TGG CAC ATC TCC CGG CAG CTA GAC

676 CCA AGC AAG GAC GAT GGT GAT GGT ATT TGC CCT GGA TAT GAG TTC
    GGT TCG TTC CTG CTA CCA CTA CCA TAA ACG GGA CCT ATA CTC AAG

721 ATC AAC ATA CCA TAT AAT GGA TGG GGC TGC AAA TCC CCA TTT GTC
    TAG TTG TAT GGT ATA TTA CCT ACC CCG ACG TTT AGG GGT AAA CAG

766 GCC GAT GTA TTA GAC ATA AAT ATC ATG TTA CTA GTT TCA TAT GAT
    CGG CTA CAT AAT CTG TAT TTA TAG TAC AAT GAT CAA AGT ATA CTA

811 GGA AAA CTA GGA GCA ACA GAC TTC TCC AAC ATA CAC GTT AAT TTT
    CCT TTT GAT CCT CGT TGT CTG AAG AGG TTG TAT GTG CAA TTA AAA
```

```
 856 CTA ATT GGT TCT TCT AAC CCT CTA ATC TAA TGC TTC ATT TGA TTA
     GAT TAA CCA AGA AGA TTG GGA GAT TAG ATT ACG AAG TAA ACT AAT

 901 TGC AAA TGG TCT ACA TAC TGT TTA ATA GAT TGG ATG TCG TCG GGT
     ACG TTT ACC AGA TGT ATG ACA AAT TAT CTA ACC TAC AGC AGC CCA

 946 TTA CTT ACG TTA GGG ACT TGA AGC GAA GAT AGA AGA GAT GTG ACG
     AAT GAA TGC AAT CCC TGA ACT TCG CTT CTA TCT TCT CTA CAC TGC

 991 TCG GTA TCG CAT GTT TGA CAA CTT TCT GGT GAC GAT CCA CCA TGT
     AGC CAT AGC GTA CAA ACT GTT GAA AGA CCA CTG CTA GGT GGT ACA

1036 ATT GTG ACA AGA ATT TCT CCT TCG TTT GAC ACA TGT AGT CCT CGT
     TAA CAC TGT TCT TAA AGA GGA AGC AAA CTG TGT ACA TCA GGA GCA

1081 ATT GTT GTT GCT CAT CGG TCG TCG GAC TCT TAA TAG CCG GCT TTA
     TAA CAA CAA CGA GTA GCC AGC AGC CTG AGA ATT ATC GGC CGA AAT

1126 GGA TAT TGT CCG GGG AGA TAT CGG TGT GAT CTT TAG AAC CGC CAT
     CCT ATA ACA GGC CCC TCT ATA GCC ACA CTA GAA ATC TTG GCG GTA

1171 TTG ATG GCC TGA GTT TTA GTA GAT CTA GAC ACA TTT CCC CAA CGG
     AAC TAC CGG ACT CAA AAT CAT CTA GAT CTG TGT AAA GGG GTT GCC

1216 AGT CGC CAA AAA GTG TGT TGG CGC CGA TCC AGG CGC GAA ACA CTG
     TCA GCG GTT TTT CAC ACA ACC GCG GCT AGG TCC GCG CTT TGT GAC

1261 GAG ATG GAC CGT TTG GCG GTG TTC TCK KCG GMR GTG AGG ACG GTC
     CTC TAC CTG GCA AAC CGC CAC AAG AGM MGC CKY CAC TCC TGC CAG

1306 CGC GAC CTG GGT CCA GCA GCG ACT CTC CTC TAC GTG TGT CCG GAC
     GCG CTG GAC CCA GGT CGT CGC TGA GAG GAG ATG CAC ACA GGC CTG

1351 GGT CCG TCG TCT GGG GCT CGG ACG GTC GCG ATG GCG NCA GAG GGT
     CCA GGC AGC AGA CCC CGA GCC TGC CAG CGC TAC CGC NGT CTC CCA

1396 CTT CTT CTT CGC AGC CGA CCT AGA TCT CGC CTC CCG GGA GGG ACC
     GAA GAA GAA GCG TCG GCT GGA TCT AGA GCG GAG GGC CCT CCC TGG

1441 GTC GGG GAG GAG AGA TTG TAG GGT GTG TCT TGG CGT CGA CAG GCC
     CAG CCC CTC CTC TCT AAC ATC CCA CAC AGA ACC GCA GCT GTC CGG

1486 ACA CAA TAC GCC TCT AGT CGA CGT AGA GCC GAA GAG AGG TGA AGG
     TGT GTT ATG CGG AGA TCA GCT GCA TCT CGG CTT CTC TCC ACT TCC

1531 ATT GAG GTA GAA GGA GGC TAA ACT TGG GCT AAA CTA GAA CTA CTG
     TAA CTC CAT CTT CCT CCG ATT TGA ACC CGA TTT GAT CTT GAT GAC

1576 CTA ATG CAT AAG GTA AAA ACG AGA AGT GGA CTT CAT TTG ATC GAT
     GAT TAC GTA TTC CAT TTT TGC TCT TCA CCT GAA GTA AAC TAG CTA

1621 TGT GGA ASG NTA ATC TGA CTG TAG CCC TTT ATC TAT ATA AAG GGG
     ACA CCT TSC NAT TAG ACT GAC ATC GGG AAA TAG ATA TAT TTC CCC

1666 AGG TAT GGA CCC GTT ACA AGC CGT TTT CCG AGC TAA TCT CAC GGT
     TCC ATA CCT GGG CAA TGT TCG GCA AAA GGC TCG ATT AGA GTG CCA

1711 TTT AGT TAA TAA ATC CTG CGA GAA ACT CGG AAC TCT AAC TGA TTC
     AAA TCA ATT ATT TAG GAC GCT CTT TGA GCC TTG AGA TTG ACT AAG

1756 TAC TCA TGC GCG AAC CAT TCG TGC GCC ACC GCT GCC CGT CCC GCG
     ATG AGT ACG CGC TTG GTA AGC ACG CGG TGG CGA CGG GCA GGG CGC

1801 ATC GCT CAG TTA ACC CTG TGT TGT GNC GCT GTG ATT TGG TGG CAT
     TAG CGA GTC AAT TGG GAC ACA ACA CNG CGA CAC TAA ACC ACC GTA
```

```
1846 ATA AAA CCA CAT TTG CAA TAA AAA TTT GTA GGG ATT TAA CAT ACC
     TAT TTT GGT GTA AAC GTT ATT TTT AAA CAT CCC TAA ATT GTA TGG

1891 AAG TGC TGC GSA AAG GAA TCG TTT TCG GAG GAC CCA AAA TTA AAG
     TTC ACG ACG CST TTC CTT AGC AAA AGC CTC CTG GGT TTT AAT TTC

1936 AGG CAG ATG CTA GAG CTC GTC CAG CTC AGC GCT GAG CAC CTG TGT
     TCC GTC TAC GAT CTC GAG CAG GTC GAG TCG CGA CTC GTG GAC ACA

1981 TGT CTT CCT CGT CCA CGC CGG CGG AGA TGA ACG GCA ACA AAG GCG
     ACA GAA GGA GCA GGT GCG GCC GCC TCT ACT TGC CGT TGT TTC CGC

2026 GAA AGG CCG AGA CGC TGA GCT CAA GGA CGT GAC ACC GCG CGT ACC
     CTT TCC GGC TCT GCG ACT CGA GTT CCT GCA CTG TGG CGC GCA TGG

2071 TCG CGT TCA GTT GGC TCA CAC AAC AGC AGC TCG CTC GCC CCA AGC
     AGC GCA AGT CAA CCG AGT GTG TTG TCG TCG AGC GAG CGG GGT TCG

2116 TCC CGC GTC CTG ATC CGT AGG TGA GCC ATG CAA AGG TCG CCG CGC
     AGG GCG CAG GAC TAG GCA TCC ACT CGG TAC GTT TCC AGC GGC GCG

2161 GCC CTG ATC CAT TGC ACC CTT CAA AGC TCG AAC CTA CAA ATA GCG
     CGG GAC TAG GTA ACG TGG GAA GTT TCG AGC TTG GAT GTT TAT CGC

2206 TGC ACC AGG CAT CCT GGC CAC ACC CAC ACA GCA AGC CAG CAG AGC
     ACG TGG TCC GTA GGA CCG GTG TGG GTG TGT CGT TCG GTC GTC TCG

2251 AGA AAG CAG CCG CAG CCC CAG CCC CCA CAA AGA CGA AGG CAA CA
     TCT TTC GTC GGC GTC GGG GTC GGG GGT GTT TCT GCT TCC GTT GT
```

Total number of bases is: 2294.
DNA sequence composition: 562 A; 554 C; 594 G; 575 T; 9 other

Fig.11.



Key to genes:
1. MFS14 promoter (5.8kb)
2. PRE-B
3. TURF-13 gene
4. NOS terminator
5. 35S-I-BAR-NOS

Fig.12.

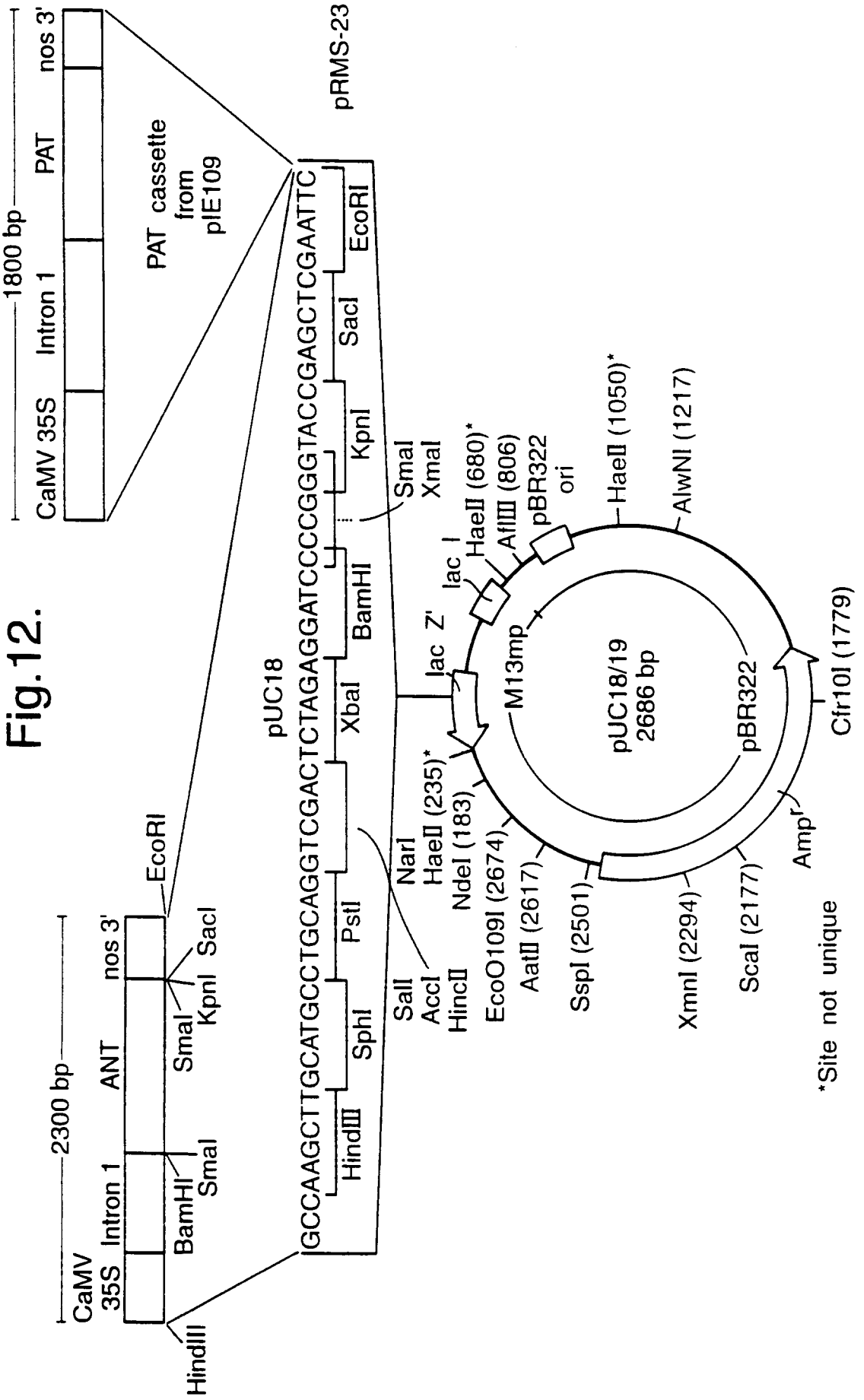

1800 bp
CaMV 35S
Intron 1
PAT
nos 3'
PAT cassette from pIE109
pRMS-23
2300 bp
CaMV 35S
Intron 1
ANT
nos 3'
EcoRI
SacI
KpnI
SmaI
BamHI
SmaI
HindIII
GCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTC
pUC18
HindIII
SphI
PstI
XbaI
BamHI
KpnI
SacI
EcoRI
SmaI
XmaI
SalI
AccI
HincII
NarI
HaeII (235)*
NdeI (183)
EcoO109I (2674)
AatII (2617)
SspI (2501)
XmnI (2294)
ScaI (2177)
Ampr
Cfr10I (1779)
lac Z'
lac I
M13mp
HaeII (680)*
AflIII (806)
pBR322 ori
HaeII (1050)*
AlwNI (1217)
pUC18/19 2686 bp
pBR322
*Site not unique

Fig.13.

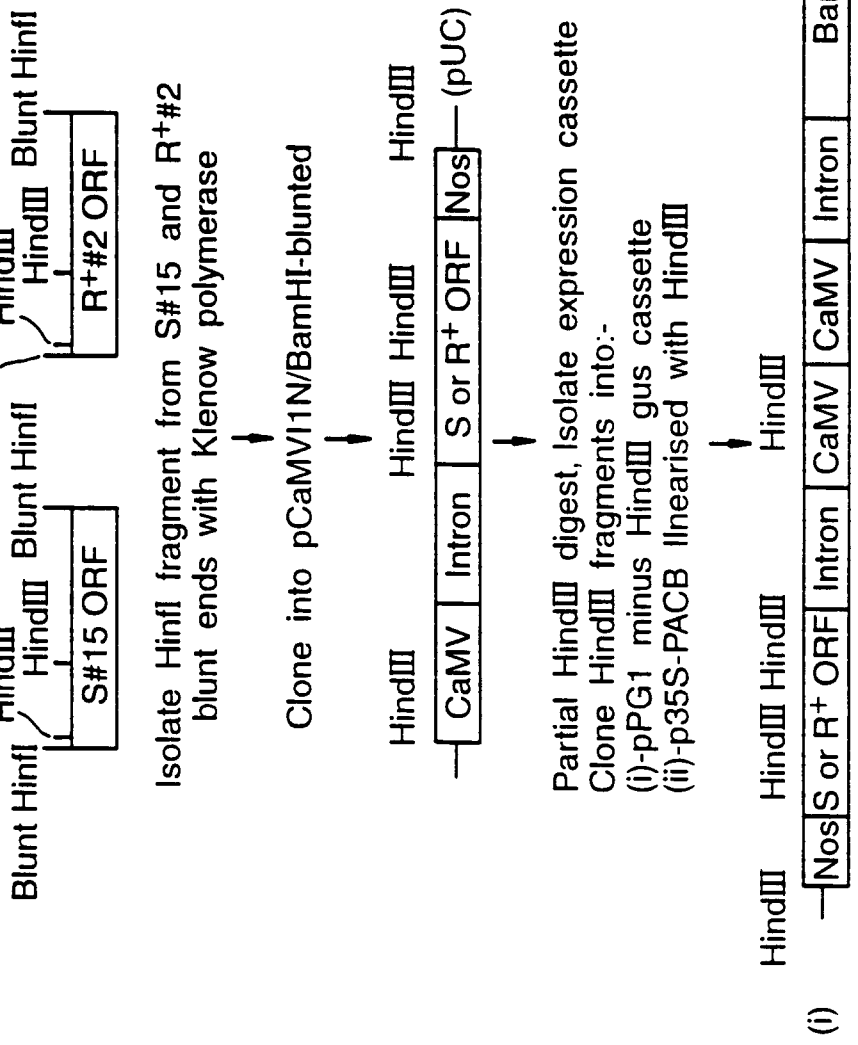
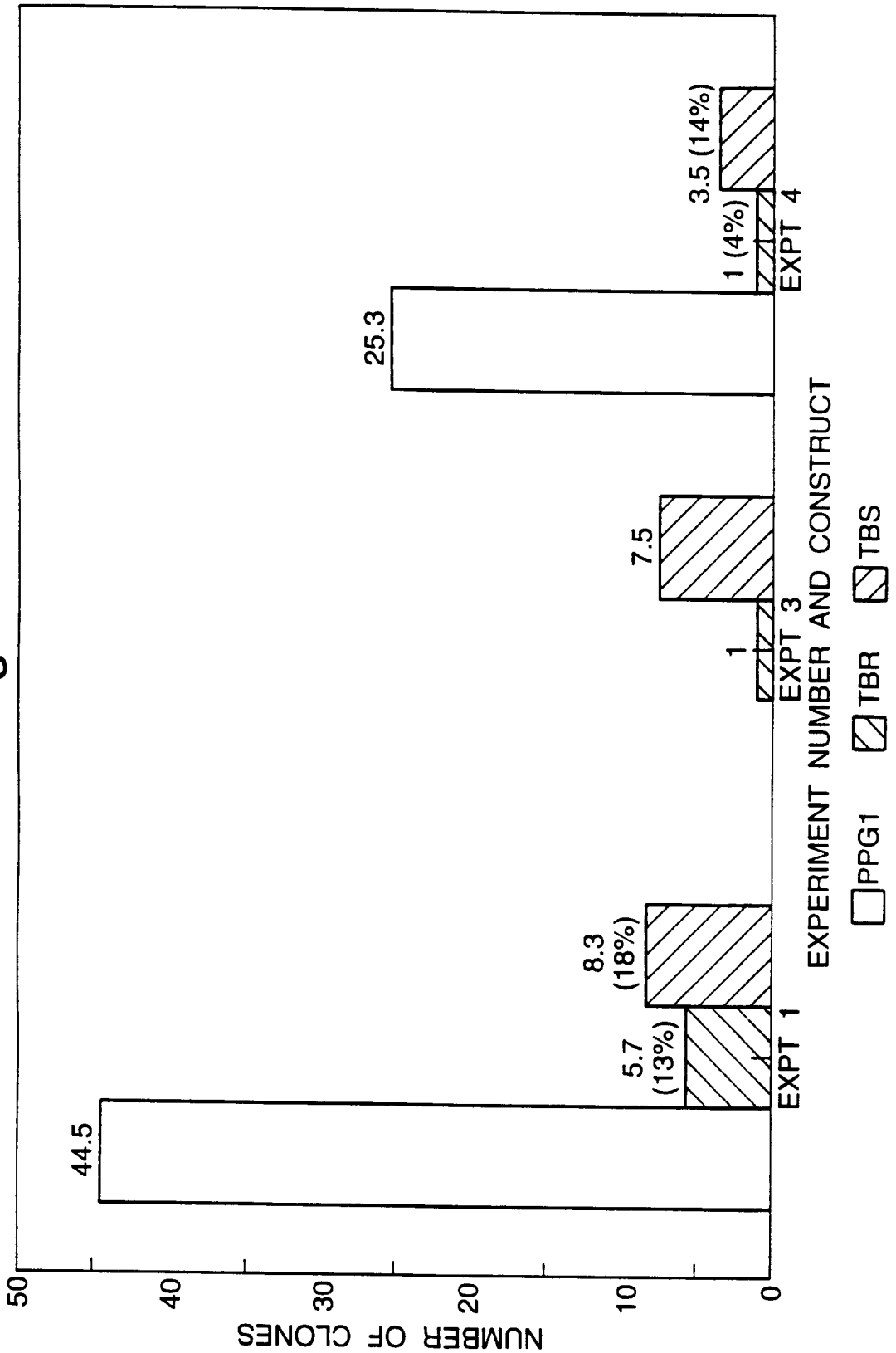

Blunt HinfI
HindIII
HindIII
Blunt HinfI
S#15 ORF
Blunt HinfI
HindIII
HindIII
Blunt HinfI
R+#2 ORF
Isolate HinfI fragment from S#15 and R+#2
blunt ends with Klenow polymerase
Clone into pCaMVI1N/BamHI-blunted
HindIII
HindIII
HindIII
CaMV
Intron
S or R+ ORF
Nos
(pUC)
pCSN/pDR+N
Partial HindIII digest, Isolate expression cassette (HindIII fragment 2.7kb)
Clone HindIII fragments into:-
(i)-pPG1 minus HindIII gus cassette
(ii)-p35S-PACB linearised with HindIII
Vectors
pTBS
and
pTBR+
(i)
HindIII
HindIII
HindIII
Nos
S or R+ ORF
Intron
CaMV
CaMV
Intron
Bar
Nos
(pUC)

Fig.14.


NUMBER OF CLONES
0
10
20
30
40
50
44.5
5.7 (13%)
8.3 (18%)
EXPT 1
1
7.5
EXPT 3
25.3
1 (4%)
3.5 (14%)
EXPT 4
EXPERIMENT NUMBER AND CONSTRUCT
PPG1
TBR
TBS