Señor
SUPERINTENDENTE DE INDUSTRIA Y COMER
DIVISION DE NUEVAS CREACIONES
E. S. D.

SUPERINDUSTRIA Y COMERCIO
Radicacion : 01100340 00000002 Folios: 51
Fecha (AMD): 2002-03-01 12:40:42
Tramite : 002 PATENTES D 1 REGISTRO/ 444 RESPUESTA
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Re: P1.-**UNILEVER N.V.**.-Solicitud de Patente de Invención para **"PROCESO PARA MODIFICAR PLANTAS"**.-Clase **C12N.**- Expediente número **01-100.340.**

1. Me refiero a mi solicitud de 22 de Noviembre de 2001 y al auto dictado por ese Despacho notificado por Estado de 21 de Febrero de 2002.

2. Anexos me permito presentar a usted:

(1) En cinco (5) hojas de papel, copia formal de los dibujos correspondientes a la solicitud;

(2) Copia auténtica debidamente certificada de la solicitud de patente presentada para el invento arriba nombrado **respecto del cual reclamo prioridad,** a saber la solicitud europea número 00310403.1, junto con su traducción simple correspondiente a las legalizaciones y reivindicaciones de dicho documento; y

(3) Documentos (2) que acreditan el traspaso que los autores del invento de la referencia y que UNILEVER PLC hacen a favor de mi representado de sus derechos sobre el mismo, debidamente apostillados, junto con las traducciones oficiales números 8423 y 8424, debidamente legalizadas por el Ministerio de Relaciones Exteriores de Colombia bajo los números 365065 y 365066 de 18 de Diciembre de 2001, correspondientes a las legalizaciones de dichos documentos de traspasos.

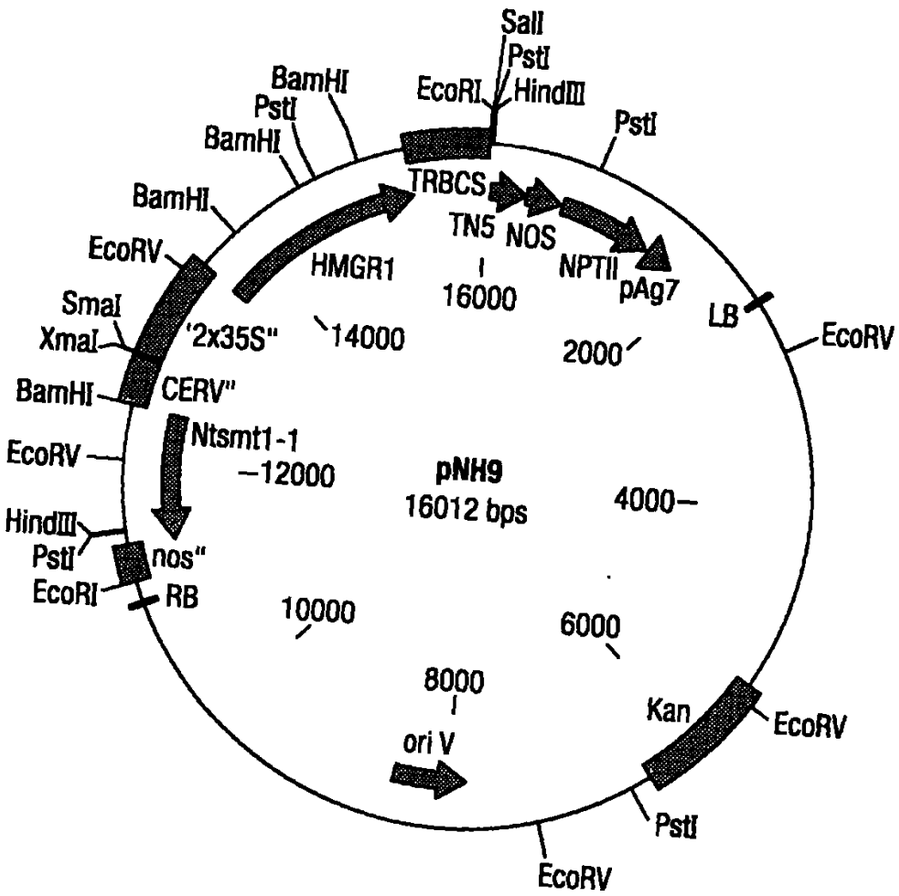
3. Anexa también acompaño copia debidamente autenticada por el Notario Sexto del Circulo de Bogotá, de los documentos que acreditan la calidad de Traductor Oficial de la señora Angela A. Pulecio Mayorga, quien elaboró la traducción de la solicitud europea 00310403.1, respecto de la cual se reivindica prioridad en el presente caso.

4. Ruego a usted tomar atenta nota de lo anterior y proceder de conformidad.

Señor Superintendente,

JOSE ANTONIO LLOREDA
T.P. 10.784
Código 231

Fig.1.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NPTII
pAg7
16000
SmaI
XmaI
"2x35S"
14000
LB
EcoRV
2000
BamHI
CERV"
Ntsmt1-1
EcoRV
12000
pNH9
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PstI
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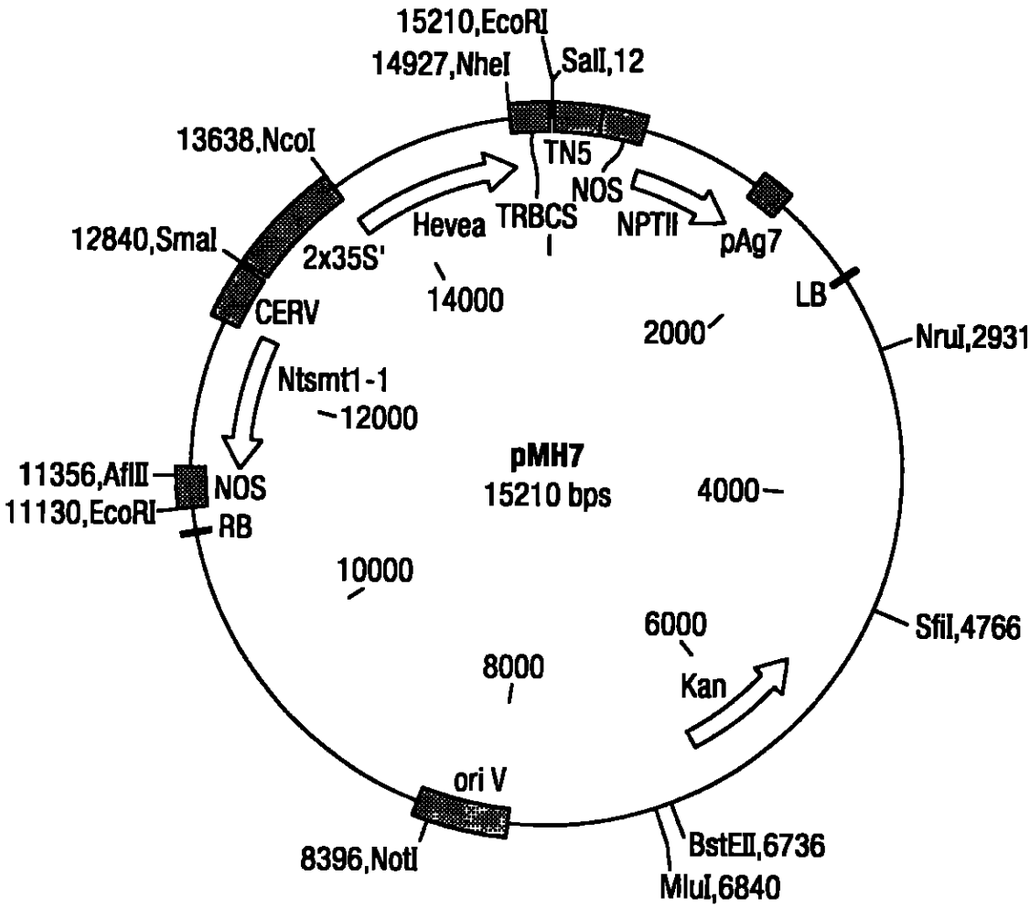
ori V
PstI
EcoRV

Fig.2.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14000
LB
CERV
2000
NruI,2931
Ntsmt1-1
12000
pMH7
15210 bps
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NOS
11130,EcoRI
RB
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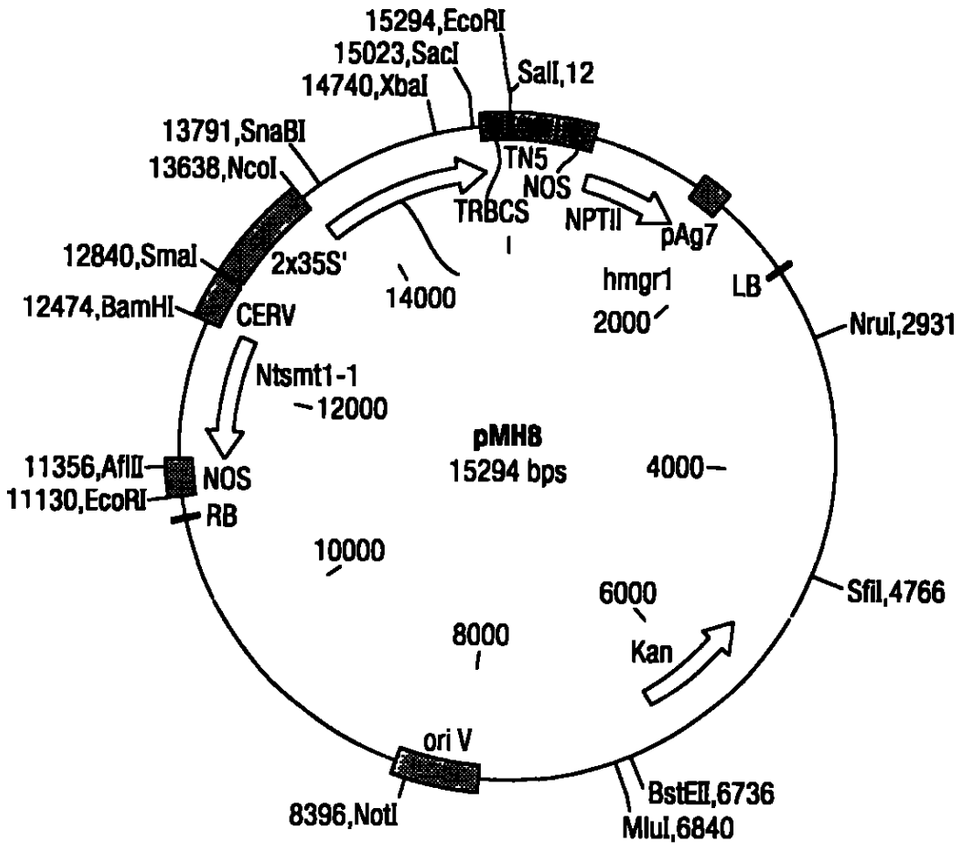
8000
Kan
ori V
8396,NotI
BstEII,6736
MluI,6840

Fig.3.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2x35S'
14000
hmgr1
LB
12474,BamHI
CERV
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Ntsmt1-1
12000
pMH8
15294 bps
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NOS
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RB
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MluI,6840

Fig.4.

Vector Binario pNH61

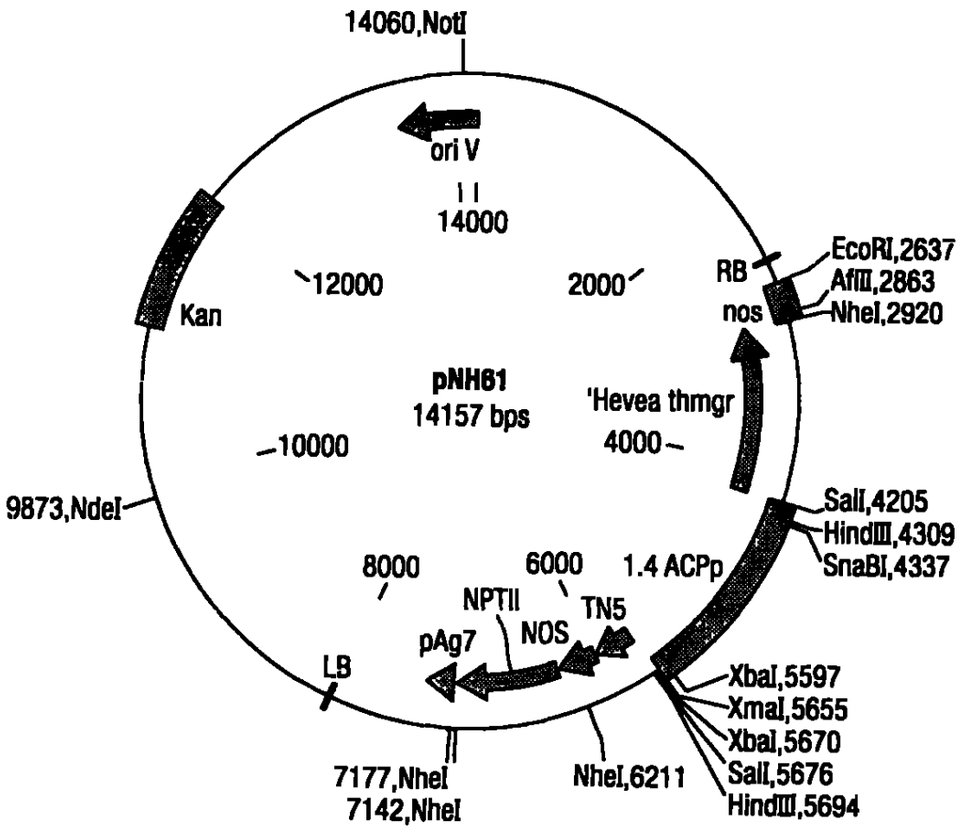
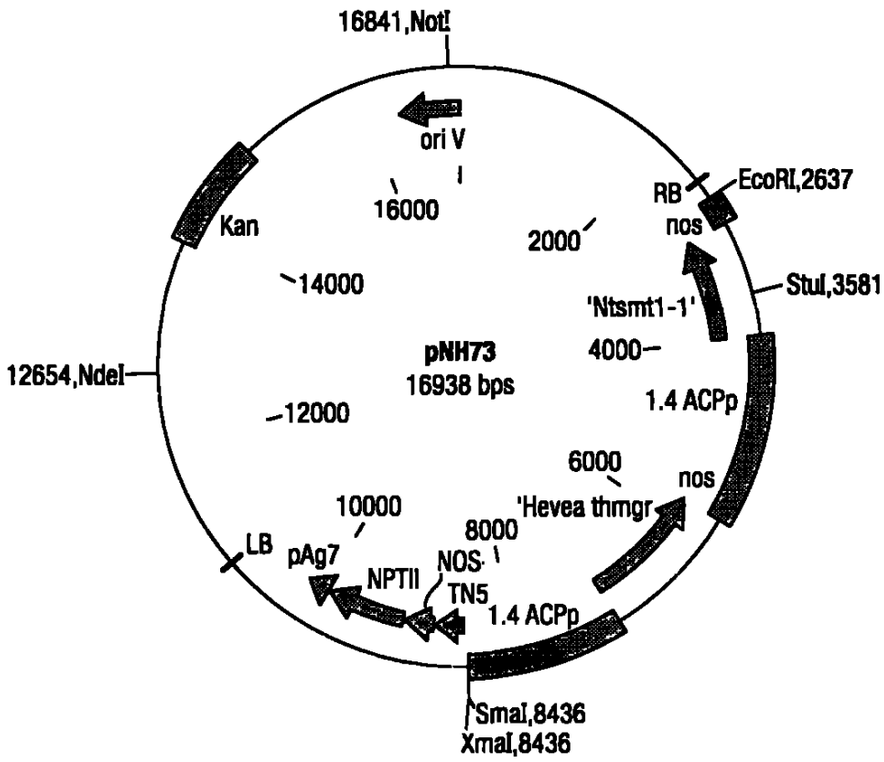


Fig.5.

Vector Binario pNH73



84 73

EL SUSCRITO JEFE DEL AREA DE LEGALIZACIONES DEL MINISTERIO DE RELACIONES EXTERIORES

C E R T I F I C A

Que revisados los registros que se llevan en la oficina de Legalizaciones aparece la firma del señora **ANGELA ALEXANDRA PULECIO MAYORGA,** como traductor e intérprete oficial para los idiomas **INGLES– ESPAÑOL, INGLES** según resolución 0177 del 11 de Marzo de 1997, del Ministerio de Justicia.

Dada en Santa Fe de Bogotá, D.C., a los veinte y dos (22) días de Diciembre de 2000

CARLOS GUILLERMO DIAZ BUITRAGO
Jefe Area de Legalizaciones

COMO NOTARIO SEXTO DE SANTAFE DE BOGOTA
HAGO CONSTAR
QUE LA FOTOCOPIA CONCIDE CON LA FOTOCOPIA AUTENTICA QUE HE TENIDO A LA VISTA
SANTAFE DE BOGOTA
9 FEB 2001
JUAN MANUEL BOTERO MEDINA
NOTARIO

NOTARIA 36-SANTAFE DE BOGOTA
Como Notaria 36 de este Circulo hago constar que este sello coincide con el documento original presentado para su autenticación
12 FEB 2001
MARIO TORRES VALDERRAMA
NOTARIO TREINTA Y SEIS

REPUBLICA DE COLOMBIA

TRIBUNAL SUPERIOR DEL DISTRITO JUDICIAL
DE SANTAFE DE BOGOTA D.C.

SALA GENERAL

ACUERDO Nro. 048

DILIGENCIA DE POSESION DE ANGELA ALEXANDRA PULECIO MAYORGA COMO TRADUCTOR E INTERPRETE OFICIAL DE LOS IDIOMAS:
INGLES - ESPAÑOL - ESPAÑOL - INGLES

EN SANTAFE DE BOGOTA D.C. A LOS SEIS (6) DIAS DEL MES DE MAYO DE MIL NOVECIENTOS NOVENTA Y SIETE (1997) COMPARECIO AL DESPACHO DEL SEÑOR PRESIDENTE DEL TRIBUNAL SUPERIOR DE SANTAFE DE BOGOTA, ANGELA ALEXANDRA PULECIO MAYORGA IDENTIFICADA CON LA CEDULA DE CIUDADANIA No. 38.872.323 EXPEDIDA EN BUGA CON EL FIN DE TOMAR POSESION DEL CARGO DE TRADUCTOR E INTERPRETE OFICIAL PARA EL CUAL FUE ADMITIDA MEDIANTE RESOLUCION NRO: 0177 DE MARZO ONCE (11) DE MIL NOVECIENTOS NOVENTA Y SIETE (1997), EMANADA DEL MINISTERIO DE JUSTICIA Y DEL DERECHO.

PARA TAL EFECTO, EL SEÑOR PRESIDENTE DE LA CORPORACION, LE RECIBIO EL JURAMENTO DE RIGOR POR CUYA GRAVEDAD PROMETIO CUMPLIR BIEN Y FIELMENTE CON SUS DEBERES.

SE FIRMA POR QUIENES EN LA DILIGENCIA INTERVINIERON PARA CONSTAR QUE LA COPIA QUE SE LE EXPIDE A LA INTERESADA [illegible] SU CALIDAD DE TRADUCTOR E INTERPRETE OFICIAL.

COMO NOTARIO SEXTO DE SANTAFE DE BOGOTA
QUE LA FOTOCOPIA CORRESPONDE A LA FOTOCOPIA
SANTAFE DE BOGOTA 0 FEB 2001
JUAN MANUEL SOTERO MEDINA
NOTARIO

RAMIRO TORRES LOZANO
PRESIDENTE

ANGELA ALEXANDRA PULECIO MAYORGA
POSESIONADA

NOTARIA 38-SANTAFE DE BOGOTA
Como Notario 34 de este Circulo hago
Constar que esta copia coincide con el
documento original presentado para
su autenticación
12 FEB 2001
MARIO TORRES VALDERRAMA
NOTARIO TREINTA Y SEIS

MARIA INES RODRIGUEZ TORRES
SECRETARIA

85 75

REPUBLICA DE COLOMBIA

MINISTERIO DE JUSTICIA Y DEL DERECHO

RESOLUCION NUMERO 0177 DE 19 11 MAR. 1997

Por la cual se expide una licencia para ejercer funciones de Traductor e Intérprete Oficial

LA SECRETARIA GENERAL DEL MINISTERIO DE JUSTICIA Y DEL DERECHO

en ejercicio de la facultad conferida por la Resolución 0601 del 30 de marzo de 1995, y

CONSIDERANDO:

Que **ANGELA ALEXANDRA PULECIO MAYORGA**, identificada con la cédula de ciudadanía No. 38.872.323 de Buga (Valle), solicita a este Ministerio le sea expedida licencia para ejercer funciones de Traductor e Intérprete oficial de conformidad con los Decretos 382 y 2257 de 1951.

Que la peticionaria ha acompañado a su solicitud los siguientes documentos:

a. Fotocopia autenticada de la cédula de ciudadanía No. 38.872.323 de Buga (Valle), en donde se establece la mayoría de edad.

b. Certificación expedida por el Jefe de la Oficina Jurídica del Ministerio de Educación Nacional, que acredita su idoneidad como traductor e Intérprete oficial en los idiomas **INGLES-ESPAÑOL-ESPAÑOL-INGLES.**

c. Tres declaraciones de personas que manifiestan que el solicitante es persona de buena conducta.

d. Fotocopia autenticada del certificado judicial registrado bajo el T.D. No. 38.872.323 expedido por el Departamento Administrativo de Seguridad, donde certifica que **ANGELA ALEXANDRA PULECIO MAYORGA** no tiene asuntos pendientes con las autoridades judiciales o de policía.

COMO NOTARIO TERCERO DEL CIRCULO
HAGO CONSTAR
QUE LA FOTOCOPIA COINCIDE CON LA COPIA AUTENTICA QUE HE TENIDO A LA VISTA
SANTAFE DE BOGOTA 19 FEB 200
JUAN JOSE ROTEPO MEDINA
NOTARIO

RESUELVE:

ARTICULO PRIMERO: Expedir licencia a **ANGELA ALEXANDRA PULECIO MAYORGA**, identificada con la cédula de ciudadanía No. 38.872.323 de Buga (Valle), para ejercer las funciones de Traductor e Intérprete Oficial en los idiomas ***INGLES- ESPAÑOL- ESPAÑOL-INGLES.***

Hoja 2 de la Resolución "Por la cual se expide una licencia para ejercer funciones de Traductor e Intérprete Oficial a **ANGELA ALEXANDRA PULECIO MAYORGA.**

ARTICULO SEGUNDO: Comunicar el texto de la Resolución a la Subsecretaria de Asuntos Administrativos del Ministerio de Relaciones Exteriores.

ARTICULO TERCERO: La interesada deberá registrar su firma y sello ante la Oficina de Autenticaciones del Ministerio de Relaciones Exteriores, e inscribir su nombre ante el Tribunal Superior del Distrito Judicial correspondiente al territorio donde vaya a desempeñarse.

ARTICULO CUARTO: La presente resolución rige a partir de la fecha de su expedición, y contra ella proceden los recursos de reposición y apelación los cuales deberán ser interpuestos dentro de los cinco días siguientes a su notificación.

NOTIFIQUESE, COMUNIQUESE Y CUMPLASE
Dada en Santa Fe de Bogotá, D.C. a

11 MAR. 1997

YOLANDA GOMEZ RESTREPO

LAR/mmc.

COMO NOTARIO SEXTO DEL CIRCULO
DE SANTAFE DE BOGOTA
HAGO CONSTAR
QUE LA FOTOCOPIA COINCIDE CON LA COPIA
AUTENTICA QUE SE TENDO A LA VISTA.
SANTAFE DE BOGOTA 10 FEB 2001
JUAN MANUEL ROTERO MEDINA
NOTARIO

85 Unilever
C-7573(V)
04100.340
77

TRADUCCION OFICIAL NO. 194-2001

Oficina de Patentes Europea

CERTIFICACIÓN

Los documentos anexos son copias exactas de la solicitud de patente Europea descrita en la siguiente página, de conformidad como fue presentada originalmente.

Solicitud de Patente No. 00310403.1

Por El Presidente de la Oficina de Patentes Europea

(Firma Ilegible)

I.L.C. HATTEN-HECKMAN

LA HAYA 22/10/01

Solicitud No. 00310403.1 Fecha de Presentación: 23/11/00

Solicitante: **UNILEVER PLC**

Londres EC4P 4BQ

REINO UNIDO

Título de la Invención: **Proceso para aumentar el nivel de esteroles en las plantas**

Prioridad Reivindicada: (En Blanco)

Clasificación Internacional de Patentes: **C12N15/82, C12N15/53, C12N15/54, C12N9/04, C12N9/10, AO1H5/00**

ANGELA A. PULECIO MAYORGA
Traductor é Intérprete Oficial
Resol No. 0177 del/97 MinJusticia

78

Estados Contratantes nombrados al momento de la presentación:

AT/BE/CH/CY/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE/TR

Observaciones: Para ver el título original de la solicitud, ver página 1 de la especificación.

Reivindicaciones:

1. El uso de un gen que comprende una HMG-reductasa inhibida sin-retroalimentación en combinación con un gen que expresa esterol metiltransferasa1 para aumentar el nivel de esteroles en las plantas.
2. El uso, de conformidad con la Reivindicación 1, donde el nivel de 4-desmetilesteroles es aumentado en las plantas por lo menos en un 10%.
3. El uso, de conformidad con la Reivindicación 1, donde los esteroles son aumentados en semillas, preferiblemente en semillas oleaginosas.
4. El uso, de conformidad con la Reivindicación 3, donde las semillas se originan del tabaco, canola, girasol, colza, soya o maní.
5. El uso, de conformidad con la Reivindicación 1, donde la HMG-reductasa inhibida sin retroalimentación es expresada por un gen HMG truncado no vegetal.
6. El uso, de conformidad con la Reivindicación 5, donde la HMG-reductasa expresado por el gen de HMG-reductasa carece de dominio de enlace de membrana..
7. El uso, de conformidad con la Reivindicación 1, donde la HMG-reductasa inhibida sin retroalimentación es expresada por un gen de HMG-reductasa de planta truncada.

ANGELA A. PULIDO MAYORGA
Traductor é Intérprete Oficial
Resol No. 0177 del/97 MinJusticia

8. El uso, de conformidad con la reivindicación 1, donde la HMG-reductasa reductasa puede derivarse de la *Asteraceae*.

9. El uso, de conformidad con la reivindicación 8, donde el gen HMGR puede derivarse de la *Hevea brasiliensis* o el gen HMGR es una versión truncada de un gen que puede derivarse de la *Hevea brasiliensis*.

10. El uso, de conformidad con la reivindicación 9, donde el gen HMGR es el gen hmg 1 derivado de la *Hevea brasiliensis* o una versión truncada de dicho gen.

11. Un método para transformar la planta mediante

A1) la transformación de una célula de una planta con un constructo de DNA recombinante que comprende un segmento de ADN que codifica un polipéptido con actividad HMGR inhibida sin retroalimentación y un polipéptido que codifica una actividad esterol metiltransferasa1 y promotores para dirigir la expresión de dichos polipéptidos en dicha célula de la planta para formar una célula transformada de planta; o

A2) re-transformación de una célula de planta que expresa una actividad HMGR inhibida sin retroalimentación con un gen que codifica una actividad esterol metiltransferasa1;

A3) re-transformación de una célula de planta que expresa una actividad esterol metiltransfersa1 con un gen que codifica una actividad HMGR inhibida sin retroalimentación; y

D) regeneración de las células transformadas de planta mencionadas en plantas transgénicas; y

E) selección de plantas transgénicas que tienen niveles mejorados de 4-desmetilesteroles comparadas con cepas de tipo silvestre de la misma planta.

ANGELA M. PULICIO MAYORGA
Traductor é Intérprete Oficial
Resol No. 0177 del/97 MinJusticia

12. Planta obtenible mediante un método de conformidad con la reivindicación 11.
13. Tejido de planta obtenido de una planta de conformidad con la reivindicación 12.
14. Tejido de planta de conformidad con la reivindicación 13, seleccionado del grupo de hojas, frutas y semillas.
15. Una planta que tiene incorporada en su genoma un gen heterólogo que codifica una actividad HMGR de polipéptido truncado en combinación con un gen heterólogo que codifica SMT1.

Es traducción fiel y completa, de la cual queda copia en el archivo del Traductor para su confrontación, a la cual me remito.

Bogotá, D.C. 3 de Diciembre de 2001

ANGELA A. PULECIO MAYORGA
Traductor é Intérprete Oficial
Resol No. 0177 del/97 MinJusticia

F 7573(V)
JOSE LLORENA CAMACHO & CO
Unilever
89 81

Europäisches Patentamt | European Patent Office | Office européen des brevets

Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

00310403.1

Der Präsident des Europäischen Patentamts:
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

I.L.C. HATTEN-HECKMAN

DEN HAAG, DEN
THE HAGUE, 22/10/01
LA HAYE, LE

Europäisches Patentamt | European Patent Office | Office européen des brevets

Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

Anmeldung Nr.: / Application no.: / Demande n°: 00310403.1

Anmeldetag: / Date of filing: / Date de dépôt: 23/11/00

Anmelder: / Applicant(s): / Demandeur(s):
UNILEVER PLC
London EC4P 4BQ
UNITED KINGDOM

Bezeichnung der Erfindung: / Title of the invention: / Titre de l'invention:
Process for increasing the level of sterols in plants

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat: / State: / Pays:	Tag: / Date: / Date:	Aktenzeichen: / File no. / Numéro de dépôt:

Internationale Patentklassifikation: / International Patent classification: / Classification internationale des brevets:
C12N15/82, C12N15/53, C12N15/54, C12N9/04, C12N9/10, A01H5/00

Am Anmeldetag benannte Vertragstaaten: / Contracting states designated at date of filing: / Etats contractants désignés lors du dépôt:
AT/BE/CH/CY/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE/TR

Bemerkungen: / Remarks: / Remarques:
See for original title of the application page 1 of the description

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Empfangsbescheinigung / Receipt for documents / [illegible]

[illegible]

UNILEVER N.V., PATENT DEPARTMENT

P.O. BOX 137

3130 AC VLAARDINGEN

THE NETHERLANDS

CORRECTED VERSION

THE PATENT OFFICE

23 NOV 2000

[illegible] BY FAX

Anmeldenummer / Application No. / N° de la demande		00310403.1	
Tag des Eingangs (Regel 24(2)) / Date of receipt (Rule 24(2)) / Date de réception (règle 24(2))	DREC	~~23 NOV 2000~~	24.11.00
Zeichen des Anmelders/Vertreters / Applicant's / Representative's ref. / Référence du demandeur ou du mandataire	AREF	F7573(V)	
Nur nach Einreichung der Anmeldung bei einer nationalen Behörde / Only after filing of the application with a national authority / Seulement après le dépôt de la demande auprès d'une autorité nationale			
Tag des Eingangs beim EPA (Regel 24(4)) / Date of receipt at EPO (Rule 24(4)) / Date de réception à l'OEB (règle 24(4))	RENA		

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PROCESS FOR MODIFYING PLANTS

Field of invention

The invention relates to a process for the modification of plants, more specifically a process for increasing the isoprenoid levels in plants.

Background of the invention

Many approaches have been suggested for modifying the isoprenoid production in plants.

Whereas only a few sterols exist in animals, with cholesterol being by far the major one, in plants a wide range of sterols are found. Structural variations between these arise from different substitutions in the side chain and the number and position of double bonds in the tetracyclic skeleton.

Plant sterols can be grouped by the presence or absence of one or more functionalities. For example they can be divided into three groups based on methylation levels at C4 as follows: 4-desmethylsterols or end product sterols, 4α-monomethylsterols and 4,4-di-methylsterols. Naturally occurring 4-desmethylsterols include sitosterol, stigmasterol, brassicasterol, Δ7-avenosterol and campesterol.

In most higher plants, sterols with a free 3β-hydroxyl group (free sterols) are the major end products. However sterols also occur as conjugates, for example, where the 3-hydroxy group is esterified by a fatty acid chain, phenolic

acids or sugar moieties to give sterol esters. For the purpose of this description the term sterol refers both to free sterols and conjugated sterols. However in this specification references to levels, amounts or percentages of sterol refer to the total weight sterol groups whereby the weight of the conjugating groups such as fatty acid, phenolic acid or sugar groups is excluded.

To date most studies aimed at manipulating sterols in plants have involved other than 4-desmethylsterols with the purpose of increasing resistance to pests or to fungicides.

WO 98/45457 describes the modulation of phytosterol compositions to confer resistance to insects, nematodes, fungi and/or environmental stresses, and/or to improve the nutritional value of plants by using a double stranded DNA molecule comprising a promoter, a DNA sequence encoding a first enzyme which binds a first sterol and produces a second sterol and a 3' non-translated region which causes polyadenylation at the 3' end of the RNA. Preferably the enzyme is selected from the group consisting of S-adenosyl-L-methionine-$\Delta^{24(25)}$-sterol methyl transferase, a C-4 demethylase, a cycloeucalenol to obtusifoliol-isomerase, a 14-α-demethylase, a Δ^8 to Δ^7- isomerase, a Δ^7-C-5-desaturase and a 24,25-reductase.

US 5,306,862 describes a method of increasing sterol accumulation in a plant by increasing the copy number of a gene encoding a polypeptide having HMG-CoA reductase activity to increase the resistance of plants to pests. Similarly US 5,349,126 discloses a process to increase the squalene and sterol accumulation in transgenic plants by increasing the amount of a gene encoding a polypeptide

having HMG-CoA reductase activity to increase the pest resistance of transgenic plants.

WO 97/48793 discloses a C-14 sterol reductase polypeptide for the genetic manipulation of a plant sterol biosynthetic pathway.

WO 96/09393 discloses a DNA sequence encoding squalene synthetase.

WO 97/34003 discloses a process of raising squalene levels in plants by introduction into a genome of a plant a DNA to suppress expression of squalene epoxidase.

WO 93/16187 discloses new plants containing in its genome one or more genes involved in the early stages of phytosterol biosynthesis, preferably the genes encode mevalonate kinase.

US 5,589,619 discloses accumulation of squalene in plants by introducing a HMG-CoA reductase gene to increase production of sterol and resistance to pests. Example 10 discloses increased squalene levels in the seeds of thes plants.

WO 00/08190 discloses a DNA sequence encoding a sterol methyltransferase isolated from *Zea mays*.

In plants, mevalonate synthesis via 3-hydroxy-3-methylglutaryl Coenzyme A reductase (HMGR) is one of the steps in isoprenoid biosynthesis.

Gondet et al in Plant Physiology (1994) 105:509-518 has isolated a tobacco mutant showing dramatically altered

sterol compositions in leaf tissue with significant increases in the proportion of cyclopropylsterols and HMGR activities increased by approximately 3-fold.

Re et al in The Plant Journal (1995) 7(5), 771-784 have shown that the over-expression of *Arabidopsis thaliana* HMG CoA reductase (HMG 1) is not sufficient to alter the bulk synthesis and accumulation of end products of the plant isoprenoid pathway.

Applicants believe that the reason for this is that the activity of HMGR in plants is subject to feedback inhibition by sterols. Some HMGR genes, however, are non-feed back inhibited. Examples of such genes are non-plant HMGR genes lacking the membrane-binding domain, such as the truncated hamster HMGR genes or the truncated *Saccharomyces cerevisiae* genes, and HMGR genes (or truncated versions thereof) from high isoprenoid producing plants such as *Hevea brasiliensis*.

A truncated hamster HMGR gene, lacking the membrane-binding domain, was expressed in tobacco plants under the control of the CaMV 35S promoter (Chappell et al., Plant Physiology (1995) 109: 1337-1343). This resulted in a 3- to 6- fold increase in total HMGR activity in leaf tissue.

Schaller et al in Plant Physiology (1995) 109:761-770 discloses the introduction of the *hmg1* gene from *Hevea brasiliensis* into tobacco leading to an enhanced sterol production, especially of cycloartenol, in leaf tissue.

Polakowski et al in Applied Microbial Biotechnology (1998) 59:66-71 describes the use of a truncated *Saccharomyces*

cerevisiae hmg 1 gene in yeast, leading to the accumulation of squalene.

In plants, 24-methylene cycloartanol production from cycloartenol via sterol methyltransferase1 (SMT1) is one of the steps in isoprenoid biosynthesis.

Bouvier-Navé et al in Eur. J. Biochem. 256, 88-96 (1988) describes two families of sterol methyl transferases (SMTs). The first (SMT1) applying to cycloartenol and the second (SMT2) to 24-methylene lophenol.

Schaller et al in Plant Physiology (1998) 118: 461-169 describes the over-expression of SMT2 from Arabidopsis in tobacco resulting in a change in the ratio of 24-methyl cholesterol to sitosterol in the tobacco leaf.

Diener et al in The Plant Cell (2000) 12: 853-870 describes the functional characterisation of an Arabidopsis SMT1 gene and show that mutants lacking the gene display poor growth and fertility.

Schaeffer et al in Lipids (2000) 35: 263-269 describe the effects of expressing *Nicotiana tabacum* SMT1 and SMT2 genes in transgenic tobacco. Overexpression of SMT1 results in variations in the level of cycloartenol and concomitant changes in the proportion of 24-ethyl sterols. Over expression of SMT 2 alters the ratio of 24-methyl cholesterol to sitosterol resulting in reduced growth.

Surprisingly it has now been found that expressing genes encoding specific HMG-reductase enzymes in combination with those encoding sterol methyltransferase1 can advantageously

be used to further increase the nutritional value of plants espacially in the seeds thereof.

Surprisingly it has been found that the use of non-feedback regulated HMGR in combination with overexpression of sterol methyltransferase1 leads to the further enhancement of nutritionally beneficial sterol for example in the seeds of said plants compared to plants where only one of the above genes has been expressed.

The present invention aims to modify sterol levels in plants, especially the seeds of plants whereby this modification can either involve an increase of the level of (beneficial) sterols or a decrease of the level of (less-desired) cholesterol.

The present invention aims to increase sterol levels in plants, whereby the sterols are preferably nutritionally attractive 4-desmethylsterols such as sitosterols, stigmasterols, brassicasterol, Δ7-avenosterol or campesterols and whereby the sterols are expressed in the seeds.

Statement of the invention

Accordingly the invention relates to the use of a gene expressing a SMT1 in combination with a non feedback inhibited HMGR gene to increase the level of sterols in plant tissue and/or decrease the level of cholesterol in plant tissue.

In another aspect, the invention relates to a modified plant having incorporated into its genome one or more genes

for increasing the expression of SMT1 and increasing the expression of non-feedback inhibited HMGR.

Detailed description of the invention

In higher plants, isoprenoids are a large family of compounds with diverse roles. They include sterols, the plant hormones gibberellins and abscisic acid, components of photosynthetic pigments, phytoalexins and a variety of other specialised terpenoids.

Sterols, especially 4-desmethylsterols are of interest because they contribute to the nutritional quality, flavour and colour of fruits and vegetable oils. Of particular interest are isoprenoid compounds of nutritional benefit such as fat-soluble sterols. These may be efficacious in reducing coronary heart disease, for example, some phytosterols have been shown to lower serum cholesterol levels when increased in the diet and vitamin E reduces atherosclerotic plaques via decreased oxidation of LDL.

Expression of such compounds in plant seeds in particular in oilseeds is commercially advantageous as generally the harvesting of such ingredients from seeds is very convenient and, in some instances, it may be possible to extract the oil in combination with the sterols from the seed, leading to an oil containing elevated levels of sterol without or with the reduced need for separate addition of sterols.

Preferred sterols are 4-desmethylsterols, most preferred sitosterol, stigmasterol, brassicasterol, avenosterol and campesterol. Also preferably, at least part of the sterols, for example at least 70 wt% based on the total of the

sterols in the seed are esters of sterols with C10-24 fatty acids. In a very preferred embodiment the sterols comprise C10-24 esters of 4-desmethylsterols.

As discussed above, several approaches have been suggested to alter levels of isoprenoids in plants.

It has now been found that for the enhancement of isoprenoid levels in plants particularly in the seeds thereof an even more preferred route is to use a non-feedback inhibited HMGR gene in combination with sterol methyltransferase1. The use of such a combination of genes is especially advantageous to enhance the levels of 4-desmethylsterols, more so than expression of either gene singularly. Even more preferred, the use of such genes enhances the level of stigmasterol, sitosterol and campesterol in seeds. Also the use of such genes is especially advantageous to enhance the levels of isoprenoids in oilseeds containing more than 10 wt% based on dry weight of triglycerides.

In a first embodiment of the invention the non-feed back inhibited HMG reductase is an enzyme which is expressed by a truncated non-plant HMGR gene, said truncation preferably leading to an enzyme lacking the membrane binding domain, but whereby the HMGR functionality of the gene is preferably maintained. Examples of such genes are the truncated hamster or yeast HMGR genes.

A second -preferred- embodiment of a non-feedback inhibited HMG reductase is an enzyme expressed by HMGR genes from high isoprenoid producing plants such as *Hevea brasiliensis*. Especially preferred are truncated versions of HMGR produced by genes from high isoprenoid producing

plants such as *Hevea brasiliensis*, most preferred truncated versions are used whereby said HMGR lacks the membrane binding domain.

The intact HMGR enzyme comprises three regions: a catalytic region, containing the active site of the enzyme, a membrane binding region, anchoring the enzyme to the endoplasmic reticulum and a linker region joining the catalytic and membrane binding regions of the enzyme. The membrane-binding domain occupies the N-terminal region of the enzyme, whereas the catalytic region occupies the C-terminal region. It is believed that feedback inhibition in most plants generally requires the presence of the membrane-binding region of the enzyme. Therefore a preferred embodiment of the invention relates to the use of an HMGR gene expressing an enzyme with an inactivated or without a membrane binding domain, whereby said gene is preferably used to increase the level of 4-desmethylsterols in plant tissue such as the seeds of plants.

An example of HMG reductase with an inactivated or without a membrane binding domain is the HMG reductase expressed by the truncated hamster HMGR gene as described by Chappell (see above). The truncation is believed to remove the membrane binding domain from the HMG reductase whereafter a significant reduction of feedback inhibition occurs. Other truncated or mutated genes whereby the membrane binding domain is removed or inactivated can equally be used. An example of this is the truncated HMGR gene as used by Polakowski (see above).

Preferred examples of HMG reductases are those expressed by HMGR genes obtained from plants which naturally have the tendancy to develop high levels of isoprenoids such as for

example triterpenes and rubber. Examples of such plants are *Asteraceae*, especially *Euphorbiaceae*. Therefore another preferred embodiment of the invention relates to the use of an HMGR gene isolated from *Asteraceae* to increase the level of sterols, particularly 4-desmethylsterols in plant tissue, particularly the seeds of plants. Preferably the HMGR gene is isolated from *Hevea brasiliensis*. Especially preferably truncated versions of such plant genes may be used. A specific promoter can be inserted into the plant genome to ensure that the HMGR gene is upregulated, preferably within the seed tissue of the plant.

Suitably the SMT1 gene can be naturally present in the plant. In accordance to the invention the circumstances are then altered such that increased expression of SMT1, preferably in the seed region of the plant will take place. Possible ways to do this may be to upregulate facilitating molecules e.g. such as transcription factors. Alternatively, a specific promoter can be inserted into the plant genome to ensure that the SMT1 gene is upregulated. Alternatively, the copy number of the "homologous" SMT1 gene may be increased to increase the expression thereof.

Alternatively, the SMT1 gene can be a heterologous gene, for example derived from other plant or microbial sources. For example, the SMT1 gene may be derived from Arabidopsis, tobacco or yeast.

Cholesterol is a less desired component of food products because consumers have a desire to reduce their cholesterol consumption. It is believed that reduced serum cholesterol levels lead to a reduced risk of cardiovascular disease. Therefore, in one embodiment the invention relates to the

reduction of the cholesterol level in plant tissue, especially the seeds of plants.

As discussed above, several approaches have been suggested to alter the levels of isoprenoids and/or cholesterol in plants. It has now been found that for the enhancement of isoprenoid levels in seeds a preferred route is to use a SMT1 gene. The use of such genes is especially advantageous to enhance the levels of 4-desmethylsterols, even more preferred the level of stigmasterol, sitosterol, brassicasterol, isofucosterol and campesterol in seeds. Also, the use of such genes is especially advantageous to enhance the levels of isoprenoids in oilseeds containing more than 10 wt% based on dry weight of triacylglycerols.

The invention also provides a method of transforming a plant by

A1) transforming a plant cell with a recombinant DNA construct comprising a DNA segment encoding a polypeptide with non feedback inhibited HMGR activity and a polypeptide encoding a sterol methyltransferase1 activity and promoters for driving the expression of said polypeptides in said plant cell to form a transformed plant cell; or

A2) re-transforming a plant cell expressing a non-feedback inhibited HMGR activity with a gene encoding a ster l methyltransferase1 activity; or

A3) re-transforming a plant cell expressing a sterol methyltransferase1 activity with a gene encoding a non-feedback inhibited HMGR activity; and

B) regenerating the above transformed plant cells into transgenic plants; and

C) selecting transgenic plants that have enhanced levels of 4-desmethylsterols compared to wild type strains f the same plant.

DNA segments encoding non-feedback inhibited HMGR or sterol methyltransferase1, for use according to the present invention, may suitably be obtained from animals, microbial sources or plants. Alternatively, equivalent genes could be isolated from gene libraries, for example by hybridisation techniques with DNA probes.

The invention will now further be illustrated in the following examples:

Example 1: Co-expression of *Hevea brasiliensis hmg1* and *Nicotiana tabacum* SMT1 in plants

E. coli strain DH5□ (Gibco BRL) was used as the host strain in all cloning and sub-cloning procedures. Binary vector pSJ34 (PCT/EP/00/09374) was created by filling in the *Bam*HI site of pGPTV-Kan[Becker et al Plant Mol Biol (1992) 20:1195-97], between the selectable marker and the p(A)g7 3'-end, with Klenow enzyme. The construction of plasmids pNH6 and pNH8 have been described in our non-pre-published patent applications PCT/EP00/09374 and EP 00303193.7 respectively. Bacteria were cultivated in LB medium (10 g/l tryptone, 5g/l yeast extract, 5 g/l NaCl) supplemented with the appropriate selection pressure (ampicillin 100 µg/ml or kanamycin 50 µg/ml) on a rotary shaker (210 rpm) at 37 °C.

Restriction endonucleases, T4 DNA ligase, shrimp alkaline phosphatase and molecular markers (X, XIV and XVII) were purchased from Roche. The enzymes were used according to the suppliers' recommendations. All chemicals and reagents used were of analytical grade and available from Fisher Scientific UK, Sigma or BDH. The following oligonucleotide primers were used: F72, 5'-GCC ATA ATA CTC GAA CTC AG-3'; 35S, 5'-TCC ACT GAC GTA AGG GAT GAC-3'; CERV1S, 5'-GTC TGT CTA AAG TAA AGT AGA TGC G-3'; NOSAS, 5'-CCG GCA ACA GGA TTC AAT CTT-3'.

The Qiagen mini prep kit was used to obtain plasmid DNA f r sequencing and sub-cloning procedures. The Qiagen gel extraction kit was used to purify DNA from agarose gels. Plasmid pNH6 was digested with *Xma*I and *Eco*RI and plasmid

pNH8 with *Xma*I and *Sal*I releasing the CERV-*Nt*sm*t*1-NOS and double CaMV35S-*Hevea hmg*1-TRBCS cassettes, respectively. The digestion reactions were separated in an agarose gel and the expression cassettes were excised and purified. Binary vector pSJ34 was digested with *Eco*RI and *Sal*I, purified and subsequently treated with shrimp alkaline phosphate to remove the terminal phosphate groups. Using a three-way ligation, both expression cassettes were inserted into pSJ34 resulting in pNH9 (Figure 1). First PCR, using gene specific primers, and second restriction enzyme digestion was used to select positive clones. Positive clones were sequenced confirming the integrity of the junctions between transgene and terminator.

Transformation of tobacco with binary vectors

Electrocompetent *Agrobacterium tumefaciens* cells (strain LBA4404) were defrosted on ice and 5ng of vector plasmid added. Cells plus plasmid were then placed into a pre-chilled electroporation cuvette and electroporated in a Bio Rad Gene Pulser at a capacitance of 25µF and at 600 ohms. Immediately after electroporation 950µl of 2X TY broth was added, the cells mixed gently and placed in a sterile vial. The cells were shaken at 28°C for 2 hours and 25µl aliquots plated on solid Lennox media containing rifampicin 50µg/ml and kanamycin 50µg/ml and incubated at 28°C for 3 days. Single colonies were used to inoculate 10µl of water (for PCR confirmation) and 500µl of Lennox media containing rifampicin 50µg/ml and kanamycin 50µg/ml.

PCR positive cultures were used to inoculate a 10 ml of Lennox media broth containing rifampicin 50µg/ml and

kanamycin 50µg/ml. The overnight culture was spun down at 3000g and resuspended in an equal volume of MS media (3% sucrose). Leaf segments were cut from young tobacco leaves from plants grown in tissue culture. Segments were placed directly into the agrobacterium solution and left for 10 minutes. The segments were then removed and placed upper surface down on feeder plates (10 per plate) and left for 2 days in low light at 22°C. The leaf segments were placed, upper surface up, on tobacco shooting media with hormones containing cefotaxime 500µg/ml and kanamycin 50µg/ml and placed in a growth room at 24°C with a 16hrs light / 8 hrs dark regime. Three weeks later, the callusing segments were transferred to Magenta tubs containing tobacco shooting media. Once formed, shoots were excised and placed on tobacco shooting media containing cefotaxime 500µg/ml and kanamycin 50µg/ml without hormones, to root. Rooted plants were then potted up into a 50% perlite / 50% compost mixture and placed in a propagator. After 1 week the plants were removed from the propagator and subsequently potted up into 5 inch pots. Once flowering had began paper bags were placed over the flowers to prevent cross pollination. When flowering had finished and pods formed the bags were removed and mature pods harvested. Mature leaves and seed from dry pods were harvested and stored for subsequent analysis.

Sterol Analysis

For sterol analysis, the plant tissue obtained as above is freeze-dried, then ground to a fine powder. 250 µl of 0.2 % w/v dihydrocholesterol dissolved in chloroform is pipetted into a screw-top septum vial. After removal of solvent, an amount of the plant tissue (50 mg) is added to the vial,

and total lipid extracted with 5 ml of a 2:1 v/v mixture of chloroform: methanol. The vial is capped and placed in a hot block maintained at 80-85°C. After 30 minutes the contents are filtered and the vial is washed out with a second 5ml aliquot of the chloroform: methanol mixture. The contents of the vial are filtered once more and the filtrates combined. The solvent portion of the filtrate is blown off using a stream of nitrogen gas to isolate the lipid residue.

The lipid fraction is then subjected to transmethylation by heating at 80-85°C in 1 ml of toluene and 2 ml of 0.5N sodium methoxide in methanol. After 30 minutes, 2 ml of a 14 % boron trifluoride solution in methanol is added and heated for a further 10 minutes at 80-85°C. After cooling, 2-3 ml of diethyl ether followed by 5 ml of deionised water are added. The ether fraction is removed and a further ether extraction carried out. The ether fractions are combined, backwashed with approx. 5 ml of water and dried overnight over anhydrous sodium sulphate. The ether phase is filtered and the solvent removed using a stream of nitrogen gas.

Sterols are dissolved in 300-400 µL of toluene and silylated by the addition of 200 µl of 95:5 N,O-bis(trimethylsilyl)acetamide:trimethylchlorosilane followed by incubation at 50°C for 10 minutes. GC analysis is carried out using a 25 m x 0.32 mm i.d. (0.25 µm film thickness) 5% BPX5 column (ex SGE) in a Perkin-Elmer 8420 GC. The temperature program is 180-240°C at 10°C/min, followed by 240-355°C at 15°C/min. and, finally, 5 min. at 355°C. The FID temperature is 380°C and the helium pressure 10 psi. A volume of 1.0 µl is injected onto the column. A GC response factor of 1.0 for each of the sterols with

respect to the dihydrocholesterol internal calibrant is assumed.

Table 1 shows the sterol analysis of leaf samples obtained from tobacco transformed with the NH9 vector co-expressing a full length Hevea HMGR and tobacco SMT1. Leaves from 12 independent transgenic plants (NH9) were analysed along with leaves from 6 independant untransformed plants (SR1) which had been generated via tissue culture, and leaves from 5 independent plants transformed with control vector lacking the gene of interest (pVEC). The total sterol content of the SR1 control leaves ranged from 0.165 - 0.268% dry weight and those of the pVEC controls from 0.175% - 0.269% dry weight. The NH9 transgenic leaves contained total sterol contents ranging from 0.176 - 0.318% dry weight, representing increases of up to 36.4% over the mean SR1 sterol content and 45.6% over the mean of 'beneficial' 4-desmethylsterols (4-desmethylsterols minus cholesterol). Also of note are the dramatically reduced levels of cholesterol in the NH9 samples, with 6 of the 12 samples having zero (or below detection) levels of cholesterol.

Table 2 shows the sterol analysis of mature seed samples from tobacco transformed with the NH9 vector co-expressing full length Hevea HMGR and tobacco SMT1. Seeds from 27 independent transgenic plants (NH9) were analysed along with seeds from 12 SR1 and 6 pVEC control plants. Seeds from the control SR1 plants contained total sterol contents ranging from 0.339 - 0.425% dry weight and those of the pVEC control plants from 0.301 - 0.413% dry weight. Seeds from the NH9 transgenic plants contained total sterol contents ranging from 0.307 - 0.545% representing increases of up to 42.0% over the mean SR1 control total sterol value

and up to 51.6% over the mean SR1 beneficial 4-desmethylsterol value. Significant decreases in the level of cycloartenol, the substrate for the sterol methyl transferase1, were found in the high sterol NH9 samples. Cholesterol levels in the high sterol NH9 samples were also significantly reduced. Of particular note are the higher levels of sitosterol in the high sterol NH9 lines compared to control levels.

Example 2: Co-expression of a truncated form of *Hevea brasiliensis hmg1* and *Nicotiana tabacum* SMT1 in plants

A truncated form of *Hevea* HMGR, lacking the N-terminal membrane-binding domain, was cloned using the *Hevea brasiliensis hmg1* as template. The *Hevea brasiliensis* (H.B.K.) Müll. Arg. *thmg1* was cloned using the primers based on the published sequence [Chye et al (1991) Plant Mol Biol 19: 473-84]. The forward primer 5'-CCTACCTCGGAAGCCATGGTTGCAC-3' incorporates a new start codon (bold) and a *Nco I* restriction site (underlined) for cloning applications. The reverse primer 5'-CATTTTACATTGCTAGCACCAGATTC-3' contains a *Nhe I* restriction site (underlined) for downstream sub-cloning purposes. The plasmid pNH8 was used as the template DNA in the PCR (30 cycles) using *Pfu* polymerase under standard conditions and produced a fragment of the expected size ~1.3 kb. The resulting *thmg1* gene codes for amino acids 153-575 of the full-length (575) *hmg1* sequence (Fig. 11b of PCT/EP/00/09374). The *thmg1* PCR product was cloned into the pGEM-T vector (Promega) according to the manufacturers' instructions and sequenced to confirm fidelity. The *H. brasiliensis tHMG1* was inserted into pNH4 (see PCT/EP/00/009374 between the *Nco I* and *Nhe I* sites of the polylinker, which lie between the CaMV 35S double promoter and nos terminator, giving pMH3 (see PCT/EP/00/09374. This chimaeric gene was isolated by digestion with *Xma CI* and *Sal I*, purified and cloned into the corresponding polylinker sites in pNH9, after removal of the chimaeric full length *hmg1* gene which previously occupied these sites, and subsequent purification of the binary vector.

The binary vector pNH9 also contains the *smt1* gene cloned from *Nicotiana tabacum*, which is under transcriptional control of the CERV viral promoter. This binary construct was named pMH7 (Fig. 2). As described in Example 1, binary vectors were transformed into *Agrobacterium tumefaciens* and these were subsequently used to transform tobacco.

Table 3 shows the sterol analysis of leaf samples obtained from tobacco transformed with the MH7 vector co-expressing the truncated *Hevea* HMGR and tobacco sterol methyltransferase1 (SMT1). Leaves from 32 independent transgenic plants (MH7) were analysed along with 4 untransformed SR1 controls and 4 vector controls (SJ34). The total sterol content of the SR1 control leaves ranged from 0.141 - 0.221% dry weight and those of the SJ34 vector control plants from 0.183 - 0.330%. The total sterol content of the MH7 transgenic plants ranged from 0.142 - 1.339% dry weight representing increases of up to 7.2-fold over the mean SR1 control value. The beneficial 4-desmethylsterol contents of the MH8 seeds were increased by up to 3.9-fold over the mean SR1 control value.

Table 4 shows the sterol analysis of mature seed samples obtained from tobacco transformed with the MH7 vector co-expressing the truncated *Hevea* HMGR and tobacco sterol methyltransferase1. Seeds from 29 independent transgenic plants (MH7) were analysed along with 9 SR1 untransformed control plants and 6 vector control plants (SJ34). Seeds from the SR1 control plants show total sterol contents ranging from 0.393 - 0.445% dry weight and those from the SJ34 vector control plants from 0.334 - 0.413% dry weight. Seeds from the MH7 plants showed total sterol contents ranging from 0.379 - 0.987% dry weight, representing

increases of up to 2.4-fold over the mean SR1 control value. The beneficial 4-desmethylsterol content of the MH8 seeds was increased by up to 1.9-fold over the mean SR1 control value. The absolute levels of the 4-desmethylsterols isofucosterol, sitosterol and campesterol were substantially enhanced in the oil control to control values. Percentage cholesterol levels were reduced by up to 73% compared to mean SR1 control values. The increase in 'beneficial' 4-desmethylsterols obtained by co-expression of truncated *Hevea* HMGR and SMT1 is greater than the corresponding increase obtained by expression of the truncated *Hevea* HMGR alone (see our non-pre-published patent applications PCT/EP00/09374 and EP 00303193.7).

Further analysis of two high sterol seed samples (MH7 53 and MH7 32) was carried out to determine the proportion of free and esterified sterol. The total lipid fraction is isolated as described in Example 2, but not subjected to the transmethylation process. The lipid residue, which contains dihydrocholesterol as internal standard, is dissolved in 40-60 petroleum ether (250 µL) and applied to a glass-backed 20 cm x 20 cm x 0.5 mm silica gel thin layer chromatography (TLC) plate. The vial that contained the lipid residue is washed out with a further 250 µL aliquot of petroleum ether, which is also applied to the plate. A 10 µL aliquot of a solution consisting of a mixture of β-sitosterol (10 mg) and cholesterol oleate (10 mg) dissolved in acetone (1 mL) is spotted to act as a marker. The plate is developed using 60-80 petroleum ether-diethyl ether-acetic acid (80:20:2, v/v/v). The sterol fractions are visualised by spraying with a 0.01 % w/v ethanolic solution of rhodamine 6G and viewing the plate under UV light. Approximate R_f values are 0.25 for free sterols and 0.9 for

steryl esters. The free sterol band is scraped off the plate and transferred to a vial. The free sterol fracti n is isolated by washing the band with three volumes of diethyl ether. The ether washings are combined and filtered. The free sterol fraction, isolated by blowing off the solvent with nitrogen gas, is silylated and analysed by gas chromatography (GC) as described in Example 1. Amounts of esterified sterol are determined by subtracting amounts of free sterol from total sterol, the latter being determined by transmethylation (see Example 1).

Table 5 shows the analyses of the free sterol and sterol ester fractions of transgenic MH7 seed samples 32 and 53, alongside that of an SR1 control sample. The additional sterol present in the transgenic samples compared to the control is primarily in the form of sterol esters. The total sterol content of the SR1 control is 0.388% dry weight, of which 52.4% is in the form of esters. The total sterol contents of MH7 32 and 53 are 0.965% and 0.987% dry weight respectively, of which 77.2% and 75.0% respectively are esterified.

Example 3: Co-expression of a truncated form of *S. cerevisiae* HMGR1 and *N.tabacum* SMT1 in plants

Saccharomyces cerevisiae NCYC 957, X2180, SUC2 was grown in liquid media (12% (w/v) glucose, 2% (w/v) Bactopeptone, 1% (w/v) yeast extract, pH 4.0) on a rotary shaker (125 rpm), at 30°C. Cells were harvested by centrifuging 50 ml of culture at 4,500 rpm for 10 minutes. To the cell pellet, 4 ml of buffer (50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 100 mM EDTA, 1% SDS) was added and heated at 60°C for 15 minutes. 40 µl RNase (1 mg/ml) and 40 mg Proteinase K were then added to the mixture prior to heating at 50°C for 15 minutes. The DNA was extracted twice with phenol/chloroform and once with chloroform. The aqueous layer was added to 0.7 volumes of isopropanol and 3 M sodium acetate, pH 5.2, incubated at room temperature for 1 minute and centrifuged at 13,000 rpm for 10 minutes. The supernatant was removed and 500 µl 70% ethanol was added to the DNA pellet and re-centrifuged. The ethanol was removed and the DNA air dried for 60 minutes. The DNA pellet was suspended in 100 µl TE buffer and the absorbance at 260nm measured and the DNA quantified. The DNA was diluted to 0.5µg/µl and frozen.

Based on the nucleotide sequence of cosmid 8248 from the *S. cerevisiae* chromosome XIII sequencing project, primers were designed to clone the *tHMG1* gene by polymerase chain reaction. The forward primer 5'-GCTTGGATAAGG **CCATG**GGTCCTTTAG-3' incorporates a new start codon (bold) and a *Nco I* restriction site (underlined) for cloning purposes. The reverse primer 5'-GAATA CCAATGAGCTCTGACTAAG-3' contains a *Sac I* restriction site (underlined) for sub-cloning applications. Prior to PCR the

genomic DNA from *S. cerevisiae*, NCYC 957, X2180, SUC2, mal, gal2, CUA was digested with *Eco RI* and the DNA fractionated on a 0.7 % agarose gel. DNA fragments ~2.0 kb in size were excised from the gel and purified using the Qiagen QIAquick gel extraction kit, according to the manufacturers protocol. This DNA was used as the template in the subsequent PCR. The PCR (35 cycles) was performed using *Taq* and *Pfu* polymerase (3:1) under standard conditions and produced a DNA fragment of the expected size ~1.4 kb. The resulting *tHMGR1* gene codes for amino acids 598-1054 of the full length (1054) *HMGR1* sequence (see Fig. 12b of PCT/EP/00/09374). The *tHMG1* PCR product was cloned into the pGEM-T vector (Promega) according to the manufacturers' instructions and sequenced to confirm fidelity.

The *S. cerevisiae tHMG1* was inserted into pNH4 between the *Nco I* and *Sac I* sites of the polylinker pMH4. This chimaeric gene was isolated by digestion with *Xma CI* and *Sal I*, purified and cloned into the corresponding polylinker sites in pNH9 as described previously for the *H. brasiliensis thmg1* chimaeric gene, to create the binary plasmid pMH8 (Fig. 3). Both pMH3 and pMH4 (see PCT/EP/00/09374) were sequenced to check that the *HMG1* genes had been inserted correctly and there were no mistakes in the promoter-initiation and terminator sequences. As described in Example 1, binary vectors were transformed into *Agrobacterium tumefaciens* and these were subsequently used to transform tobacco.

Table 6 shows the sterol analysis of mature seed samples obtained from tobacco plants transformed with the MH8 vector expressing the truncated *S.cerevisiae* HMGR and

tobacco SMT1 genes. Seeds from 23 independent transgenic plants (MH8) were analysed along with seeds from 4 SR1 control and 4 SJ34 vector control plants. The total sterol content of seeds from the SR1 control plants ranged from 0.363% - 0.428% (average = 0.388%) and those from the vector control plants from 0.213 - 0.428%. The total sterol content of the MH8 transgenic seeds ranged from 0.251% - 0.526% representing increases of up to 35% over the SR1 average. The 4-desmethylsterol content of the MH8 seeds was increased by up to 41% compared to the SR1 average.

Example 4: Re-transformation of ACP - Ntsmt-1 transgenic tobacco plant #27 with an N-truncated form of *Hevea brasiliensis* hmg1 gene driven by a constitutive promoter.

Nicotiana tabacum plants (NH19 series) transformed with the *N. tabacum* Ntsmt-1 gene (SMT1) were generated as described in EP 00303193.7 Seeds from NH19 plant #27 EP 00303193.7 were germinated on MS agar containing 25mg/L hygromycin. From the resulting seedlings, leaf segments were cut and transformed with 35S - truncated Hevea HMGR construct (MH 5, PCT/EP/00/09374) as described hereabove.

Example 5: Re-transformation of ACP - Ntsmt 1 transgenic tobacco plant 27 with an N-truncated *Hevea brasiliensis hmg1* gene driven by a seed-specific promoter (MH15)

Nicotiana tabacum plants (NH19 series) transformed with the *N. tabacum* Ntsmt-1 gene (SMT1) were generated as described in EP 00303193.7. Seeds from NH19 plant #27 EP 00303193.7 were germinated on MS agar containing 25mg/L hygromycin. From the resulting seedlings, leaf segments were cut and transformed with the truncated *Hevea hmg1* gene driven by the ACP promoter (MH15, as in PCT/EP/00/09374) as described hereabove.

Example 6: Re-transformation of a *Hevea brasiliensis* truncated *hmg1* transgenic tobacco plant with *Nicotiana tabacum* sterol methyltransferase1 (SMT1)

Nicotiana tabacum plants (MH5 series) transformed with a truncated Hevea *hmg1* gene were generated as described in PCT/EP/00/09374. Seeds from MH 5 plants #6 and 33 were germinated on MS containing 50mg/L kanamycin. Leaf segments were cut from the resulting seedlings and transformed with the tobacco SMT1 gene (NH19 - construction described in EP00303193.7) as described in Example 1.

Example 7: Co-transformation of *Brassica napus* (oil seed rape) with a truncated form of *Hevea brasiliensis* hmg1 and *Nicotiana tabacum* SMT 1

Electrocompetent *Agrobacterium tumefaciens* cells (strain LBA4404) were defrosted on ice and 5ng of pMH7 plasmid (see Example 2) added. Cells plus plasmid were then placed into a pre-chilled electroporation cuvette and electroporated in a Bio Rad Gene Pulser at a capacitance of 25µF and at 600 ohms. Immediately after electroporation 950µl of 2X TY broth was added, the cells mixed gently and placed in a sterile vial. The cells were shaken at 28°C for 2 hours and 25µl aliquots plated on solid Lennox media containing rifampicin 50µg/ml and kanamycin 50µg/ml and incubated at 28°c for 3 days. Single colonies were used to inoculate 10µl of water (for PCR confirmation) and 500µl of Lennox media containing rifampicin 50µg/ml and kanamycin 50µg/ml.

Seeds were surface sterilised in 1% sodium hypochlorite for 20 mins. The seeds were washed in sterile distilled water 3 times and plated at a density of 10 seeds per plate on MSMO with 3% sucrose pH 5.8. Seeds were germinated at 24°C in a 16 h light / 8 h dark photoperiod. After 3-4 days, the cotyledons, including 2mm of petiole, were excised. Care was taken to remove the apical meristem and to keep the cotyledon out of the medium. The excised cotyledons were placed on MS medium, 3% sucrose and 0.7% agar with 20 µM 6-benzylaminopurine (BAP). Petioles with attached cotyledons were embedded in this medium to a depth of approximately 2mm at 10 per plate.

For transformation, individual excised cotyledons were taken from the plates and the cut surface of their petiole immersed into the agrobacterium suspension for a few seconds. They were then returned to the MS plates and co-cultivated with the agrobacterium for 72 h. After co-cultivation, the cotyledons were transferred to regeneration medium (MS medium with 20μM BAP, 3% sucrose, 0.7% agar, pH 5.8 with 400mg/l augmentin and 20 mg/l kanamycin sulphate). The petioles were, as before, embedded to a depth of 2mm at a density of 10 explants per plate, and again the cotyledon was kept out of the medium. After 2 or 3 weeks, shoots had appeared, some of which bleached by the fourth week, the remaining green shoots were sub-cultured onto shoot elongation medium (regeneration medium minus BAP). After 1 or 2 weeks, when apical dominance had been established, the shoots were transferred to rooting medium [MS medium, 3% sucrose, 0.7% agar and 400mg/l augmentin and 25mg/L kanamycin sulphate]. As soon as a small root mass was obtained, the plantlets were transferred to potting mix supplemented with fertiliser granules. The plants were grown in a propagator (average humidity 75%) for 2-3 weeks at 24°C, 16h light / 8h dark photoperiod. After 3 weeks the plants were transferred to the glasshouse and allowed to flower and set seed.

Table 1
Sterol Analysis of Leaf from Tobacco transformed with Hevea HMGR + N. tabacum SMT 1 (NH9)

Total sterols as % of smpl wt

Smpl code	squalene	cycloart	24mca	24mdoph	24doph	d7-aven	isofuc	sito	stig	camp	chol	Total
NH9 36	0.0000	0.0061	0.0000	0.0000	0.0068	0.0000	0.0135	0.0700	0.1077	0.1086	0.0041	0.316
NH9 37	0.0000	0.0225	0.0000	0.0127	0.0000	0.0000	0.0343	0.0262	0.0811	0.0834	0.0284	0.260
NH9 40	0.0000	0.0072	0.0000	0.0055	0.0000	0.0000	0.0187	0.0227	0.0802	0.0855	0.0202	0.251
NH9 22	0.0000	0.0000	0.0000	0.0103	0.0000	0.0000	0.0100	0.0488	0.0857	0.0891	0.0000	0.252
NH9 11	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0093	0.0473	0.0963	0.0863	0.0000	0.249
NH9 7	0.0000	0.0067	0.0000	0.0113	0.0000	0.0000	0.0228	0.0228	0.0732	0.0842	0.0269	0.248
NH9 30	0.0000	0.0000	0.0000	0.0028	0.0000	0.0000	0.0077	0.0410	0.0891	0.1022	0.0033	0.245
NH9 28	0.0000	0.0000	0.0000	0.0036	0.0000	0.0000	0.0108	0.0398	0.0891	0.0962	0.0029	0.241
NH9 21	0.0000	0.0000	0.0000	0.0024	0.0000	0.0000	0.0101	0.0416	0.0787	0.0871	0.0000	0.221
NH9 31	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0043	0.0360	0.0767	0.0816	0.0000	0.201
NH9 18	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0035	0.0259	0.0694	0.0838	0.0000	0.182
NH9 25	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0026	0.0161	0.0758	0.0783	0.0000	0.176
SR1 6	0.0000	0.0088	0.0000	0.0036	0.0000	0.0000	0.0212	0.0303	0.0880	0.0958	0.0204	0.268
SR1 7	0.0000	0.0062	0.0000	0.0018	0.0000	0.0000	0.0158	0.0267	0.0851	0.0959	0.0202	0.250
SR1 9	0.0000	0.0000	0.0000	0.0057	0.0000	0.0000	0.0244	0.0377	0.0783	0.0838	0.0206	0.249
SR1 8	0.0000	0.0031	0.0000	0.0032	0.0000	0.0000	0.0161	0.0258	0.0797	0.0880	0.0189	0.239
SR1 10	0.0000	0.0070	0.0000	0.0045	0.0000	0.0000	0.0154	0.0173	0.0802	0.0807	0.0200	0.225
SR1 3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0129	0.0453	0.0472	0.0457	0.0108	0.165
Average	0.0000	0.0042	0.0000	0.0031	0.0000	0.0000	0.0180	0.0305	0.0761	0.0822	0.0185	0.239
pVEC 1	0.0000	0.0059	0.0000	0.0088	0.0000	0.0000	0.0291	0.0409	0.0760	0.0875	0.0208	0.269
pVEC 18	0.0000	0.0094	0.0027	0.0054	0.0000	0.0000	0.0308	0.0254	0.0766	0.0702	0.0174	0.239
pVEC 5	0.0000	0.0019	0.0000	0.0040	0.0000	0.0000	0.0197	0.0349	0.0710	0.0784	0.0186	0.224
pVEC 17	0.0000	0.0055	0.0000	0.0029	0.0000	0.0000	0.0175	0.0265	0.0710	0.0822	0.0186	0.224
pVEC 10	0.0000	0.0000	0.0000	0.0024	0.0000	0.0000	0.0106	0.0226	0.0653	0.0845	0.0064	0.175
Average	0.0000	0.0045	0.0005	0.0046	0.0000	0.0000	0.0215	0.0301	0.0720	0.0761	0.0166	0.228

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Table 2
Sterol Analysis of Seed from Tobacco transformed with Hirvea HMGR + N. tabacum SMT 1 (NH9)

Total sterols as % of smpl wt.

Smpl code	squalene	cycloart.	24mca	24mbph	24ebph	d7-avena	isoluc	sito	stig	camp	chol	Total
NH9 34	0.0000	0.0108	0.0078	0.0142	0.0025	0.0046	0.0035	0.2288	0.0377	0.0692	0.0158	0.545
NH9 40	0.0000	0.0086	0.0069	0.0128	0.0586	0.0037	0.1095	0.2062	0.0344	0.0714	0.0168	0.528
NH9 21	0.0000	0.0115	0.0081	0.0118	0.0505	0.0027	0.0913	0.2074	0.0451	0.0704	0.0218	0.521
NH9 19	0.0000	0.0184	0.0086	0.0085	0.0543	0.0030	0.0798	0.1855	0.0382	0.0586	0.0548	0.507
NH9 38	0.0000	0.0089	0.0081	0.0068	0.0437	0.0025	0.0868	0.2022	0.0384	0.0615	0.0164	0.476
NH9 11	0.0000	0.0163	0.0086	0.0119	0.0521	0.0034	0.0870	0.1840	0.0342	0.0585	0.0217	0.476
NH9 22	0.0000	0.0113	0.0083	0.0101	0.0474	0.0031	0.0804	0.1940	0.0396	0.0589	0.0157	0.468
NH9 31	0.0000	0.0168	0.0091	0.0112	0.0516	0.0038	0.0949	0.1731	0.0331	0.0559	0.0256	0.464
NH9 10	0.0000	0.0163	0.0089	0.0094	0.0484	0.0031	0.0916	0.1819	0.0320	0.0548	0.0187	0.462
NH9 33	0.0000	0.0157	0.0074	0.0083	0.0465	0.0031	0.0790	0.1703	0.0397	0.0531	0.0247	0.447
NH9 13	0.0000	0.0146	0.0075	0.0069	0.0417	0.0028	0.0722	0.1788	0.0418	0.0583	0.0187	0.444
NH9 32	0.0000	0.0114	0.0074	0.0061	0.0400	0.0023	0.0782	0.1881	0.0401	0.0537	0.0244	0.434
NH9 27	0.0000	0.0149	0.0083	0.0071	0.0408	0.0026	0.0702	0.1594	0.0381	0.0525	0.0179	0.418
NH9 26	0.0000	0.0133	0.0085	0.0000	0.0382	0.0026	0.0830	0.1761	0.0422	0.0571	0.0152	0.416
NH9 29	0.0000	0.0087	0.0056	0.0059	0.0307	0.0018	0.0885	0.1835	0.0403	0.0589	0.0136	0.416
NH9 25	0.0000	0.0161	0.0079	0.0067	0.0352	0.0031	0.0676	0.1546	0.0383	0.0534	0.0268	0.410
NH9 35	0.0000	0.0178	0.0058	0.0048	0.0372	0.0027	0.0719	0.1504	0.0361	0.0487	0.0269	0.403
NH9 39	0.0000	0.0247	0.0082	0.0048	0.0323	0.0019	0.0718	0.1460	0.0343	0.0461	0.0264	0.386
NH9 8	0.0000	0.0280	0.0102	0.0044	0.0278	0.0013	0.0911	0.1423	0.0411	0.0505	0.0276	0.394
NH9 30	0.0000	0.0110	0.0054	0.0065	0.0366	0.0024	0.0711	0.1485	0.0376	0.0539	0.0183	0.381
NH9 12	0.0000	0.0107	0.0055	0.0053	0.0325	0.0025	0.0663	0.1658	0.0428	0.0529	0.0143	0.385
NH9 37	0.0000	0.0356	0.0086	0.0000	0.0252	0.0018	0.0557	0.1410	0.0366	0.0448	0.0239	0.377
NH9 14	0.0000	0.0288	0.0078	0.0045	0.0315	0.0019	0.0608	0.1335	0.0314	0.0421	0.0205	0.363
NH9 24	0.0000	0.0297	0.0088	0.0040	0.0207	0.0018	0.0572	0.1302	0.0351	0.0445	0.0297	0.362
NH9 18	0.0000	0.0118	0.0054	0.0000	0.0382	0.0026	0.0578	0.1470	0.0370	0.0465	0.0148	0.361
NH9 23	0.0000	0.0077	0.0033	0.0039	0.0152	0.0024	0.0383	0.1371	0.0381	0.0559	0.0127	0.333
NH9 16	0.0000	0.0076	0.0000	0.0000	0.0169	0.0000	0.0481	0.1236	0.0432	0.0468	0.0173	0.307

SR1 16	0.0000	0.0330	0.0085	0.0058	0.0388	0.0020	0.0768	0.1633	0.0325	0.0497	0.0235	0.425	pVEC 17	0.0000	0.0818	0.0091	0.0027	0.0382	0.0027	0.0735	0.1418	0.0331	0.0465	0.413
SR1 8	0.0000	0.0337	0.0077	0.0057	0.0347	0.0014	0.0694	0.1416	0.0347	0.0497	0.0230	0.409	pVEC 10	0.0000	0.0284	0.0111	0.0028	0.0377	0.0028	0.0963	0.1453	0.0334	0.0474	0.407
SR1 3	0.0000	0.0306	0.0093	0.0070	0.0349	0.0029	0.0725	0.1427	0.0317	0.0498	0.0244	0.407	pVEC 15	0.0000	0.0257	0.0081	0.0017	0.0314	0.0017	0.0717	0.1443	0.0339	0.0469	0.405
SR1 17	0.0000	0.0346	0.0088	0.0043	0.0336	0.0025	0.0678	0.1471	0.0305	0.0458	0.0244	0.400	pVEC 8	0.0000	0.0235	0.0081	0.0028	0.0347	0.0028	0.0761	0.1409	0.0340	0.0462	0.403
SR1 1	0.0000	0.0310	0.0079	0.0053	0.0337	0.0023	0.0654	0.1357	0.0329	0.0448	0.0271	0.386	pVEC 11	0.0000	0.0242	0.0098	0.0028	0.0312	0.0028	0.0841	0.1537	0.0361	0.0429	0.394
SR1 9	0.0000	0.0292	0.0071	0.0046	0.0332	0.0021	0.0661	0.1391	0.0312	0.0458	0.0235	0.384	pVEC 5	0.0000	0.0131	0.0050	0.0000	0.0147	0.0025	0.0373	0.1221	0.0485	0.0425	0.301
SR1 7	0.0000	0.0305	0.0093	0.0048	0.0307	0.0025	0.0647	0.1334	0.0385	0.0468	0.0243	0.384	Average	0.0000	0.0245	0.0085	0.0025	0.0313	0.0025	0.0548	0.1413	0.0352	0.0457	0.387
SR1 2	0.0000	0.0212	0.0062	0.0040	0.0285	0.0024	0.0627	0.1391	0.0400	0.0488	0.0267	0.380												
SR1 6	0.0000	0.0334	0.0083	0.0053	0.0350	0.0019	0.0627	0.1261	0.0284	0.0427	0.0274	0.372												
SR1 4	0.0000	0.0176	0.0062	0.0000	0.0218	0.0012	0.0598	0.1357	0.0367	0.0431	0.0220	0.344												
SR1 5	0.0000	0.0125	0.0048	0.0000	0.0177	0.0014	0.0547	0.1413	0.0413	0.0442	0.0228	0.341												
SR1 20	0.0000	0.0246	0.0084	0.0000	0.0232	0.0012	0.0485	0.1348	0.0407	0.0427	0.0160	0.339												
Average	0.0000	0.0277	0.0078	0.0039	0.0301	0.0020	0.0644	0.1392	0.0348	0.0459	0.0261	0.381												

Table 3
Sterol Analysis of Leaf from Tobacco transformed with truncated Hevea HMGR and Nicotiana SMT1 (MH7)

Total sterols as % of dry weight

Smpl code	squalene	cycbert	24mca	24mieph	24sieph	d7-avena	kotuc	sito	stig	camp	chol	Total
MH7 53	0.1504	0.1082	0.2321	0.1357	0.1273	0.0229	0.1400	0.2241	0.1079	0.0714	0.0095	1.339
MH7 31	0.0527	0.4200	0.0282	0.0444	0.0509	0.0236	0.1148	0.1010	0.0872	0.0424	0.0315	0.878
MH7 32	0.0276	0.0830	0.1479	0.0864	0.1132	0.0194	0.1161	0.1716	0.0899	0.0512	0.0152	0.845
MH7 3	0.0169	0.0891	0.1544	0.0891	0.0973	0.0212	0.1346	0.1406	0.0929	0.0460	0.0059	0.890
MH7 35	0.0262	0.0825	0.2329	0.0794	0.0802	0.0229	0.1012	0.1208	0.0640	0.0383	0.0110	0.861
MH7 19	0.0136	0.0838	0.1311	0.0836	0.0953	0.0201	0.1253	0.1458	0.0776	0.0470	0.0042	0.827
MH7 54	0.0076	0.0912	0.1063	0.0825	0.0884	0.0179	0.1260	0.1476	0.0882	0.0853	0.0045	0.805
MH7 6	0.0096	0.1312	0.0716	0.0831	0.0841	0.0119	0.1127	0.1167	0.0800	0.0504	0.0058	0.707
MH7 4	0.0088	0.1628	0.0201	0.0328	0.0275	0.0042	0.0462	0.0857	0.1042	0.0518	0.0280	0.558
MH7 46	0.0000	0.0387	0.0158	0.0170	0.0044	0.0024	0.0175	0.0190	0.1044	0.0279	0.0288	0.276
MH7 48	0.0014	0.0215	0.0121	0.0179	0.0108	0.0032	0.0145	0.0398	0.0850	0.0481	0.0087	0.273
MH7 43	0.0000	0.0167	0.0115	0.0118	0.0119	0.0059	0.0428	0.0963	0.0580	0.0486	0.0053	0.273
MH7 45	0.0000	0.0388	0.0154	0.0188	0.0064	0.0024	0.0121	0.0285	0.0988	0.0366	0.0185	0.265
MH7 11	0.0000	0.0070	0.0138	0.0133	0.0058	0.0033	0.0258	0.0327	0.1027	0.0429	0.0177	0.264
MH7 42	0.0000	0.0283	0.0093	0.0130	0.0059	0.0016	0.0027	0.0485	0.0994	0.0488	0.0071	0.254
MH7 24	0.0000	0.0216	0.0122	0.0116	0.0042	0.0028	0.0157	0.0305	0.0945	0.0371	0.0208	0.251
MH7 27	0.0000	0.0074	0.0128	0.0104	0.0047	0.0030	0.0163	0.0902	0.1023	0.0380	0.0225	0.249
MH7 33	0.0000	0.0345	0.0140	0.0119	0.0053	0.0026	0.0083	0.0280	0.0917	0.0388	0.0120	0.247
MH7 37	0.0013	0.0276	0.0131	0.0106	0.0039	0.0029	0.0162	0.0213	0.0853	0.0316	0.0209	0.235
MH7 22	0.0000	0.0226	0.0077	0.0081	0.0038	0.0020	0.0125	0.0191	0.1011	0.0364	0.0216	0.234
MH7 34	0.0000	0.0182	0.0062	0.0073	0.0048	0.0028	0.0128	0.0328	0.0914	0.0395	0.0145	0.229
MH7 26	0.0000	0.0159	0.0111	0.0105	0.0037	0.0027	0.0164	0.0249	0.0873	0.0408	0.0142	0.227
MH7 38	0.0000	0.0130	0.0080	0.0068	0.0068	0.0032	0.0169	0.0310	0.0877	0.0381	0.0163	0.227
MH7 21	0.0000	0.0122	0.0041	0.0058	0.0066	0.0033	0.0227	0.0398	0.0726	0.0441	0.0051	0.216
MH7 47	0.0008	0.0246	0.0080	0.0096	0.0032	0.0015	0.0126	0.0167	0.0855	0.0258	0.0255	0.216
MH7 40	0.0000	0.0258	0.0119	0.0067	0.0044	0.0026	0.0123	0.0230	0.0763	0.0325	0.0160	0.212
MH7 39	0.0000	0.0131	0.0084	0.0069	0.0062	0.0019	0.0078	0.0324	0.0734	0.0358	0.0039	0.194
MH7 7	0.0000	0.0275	0.0076	0.0064	0.0030	0.0020	0.0146	0.0172	0.0686	0.0282	0.0121	0.175

MH7 40	0.0000	0.0088	0.0045	0.0060	0.0025	0.0024	0.0201	0.0141	0.0586	0.0296	0.0184	0.164	
MH7 5	0.0000	0.0038	0.0023	0.0031	0.0036	0.0025	0.0152	0.0216	0.0840	0.0345	0.0067	0.160	
MH7 23	0.0000	0.0134	0.0056	0.0053	0.0017	0.0019	0.0131	0.0126	0.0609	0.0243	0.0151	0.157	
MH7 39	0.0000	0.0089	0.0038	0.0058	0.0037	0.0021	0.0117	0.0182	0.0553	0.0276	0.0085	0.142	
SR1 6	0.0000	0.0168	0.0055	0.0079	0.0039	0.0017	0.0088	0.0216	0.0721	0.0264	0.0132	0.178	
SR1 7	0.0000	0.0086	0.0029	0.0038	0.0039	0.0023	0.0088	0.0273	0.0988	0.0362	0.0115	0.201	
SR1 8	0.0000	0.0163	0.0061	0.0074	0.0035	0.0023	0.0175	0.0321	0.0884	0.0320	0.0162	0.221	
SR1 10	0.0000	0.0044	0.0024	0.0035	0.0038	0.0024	0.0121	0.0187	0.0586	0.0247	0.0108	0.141	
Average	0.0000	0.0110	0.0042	0.0057	0.0037	0.0022	0.0122	0.0248	0.0790	0.0298	0.0127	0.185	
SJ34 1	0.0000	0.0116	0.0060	0.0100	0.0083	0.0017	0.0123	0.0250	0.0988	0.0286	0.0127	0.183	
SJ34 2	0.0014	0.0280	0.0102	0.0129	0.0078	0.0031	0.0241	0.0340	0.1438	0.0368	0.0284	0.330	
SJ34 3	0.0000	0.0072	0.0090	0.0054	0.0044	0.0028	0.0180	0.0262	0.1013	0.0315	0.0141	0.216	
SJ34 4	0.0000	0.0101	0.0042	0.0044	0.0033	0.0023	0.0119	0.0260	0.0915	0.0305	0.0137	0.198	
Average	0.0004	0.0137	0.0058	0.0082	0.0054	0.0025	0.0168	0.0290	0.1013	0.0327	0.0167	0.232	

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Table 4
Sterol Analysis of Seed from Tobacco transformed with truncated Hevea HMGR and Nicotiana SMT1 (MH7)

Total sterols as % of dry weight

Smpl code	squalene	cycloart	24mca	24mbph	24ebph	d7-aven	isofuc	sito	stig	camp	chol	Total	
MH7 53	0.0204	0.0978	0.1707	0.0473	0.0735	0.0242	0.1345	0.2463	0.0462	0.0988	0.0233	0.987	
MH7 3	0.0188	0.1082	0.1849	0.0625	0.0714	0.0216	0.1254	0.2346	0.0573	0.0940	0.0176	0.972	
MH7 32	0.0127	0.1004	0.1281	0.0569	0.0783	0.0286	0.1295	0.2484	0.0636	0.0863	0.0232	0.965	
MH7 35	0.0144	0.0832	0.1349	0.0445	0.0839	0.0185	0.1228	0.2301	0.0515	0.0807	0.0194	0.874	
MH7 54	0.0139	0.0838	0.1341	0.0401	0.0705	0.0142	0.1191	0.2253	0.0464	0.0786	0.0202	0.850	
MH7 31	0.0178	0.1770	0.0448	0.0192	0.0476	0.0133	0.1060	0.1854	0.0488	0.0892	0.0245	0.795	
MH7 7	0.0272	0.0302	0.0100	0.0263	0.1234	0.0104	0.1248	0.2123	0.0337	0.0674	0.0245	0.890	
MH7 21	0.0162	0.0217	0.0142	0.0262	0.1089	0.0111	0.1203	0.2298	0.0346	0.0712	0.0191	0.873	
MH7 28	0.0152	0.0179	0.0113	0.0245	0.1115	0.0101	0.1104	0.2414	0.0321	0.0681	0.0163	0.859	
MH7 23	0.0147	0.1047	0.0248	0.0161	0.0488	0.0078	0.0873	0.1818	0.0413	0.0842	0.0220	0.835	
MH7 37	0.0114	0.0549	0.0228	0.0237	0.0594	0.0080	0.1020	0.2008	0.0416	0.0757	0.0230	0.823	
MH7 47	0.0268	0.0539	0.0078	0.0153	0.1037	0.0098	0.1009	0.1930	0.0312	0.0620	0.0263	0.821	
MH7 40	0.0122	0.0627	0.0224	0.0188	0.0538	0.0085	0.0895	0.1868	0.0378	0.0868	0.0254	0.594	
MH7 33	0.0143	0.0276	0.0105	0.0183	0.0795	0.0083	0.0958	0.1968	0.0351	0.0861	0.0205	0.573	
MH7 49	0.0148	0.0846	0.0070	0.0122	0.0698	0.0067	0.1008	0.1931	0.0307	0.0698	0.0268	0.573	
MH7 5	0.0180	0.0213	0.0076	0.0175	0.0797	0.0079	0.0978	0.2009	0.0317	0.0838	0.0205	0.668	
MH7 24	0.0098	0.0810	0.0095	0.0136	0.0433	0.0063	0.0844	0.1698	0.0401	0.0820	0.0230	0.643	
MH7 39	0.0115	0.0463	0.0091	0.0116	0.0545	0.0068	0.0780	0.1847	0.0405	0.0598	0.0217	0.524	
MH7 45	0.0112	0.0550	0.0150	0.0138	0.0435	0.0050	0.0805	0.1710	0.0362	0.0694	0.0273	0.517	
MH7 27	0.0124	0.0184	0.0068	0.0142	0.0735	0.0078	0.0871	0.1915	0.0299	0.0555	0.0177	0.515	
MH7 48	0.0089	0.0488	0.0103	0.0115	0.0447	0.0063	0.0813	0.1682	0.0403	0.0582	0.0274	0.510	
MH7 22	0.0124	0.0239	0.0076	0.0139	0.0716	0.0065	0.0834	0.1780	0.0320	0.0535	0.0217	0.505	
MH7 36	0.0087	0.0205	0.0068	0.0098	0.0540	0.0061	0.0718	0.1677	0.0393	0.0576	0.0177	0.460	
MH7 46	0.0097	0.0341	0.0047	0.0086	0.0453	0.0051	0.0788	0.1565	0.0333	0.0533	0.0265	0.457	
MH7 4	0.0109	0.0492	0.0128	0.0101	0.0380	0.0048	0.0651	0.1549	0.0369	0.0512	0.0215	0.456	
MH7 34	0.0053	0.0147	0.0054	0.0040	0.0534	0.0060	0.0897	0.1728	0.0358	0.0526	0.0168	0.432	
MH7 43	0.0073	0.0369	0.0062	0.0067	0.0397	0.0045	0.0848	0.1494	0.0367	0.0480	0.0240	0.421	
MH7 11	0.0091	0.0368	0.0037	0.0057	0.0327	0.0044	0.0723	0.1388	0.0362	0.0458	0.0303	0.416	
MH7 38	0.0056	0.0356	0.0042	0.0051	0.0338	0.0038	0.0535	0.1363	0.0347	0.0449	0.0204	0.378	

SR19	0.0083	0.0458	0.0055	0.0062	0.0676	0.1601	0.0392	0.0512	0.0255	0.445	0.0051	0.0430	0.0062
SR14	0.0075	0.0460	0.0042	0.0069	0.0735	0.1423	0.0342	0.0488	0.0272	0.436	0.0047	0.0414	0.0069
SR13	0.0067	0.0453	0.0030	0.0063	0.0673	0.1407	0.0316	0.0469	0.0249	0.420	0.0046	0.0404	0.0063
SR12	0.0108	0.0400	0.0044	0.0049	0.0607	0.1485	0.0357	0.0484	0.0214	0.419	0.0046	0.0398	0.0049
SR18	0.0075	0.0431	0.0053	0.0065	0.0628	0.1439	0.0347	0.0457	0.0232	0.417	0.0049	0.0378	0.0065
SR17	0.0078	0.0400	0.0048	0.0055	0.0628	0.1403	0.0342	0.0460	0.0234	0.412	0.0045	0.0421	0.0055
SR15	0.0085	0.0349	0.0051	0.0026	0.0605	0.1437	0.0311	0.0404	0.0268	0.394	0.0048	0.0386	0.0026
SR110	0.0062	0.0843	0.0051	0.0042	0.0603	0.1421	0.0328	0.0455	0.0224	0.394	0.0052	0.0354	0.0042
SR11	0.0051	0.0668	0.0048	0.0048	0.0620	0.1372	0.0268	0.0410	0.0258	0.393	0.0042	0.0363	0.0048
Average	0.0080	0.0407	0.0047	0.0052	0.0641	0.1432	0.0334	0.0461	0.0244	0.414	0.0048	0.0398	0.0052
SJ348	0.0091	0.0426	0.0044	0.0058	0.0659	0.1407	0.0307	0.0451	0.0247	0.413	0.0045	0.0383	0.0058
SJ345	0.0082	0.0439	0.0040	0.0061	0.0625	0.1408	0.0344	0.0474	0.0223	0.413	0.0044	0.0380	0.0061
SJ343	0.0081	0.0345	0.0048	0.0060	0.0626	0.1484	0.0357	0.0483	0.0234	0.410	0.0041	0.0386	0.0060
SJ342	0.0084	0.0382	0.0048	0.0060	0.0592	0.1437	0.0333	0.0460	0.0225	0.401	0.0041	0.0373	0.0060
SJ346	0.0079	0.0387	0.0045	0.0044	0.0584	0.1471	0.0363	0.0445	0.0185	0.401	0.0048	0.0375	0.0044
SJ344	0.0049	0.0268	0.0027	0.0028	0.0414	0.1293	0.0415	0.0429	0.0160	0.334	0.0036	0.0218	0.0028
Average	0.0081	0.0375	0.0042	0.0049	0.0583	0.1416	0.0351	0.0462	0.0212	0.396	0.0042	0.0351	0.0049

Table 5
Analysis of free sterol and sterol ester fractions of MH7 transgenic seed samples

Sterols as % dry wt

Sample / Fraction	cycloart	24mca	24mioph	24ioph	d7-avena	isofuc	sito	stig	camp	chol	Total
MH7 32											
Total sterol (TS)	0.1004	0.1281	0.0559	0.0783	0.0286	0.1285	0.2494	0.0836	0.0863	0.0232	0.986
Free sterol (FS)	0.0097	0.0243	0.0047	0.0152	0.0019	0.0239	0.0702	0.0362	0.0261	0.0060	0.217
Sterol ester (TS-FS)	0.0906	0.1039	0.0512	0.0631	0.0267	0.1055	0.1782	0.0274	0.0702	0.0182	0.748
MH7 53											
Total sterol (TS)	0.0976	0.1707	0.0473	0.0735	0.0242	0.1345	0.2483	0.0482	0.0989	0.0233	0.957
Free sterol (FS)	0.0122	0.0307	0.0064	0.0222	0.0024	0.0300	0.0774	0.0308	0.0253	0.0044	0.242
Sterol ester (TS-FS)	0.0854	0.1400	0.0410	0.0513	0.0218	0.1044	0.1709	0.0174	0.0735	0.0189	0.745
SR1 control											
Total sterol (TS)	0.0260	0.0161	0.0000	0.0237	0.0017	0.0534	0.1615	0.0386	0.0485	0.0205	0.388
Free sterol (FS)	0.0126	0.0032	0.0000	0.0158	0.0000	0.0191	0.0726	0.0314	0.0244	0.0060	0.185
Sterol ester (TS-FS)	0.0134	0.0129	0.0000	0.0081	0.0017	0.0343	0.0889	0.0052	0.0241	0.0145	0.203

% FS vs. SE for sterol components

Sample / Fraction	cycloart	24mca	24mioph	24ioph	d7-avena	isofuc	sito	stig	camp	chol	Total
MH7 32											
FS	9.7	18.9	8.4	19.4	6.7	18.5	28.3	56.9	27.1	21.5	22.8
SE	90.3	81.1	91.6	80.6	93.3	81.5	71.7	43.1	72.9	78.5	77.2
MH7 53											
FS	12.5	18.0	13.5	30.2	9.9	22.3	31.2	83.9	25.0	18.8	25.0
SE	87.5	82.0	86.5	69.8	90.1	77.7	68.8	38.1	74.4	81.2	75.0
SR1 control											
FS	48.6	19.9	0.0	65.9	0.0	35.8	45.0	85.7	50.3	29.1	47.6
SE	51.4	80.1	0.0	34.1	100.0	64.2	55.0	14.3	49.7	70.9	52.4

Table 6
Sterol Analysis of seed from Tobacco transformed with truncated yeast HMGR + N. tabacum SMT1 (MH8)

Total sterols as % of dry wt
smpl wt

Smpl code	squalene	cycloart	24mea	24methph	24ethph	d7-aven	lsofuc	sito	stig	camp	chol	Total
MH8 16	0.0143	0.0092	0.0058	0.0070	0.0647	0.0073	0.0084	0.2108	0.0371	0.0589	0.0143	0.528
MH8 53	0.0084	0.0111	0.0081	0.0080	0.0605	0.0071	0.0076	0.1931	0.0360	0.0588	0.0162	0.505
MH8 48	0.0080	0.0243	0.0060	0.0125	0.0614	0.0065	0.0740	0.1736	0.0368	0.0567	0.0174	0.490
MH8 54	0.0089	0.0172	0.0063	0.0103	0.0567	0.0061	0.0760	0.1741	0.0368	0.0630	0.0158	0.476
MH8 56	0.0088	0.0167	0.0064	0.0083	0.0542	0.0068	0.0737	0.1763	0.0408	0.0611	0.0161	0.474
MH8 38	0.0088	0.0124	0.0077	0.0062	0.0675	0.0072	0.0749	0.1732	0.0405	0.0566	0.0167	0.472
MH8 18	0.0063	0.0129	0.0070	0.0053	0.0653	0.0068	0.0719	0.1636	0.0433	0.0569	0.0148	0.466
MH8 19	0.0081	0.0173	0.0062	0.0078	0.0533	0.0065	0.0805	0.1702	0.0348	0.0571	0.0186	0.462
MH8 32	0.0062	0.0098	0.0056	0.0055	0.0466	0.0070	0.0683	0.1669	0.0348	0.0901	0.0182	0.453
MH8 46	0.0072	0.0463	0.0056	0.0053	0.0334	0.0048	0.0838	0.1459	0.0356	0.0457	0.0280	0.421
MH8 14	0.0044	0.0284	0.0075	0.0047	0.0330	0.0058	0.0670	0.1604	0.0348	0.0454	0.0284	0.408
MH8 44	0.0065	0.0300	0.0061	0.0060	0.0328	0.0070	0.0688	0.1388	0.0348	0.0448	0.0271	0.403
MH8 40	0.0041	0.0066	0.0046	0.0037	0.0396	0.0057	0.0551	0.1606	0.0459	0.0585	0.0069	0.395
MH8 12	0.0042	0.0150	0.0048	0.0025	0.0396	0.0054	0.0500	0.1536	0.0458	0.0505	0.0163	0.388
MH8 43	0.0060	0.0340	0.0041	0.0049	0.0316	0.0041	0.0626	0.1346	0.0307	0.0430	0.0243	0.380
MH8 16	0.0044	0.0244	0.0058	0.0038	0.0282	0.0050	0.0516	0.1403	0.0414	0.0446	0.0220	0.371
MH8 11	0.0030	0.0075	0.0058	0.0018	0.0299	0.0045	0.0448	0.1510	0.0558	0.0520	0.0116	0.367
MH8 23	0.0050	0.0055	0.0061	0.0024	0.0378	0.0051	0.0491	0.1500	0.0436	0.0623	0.0104	0.365
MH8 34	0.0054	0.0289	0.0039	0.0049	0.0318	0.0040	0.0511	0.1322	0.0361	0.0455	0.0178	0.364
MH8 28	0.0024	0.0086	0.0054	0.0039	0.0280	0.0041	0.0497	0.1443	0.0488	0.0525	0.0145	0.360
MH8 33	0.0027	0.0167	0.0037	0.0025	0.0195	0.0040	0.0377	0.1175	0.0501	0.0474	0.0140	0.318
MH8 7	0.0000	0.0036	0.0047	0.0014	0.0088	0.0025	0.0208	0.0980	0.0601	0.0487	0.0068	0.257
MH8 8	0.0000	0.0020	0.0050	0.0000	0.0098	0.0019	0.0208	0.0982	0.0804	0.0492	0.0068	0.251
SR1 3	0.0085	0.0403	0.0058	0.0059	0.0385	0.0051	0.0589	0.1511	0.0481	0.0505	0.0188	0.429
SR1 2	0.0063	0.0287	0.0052	0.0040	0.0311	0.0041	0.0511	0.1448	0.0480	0.0493	0.0161	0.388
SR1 4	0.0038	0.0252	0.0051	0.0032	0.0232	0.0043	0.0516	0.1361	0.0468	0.0505	0.0213	0.371
SR1 1	0.0065	0.0312	0.0046	0.0046	0.0306	0.0039	0.0514	0.1321	0.0367	0.0432	0.0179	0.363

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Average	0.0062	0.0313	0.0052	0.0044	0.0306	0.0043	0.0833	0.1410	0.0443	0.0484	0.0185	0.398							
SJ34 13	0.0094	0.0335	0.0057	0.0058	0.0323	0.0067	0.0758	0.1462	0.0332	0.0476	0.0326	0.428							
SJ34 11	0.0078	0.0252	0.0055	0.0053	0.0341	0.0048	0.0724	0.1430	0.0369	0.0470	0.0300	0.414							
SJ34 16	0.0017	0.0143	0.0047	0.0020	0.0165	0.0034	0.0315	0.1068	0.0515	0.0447	0.0134	0.291							
SJ34 17	0.0000	0.0038	0.0027	0.0000	0.0041	0.0021	0.0143	0.0714	0.0832	0.0431	0.0084	0.213							

Claims

1. The use of a gene expressing a non-feed back inhibited HMG-reductase in combination with a gene expressing sterol methyltransferase1 to increase the level of sterols in plants.

2. The use according to claim 1, wherein the level of 4-desmethylsterols is increased in the plants by at least 10%.

3. The use according to claim 1, wherein the sterols are increased in seeds, more preferred in oilseeds.

4. The use according to claim 3, wherein the seeds are from tobacco, canola, sunflower, rape, soy or peanut.

5. The use according to claim 1, wherein the non feedback inhibited HMG-reductase is expressed by a truncated non-plant HMG gene.

6. The use according to claim 5, wherein the HMG-reductase expressed by the truncated HMG-reductase gene lacks the membrane-binding domain.

7. The use according to claim 1, wherein the non-feedback inhibited HMG-reductase is expressed by a truncated plant HMG-reductase gene.

8. The use according to claim 1, wherein the HMG-reductase can be derived from *Asteraceae*.

9. The use according to claim 8, wherein the HMGR gene can be derived from *Hevea brasiliensis* or the HMGR gene is

a truncated version of a gene which can be derived from *Hevea brasiliensis*.

10. Use according to claim 9, wherein the HMGR gene is the hmg 1 gene derived from *Hevea brasiliensis* or a truncated version of said gene.

11. A method of transforming a plant by

A1) transforming a plant cell with a recombinant DNA construct comprising a DNA segment encoding a polypeptide with non feedback inhibited HMGR activity and a polypeptide encoding a sterol methyltransferase1 activity and promoters for driving the expression of said polypeptides in said plant cell to form a transformed plant cell; or

A2) re-transforming a plant cell expressing a non-feedback inhibited HMGR activity with a gene encoding a sterol methyltransferase1 activity; or

A3) re-transforming a plant cell expressing a sterol methyltransferase1 activity with a gene encoding a non-feedback inhibited HMGR activity; and

D) regenerating the above transformed plant cells into transgenic plants; and

E) selecting transgenic plants that have enhanced levels of 4-desmethylsterols compared to wild type strains of the same plant.

12. Plant obtainable by a method according to claim 11.

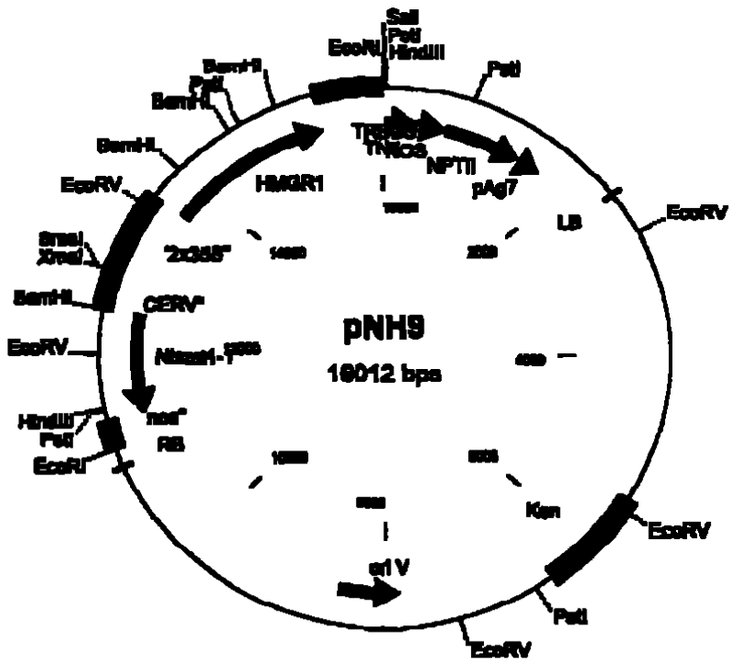
13. Plant tissue obtained from a plant according to claim 12.

14. Plant tissue according to claim 13, selected from the group of leaves, fruit and seeds.

15. Plant having incorporated in its genome a heterologous gene encoding a truncated polypeptide HMGR activity in combination with an heterologous gene encoding SMT1.

Abstract

The use of a gene expressing a non-feed back inhibited HMG-reductase in combination with a gene expressing sterol methyltransferase1 to increase the level of sterols in plants.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pAg7
EcoRV
LB
EcoRV
SmaI
XbaI
"2x35S"
BamHI
CERV
pNH9
EcoRV
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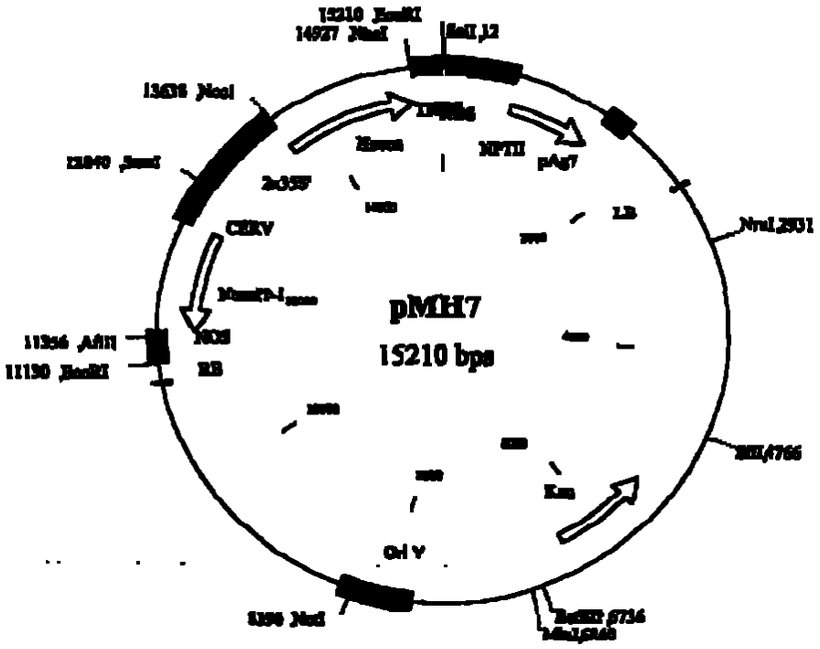
HindIII
PstI
EcoRI
RB
Kan
EcoRV
ori V
PstI
EcoRV

Figure 1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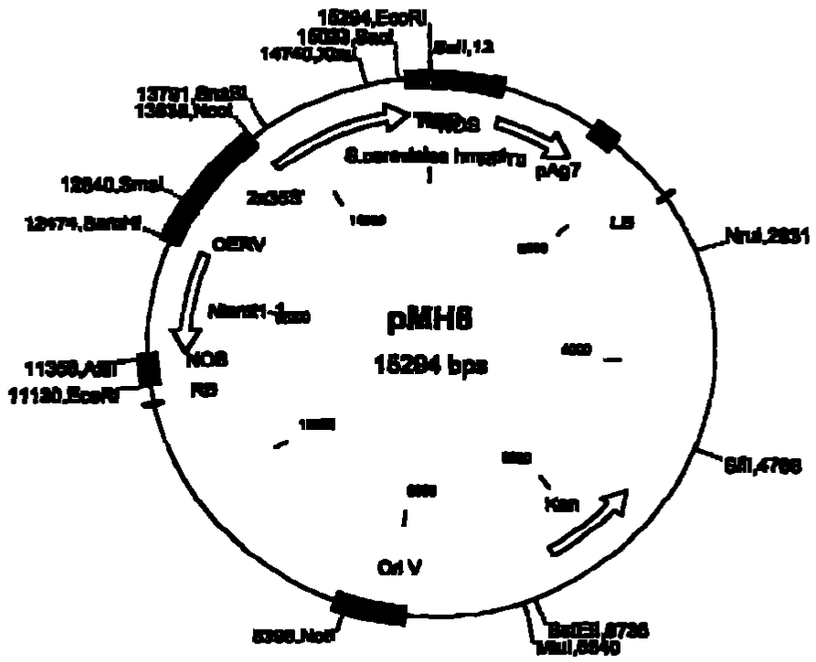
NOS
RB
ori V
5390 NcoI

Figure 2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2u ORI
LEU
12474,BamHI
OERV
NruI,2851
pMH6
15294 bps
NOS
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11120,EcoRI
RB
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Kan
Ori V
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BstEII,6736
MluI,6840

Figure 3

Unilever
01-100.340
107

TRADUCCION OFICIAL NO. 8423

De un documento escrito en Inglés.

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Expediente No. 01-100.340 F 7573 (V) / CO

TRASPASO DE INVENTO

Nosotros, los suscritos, Mark HARKER, Susan Amanda HELLYER, Niklas HOLMBERG y Dick Safford, todos ciudadanos británicos de Unilever Research Colworth, Colworth House, Sharnbrook Bedford MK 44 1LQ, Reino Unido;

por el presente traspasamos a:

UNILEVER PLC.
una compañía británica
Unilever House
Blackfriars, Londres EC4P 4BQ
Gran Bretaña

la totalidad de nuestro derecho para solicitar y obtener una patente en:

COLOMBIA

sobre un invento hecho por nosotros denominado:

Proceso para modificar plantas

Y SUJETO A UN PRIMER REGISTRO EN Europa, A SABER.

SOLICITUD NÚMERO 00310403.1 de fecha noviembre 23 de 2000

A nombre de UNILEVER PLC

Fechado el día 7 de noviembre de 2001

Firmado: Mark Harker

Firmado: Susan Amanda Hellyer

Firmado: Niklas Holmberg

Firmado: Dick Safford

= = = = = =

Visto por mí, Victor Joseph Antonius Johannes Clemens van Heeswijk, notario de ley civil en Rotterdam, Países Bajos, para legalización de las firmas puestas en el documento adjunto de:

- Sr. Mark Harker, c/o Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, MK44 1LQ, Reino Unido;
- Sra. Susan Amanda Hellyer, c/o Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, MK44 1LQ, Reino Unido;
- Sr. Niklas Holmberg, c/o Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, MK44 1LQ, Reino Unido;
- Sr. Dick Safford, c/o Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, MK44 1LQ, Reino Unido.

Roterdam, Noviembre 12 de 2001.

Firma ilegible

Sello de caucho: Sr. V.J.A.J.C van HEESWIJK NOTARIS TE ROTTERDAM – Con escudo en el centro.

= = = = = =

APOSTILLA

(Convención de La Haya de octubre 5 de 1961)

1. País: PAÍSES BAJOS
2. Este Documento Público
2. Ha sido firmado por el Sr. V.J.A.J.C. van Heeswijk
3. Actuando en su condición de Notario
4. Tiene el sello de el Sr. V.J.A.J.C. van Heeswijk

CERTIFICADO

5. En ROTTERDAM 6. El 12/11/ 2001
7. Por el oficial de registro del Tribunal del Distrito

(Griffier van de Arrondissementsrechtbank)

8. Número 113547
9. Sello: Sello en relieve estampado sobre lámina plástica: Griffier van de Arrondissementsrechtbank – Rotterdam

10 Firma: Firmado ilegiblemente

= = = = = =

Es traducción fiel y competa, de la cual queda copia en el archivo de esta oficina de traducciones para su confrontación, a la cual me remito.

Derechos de traducción: $27.000.

Bogotá, D.C. 17 de diciembre de 2001.

= = = = = =

MINISTERIO DE RELACIONES EXTERIORES

365065

CUYA FIRMA APARECE EN EL DOCUMENTO DESEMPEÑA LAS FUNCIONES INDICADAS

NO SE ASUME RESPONSABILIDAD DEL TEXTO

2001 DEC 18 A 11:58

JEFE DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.

JOSE LLORENTA CAMACHO

110

Unilever NV
F 7573 (V) / CO

ASSIGNMENT OF INVENTION

I/We, the undersigned,

Mark HARKER, Susan Amanda HELLYER, Niklas HOLMBERG and Dick Safford, all British subjects of Unilever Research Colworth, Colworth House, Sharnbrook Bedford, MK44 1LQ, United Kingdom;

do hereby assign to:

Unilever PLC
a British company
Unilever House
Blackfriars
London EC4P 4BQ
Great Britain

my/our entire right to apply for and to obtain a Patent in:

COLOMBIA

on an invention made by me/us, entitled:

Process for modifying plants

AND SUBJECT TO A FIRST FILING IN Europe, VIZ.
APPLICATION NUMBER 00310403.1 dated 23 November 2000
in the name of UNILEVER PLC

Dated the 7th day of November 2001

Mark Harker

Susan Amanda Hellyer

Niklas Holmberg

Dick Safford

Notarise/Apostile/Legalise

111

Seen by me, Victor Joseph Antonius Johannes Clemens van Heeswijk, civil law notary in Rotterdam, the Netherlands, for legalisation of the signatures placed on the attached document of:

- Mr Mark Harker, c/o Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, MK44 1LQ, United Kingdom;
- Mrs Susan Amanda Hellyer, c/o Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, MK44 1LQ, United Kingdom;
- Mr Niklas Holmberg, c/o Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, MK44 1LQ, United Kingdom; and
- Mr Dick Safford, c/o Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, MK44 1LQ, United Kingdom.

Rotterdam, 12 November 2001

APOSTILLE

(Convention de La Haye du 5 Octobre 1961)

1. Country: The NETHERLANDS
   This public document
2. has been signed by mr V.J.A.J.C. van Heeswijk
3. acting in the capacity of
   notary
4. bears the stamp of
   mr V.J.A.J.C. van Heeswijk
   Certified
5. at ROTTERDAM 6. on 12/11/2001
7. by the clerk of the District Court
   (Griffier van de Arrondissementsrechtbank)
8. N° 113547
9. Stamp: 10. Signature:

Unilever
01-00340
112

TRADUCCIÓN OFICIAL NO. 8424

DE UN DOCUMENTO ESCRITO EN INGLÉS

= = = = = =

Expediente 01.100.340 **(F 7573 (V))**

TRASPASO

Nosotros, UNILEVER PLC, Unilever House, Blackfrairs Londres EC4P 4BQ Reino Unido, por el presente traspasamos a: UNILEVER N.V. domiciliada en Weena 455 3013 AL Rotterdam, Países Bajos la totalidad de nuestros derechos para solicitar y obtener una patente en su nombre, junto con el derecho de prioridad en Colombia de conformidad con las estipulaciones de la Convención Internacional para la Protección de la Propiedad Industrial sobre la base de la solicitud de patente número 00310403.1 presentada por la citada UNILEVER PLC en Europa el 24 de noviembre de 2000.

Fechado el día 7de noviembre de 2001.

Firmado ilegiblemente por H. de Rooij

(firmante autorizado)

= = = = =

Yo, Victor Joseph Antonius Johannes Clemens van Heeswijk, notario de ley civil en Rotterdam, Países Bajos, certifico que la firma puesta en el documento adjunto es la firma del Sr. H. De Rooij quien esta debidamente autorizado para firmar en asuntos de patentes por y en nombre de Unilever PLC.

Roterdam, Noviembre 12 de 2001.

Firma ilegible

Sello de caucho: Sr. V.J.A.J.C van HEESWIJK NOTARIS TE ROTTERDAM – Con escudo en el centro.

======

APOSTILLA

(Convención de La Haya de octubre 5 de 1961)

1. País: PAÍSES BAJOS

   Este documento público

2. Ha sido firmado por el Sr. V.J.A.J.C. van Heeswijk
3. Actuando en su condición de Notario
4. Tiene el sello del Sr. V.J.A.J.C. van Heeswijk

CERTIFICADO

5. En ROTTERDAM 6. El 12/11/ 2001
7. Por el oficial de registro del Tribunal del Distrito

   (Griffier van de Arrondissementsrechtbank)

8. Número 113545
9. Sello: Sello en relieve estampado sobre lámina plástica: Griffier van de

   Arrondissementsrechtbank – Rotterdam

10 Firma: Firmado ilegiblemente

======

Es traducción fiel y completa, de la cual queda copia en el archivo de esta oficina de traducciones para su confrontación, a la cual me remito.

Derechos de traducción: $18.000.

Bogotá, D.C. 17 de diciembre de 2001.

TRADUCTOR OFICIAL
Res. 0987 Ministerio de Justicia

MINISTERIO DE RELACIONES EXTERIORES

365066

CUYA FIRMA APARECE EN EL DOCUMENTO DESEMPEÑA LAS FUNCIONES INDICADAS

NO SE ASUME RESPONSABILIDAD DEL TEXTO

2001 DEC 18 A 11:58

JEFE DE LEGALIZACIONES SANTAFE DE BOGOTA D.C.

ASSIGNMENT

We, UNILEVER PLC, Unilever House Blackfriars London EC4P 4BQ United Kingdom, do hereby assign to UNILEVER N.V. of Weena 455, 3130 AL Rotterdam, The Netherlands, the entire right to apply for and to obtain a patent in their name, together with the right of priority, in Colombia in conformity with the provisions of the International Convention for the Protection of Industrial Property on the basis of patent application number 00310403.1 filed by said UNILEVER PLC in Europe on 24 November 2000.

Dated the 7 st day of November 2001

on behalf of
UNILEVER PLC

....

H. de Rooij
(authorised signatory)

115

I, Victor Joseph Antonius Johannes Clemens van Heeswijk, civil law notary in Rotterdam, the Netherlands, certify that the signature placed on the attached document is the signature of Mr H. de Rooij who is duly authorized to sign in patent affairs for and on behalf of Unilever PLC.

Rotterdam, 12 November 2001

APOSTILLE

(Convention de La Haye du 5 Octobre 1961)

1. Country: The NETHERLANDS
   This public document
2. has been signed by mr V.J.A.J.C. van Heeswijk
3. acting in the capacity of notary
4. bears the stamp of mr V.J.A.J.C. van Heeswijk Certified
5. at ROTTERDAM 6. on 12/11/2001
7. by the clerk of the District Court (Griffier van de Arrondissementsrechtbank)
   N° 113545
8. Stamp: 10. Signature:

Industria y Comercio
SUPERINTENDENCIA

2do. EXAMEN DE FORMA PATENTE DE INVENCION

Folio 125

Radicación no 01-100340
Concepto Técnico no. 0546
Clasificación Internacional de Patentes : C 12 N 15/82

Practicado el examen de acuerdo a lo establecido en el Art. 38 de la Decisión 486 se determinó que esta solicitud de **Patente de Invención:**

SI [✔] NO [ ]	Contiene un resumen de la invención (Art.26-e).
SI [✔] NO [ ]	Contiene el título o nombre de la invención (Art.27-d).
SI [✔] NO [ ]	Contiene la descripción de la invención (Art.26-b).
SI [✔] NO [ ]	NO ES APLICABLE [ ] Contiene los dibujos necesarios para comprender la invención (Art.26-d)
SI [✔] NO [ ]	Contiene una o más reivindicaciones. (Art.26-c).
SI [ ] NO [ ]	NO ES APLICABLE [✔] Han sido desarrollados los productos y/o procedimientos a partir de recursos genéticos o de sus productos derivados de los que cualquiera de los Países Miembros es país de origen. (Art. 26- h).
SI [ ] NO [ ]	NO ES APLICABLE [✔]Los productos y/o procedimientos han sido obtenidos o desarrollados a partir de los conocimientos tradicionales de las comunidades indígenas, afroamericanas, o locales de los países miembros, de acuerdo a lo establecido en la Decisión 391 y sus modificaciones y reglamentaciones vigentes.(Art.26-I).
SI [ ] NO [✔]	La invención se refiere a un producto relativo a un material biológico. (Art.26-J).
SI [✔] NO [ ]	La prioridad coincide con la materia reivindicada.
SI [✔] NO [ ]	**CUMPLE LOS REQUISITOS TÉCNICOS**

EXAMINADOR TÉCNICO
202048

Bogotá D. C. Junio 11 2003.

Concepto técnico no. 546
OBSERVACIONES

- Anexa copia auténtica certificada de la solicitud, con la cúal invoca prioridad, con sus debida traduccion. Folios del 81 al 115
- Envia copia formal de los dibujos, folios del 71 al 80

EXAMINADOR TECNICO
202048

Bogotá, Junio 11 de 2003.

Carrera 13 No. 27-00 Piso 5 y 10
Conmutador: 334 12 21-2-3-4
Fax: 281 31 25 e-mail: info@dic.gov.co
Santa Fe de Bogotá D.C. - Colombia

118

Industria y Comercio
SUPERINTENDENCIA

Folio 127

2020
Bogotá DC

Doctor(a)
JOSE ANTONIO LLOREDA
Apoderado(a)

REFERENCIA:	Radicación No.	01 100340
	Trámite	002
	Evento	001
	Actuación	416
	Oficio No.	0833
	Folios	001

Teniendo en cuenta que la solicitud de privilegio de patente que se tramita bajo el expediente indicado en la referencia, reúne los requisitos de forma establecidos en los artículos 26 y 27 de la Decisión 486 de la Comisión de la Comunidad Andina, y en las demás disposiciones vigentes sobre la materia, se envía el extracto de la solicitud a la Oficina de Comunicaciones para efecto de su publicación en la Gaceta de Propiedad Industrial, conforme lo establece el artículo 40 de la misma Decisión.

Cúmplase
Dado en Bogotá DC, a los 12 JUN. 2003

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

202048

Carrera 13 No. 27-00 Piso 5 y 10
Conmutador: 334 12 21-2-3-4
Fax: 281 31 25 e-mail: info@sic.gov.co
Santa Fe de Bogotá D.C. - Colombia

Industria y Comercio
SUPERINTENDENCIA

DIVISION DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No 01-100340

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION** FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **531**, DE FECHA **29 TDE AGOSTO DE 2003**, EL TERMINO HABIL SEÑALADO POR LA LEY EXPIRA EL **26 DE NOVIEMBRE DE 2003.**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____ NO ___X_____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RADICACIÓN: 1-100340 **EDICTO No. 4158**

LA SECRETARÍA GENERAL AD-HOC
HACE SABER:

Que esta Superintendencia profirió Resolución No. 5148 de 19-MAR-2004. Que el contenido de la parte resolutiva es como sigue: El Superintendente de Industria y Comercio.

RESUELVE

ARTICULO PRIMERO: Declarar abandonada la solicitud de privilegio de patente de invención contenida en el expediente de la referencia.

ARTICULO SEGUNDO: Notificar personalmente el contenido de la presente resolución al doctor José Antonio Llareda, o a quien haga sus veces, en su calidad de apoderado de Unilever N.V., entregándole copia de la misma, advirtiéndole que contra ella procede el recurso de reposición interpuesto ante el Superintendente de Industria y Comercio, al momento de la notificación o dentro de los 5 días hábiles siguientes a ella.

NOTIFÍQUESE Y CUMPLASE
Dada en Bogotá, D.C., a los 19 MAR 2004

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO (E).

GIANCARLO MARCENARO JIMENEZ

Para notificar a los interesados, se fija el presente EDICTO en lugar visible al público en la SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO, por el término de (10) diez días hábiles contados a partir de las 8:30 a.m. de hoy.

Secretaría General Ad-Hoc 16-ABR-2004 _____ **Este edicto se desfija hoy 29-ABR-2004 a las cinco (5:00 p.m.)** _____
Secretaría General Ad-Hoc
EDICTO No. 4158

121

00004158

AVISO DE NOTIFICACION

0103/

CORREO CERTIFICADO

Bogotá D.C.

Radicacion : 01100340 00000004 Folios :001
Fecha (AMD) : 2004-04-02 12:48:35
Tramite : 002 PATENTES DE INVEN
Evento : 001 REGISTRO/DEPOSITO
Actuacion: 432 RESOLUCION

Doctor(a)
LLOREDA JOSE ANTONIO
CALLE 72 NO. 5-83 PISO 6
BOGOTA

REF: RESOLUCION No. 5146
FECHA: 19/03/2004
EXPEDIENTE No. 1 100340 000
Tramite: 2 PATENTES DE INVENCION
Evento : 1 REGISTRO/DEPOSITO/CONCESION
Actuac.: 432 RESOLUCION

Estimado doctor(a):

Comedidamente le solicito presentarse a este Despacho ubicado en la Carrera 13 No. 27 - 00 Piso Quinto(5), de Bogotá D.C., dentro del término de cinco (5) días hábiles contados a partir del envío de la presente citación, en horario de 8:30 a.m. a 5:00 p.m., jornada continua con el fin de notificarle personalmente el contenido de la resolución y expediente citados en la referencia.

De no hacerse presente en el téraino indicado anteriormente, la providencia en mención se notificará por edicto.

Secretaría General
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

122

Industria y Comercio
SUPERINTENDENCIA

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RESOLUCION 05146

Por la cual se declara abandonada una solicitud

Rad. Exp. N° 01-100340

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO (E)
en ejercicio de sus facultades legales, en especial de las que se confirieron en el artículo 4, numeral 5 del decreto 2153 de 1992, y

CONSIDERANDO:

PRIMERO: Que el artículo 44 de la Decisión 486 de la Comisión de la Comunidad Andina, establece que: "Dentro del plazo de seis meses contados desde la publicación de la solicitud, independientemente que se hubieren presentado oposiciones, el solicitante deberá pedir que se examine si la invención es patentable. Los Países Miembros podrán cobrar una tasa para la realización de este examen. Si transcurriera dicho plazo sin que el solicitante hubiera pedido que se realice el examen, la solicitud caerá en abandono".

SEGUNDO: Que el extracto de esta solicitud fue publicado en la Gaceta de la Propiedad Industrial N° 531 del 29 de agosto de 2003.

TERCERO: Que contados seis meses desde la publicación de la solicitud en la Gaceta de la Propiedad Industrial, no se presentó petición de parte del interesado, para efectuar el examen de fondo, razón por la cual se considera abandonada la solicitud en los términos del mencionado artículo 44.

RESUELVE:

ARTICULO PRIMERO: Declarar abandonada la solicitud de privilegio de patente de invención contenida en el expediente de la referencia.

ARTICULO SEGUNDO: Notificar personalmente el contenido de la presente resolución al doctor José Antonio Lloreda, o a quien haga sus veces, en su calidad de apoderado de Unilever N.V., entregándole copia de la misma, advirtiéndole que contra ella procede el recurso de reposición interpuesto ante el Superintendente de Industria y Comercio, al momento de la notificación o dentro de los 5 días hábiles siguientes a ella.

NOTIFÍQUESE Y CUMPLASE
Dada en Bogotá, D.C., a los 19 MAR. 2004

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO (E),

GIANCARLO MARCENARO JIMENEZ

Doctor
JOSÉ ANTONIO LLOREDA
Calle 72 N° 5 – 83 Piso 5.
Bogotá, D.C.

202021

Carrera 13 No. 27-00 Pisos 5, 7 y 10
Conmutador: 382 0840
Fax: 350 5220
E-mail: info@sic.gov.co
www.sic.gov.co
Bogotá, D.C., Colombia

Industria y Comercio
SUPERINTENDENCIA

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RESOLUCION 05146

Por la cual se declara abandonada una solicitud

Rad. Exp. N° 01-100340

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO (E)
en ejercicio de sus facultades legales, en especial de las que se confirieron en el artículo 4, numeral 5 del decreto 2153 de 1992, y

CONSIDERANDO:

PRIMERO: Que el artículo 44 de la Decisión 486 de la Comisión de la Comunidad Andina, establece que: "Dentro del plazo de seis meses contados desde la publicación de la solicitud, independientemente que se hubieren presentado oposiciones, el solicitante deberá pedir que se examine si la invención es patentable. Los Paises Miembros podrán cobrar una tasa para la realización de este examen. Si transcurriera dicho plazo sin que el solicitante hubiera pedido que se realice el examen, la solicitud caerá en abandono".

SEGUNDO: Que el extracto de esta solicitud fue publicado en la Gaceta de la Propiedad Industrial N° 531 del 29 de agosto de 2003.

TERCERO: Que contados seis meses desde la publicación de la solicitud en la Gaceta de la Propiedad Industrial, no se presentó petición de parte del interesado, para efectuar el examen de fondo, razón por la cual se considera abandonada la solicitud en los términos del mencionado artículo 44.

RESUELVE:

ARTICULO PRIMERO: Declarar abandonada la solicitud de privilegio de patente de invención contenida en el expediente de la referencia.

ARTICULO SEGUNDO: Notificar personalmente el contenido de la presente resolución al doctor José Antonio Lloreda, o a quien haga sus veces, en su calidad de apoderado de Unilever N.V., entregándole copia de la misma, advirtiéndole que contra ella procede el recurso de reposición interpuesto ante el Superintendente de Industria y Comercio, al momento de la notificación o dentro de los 5 dias hábiles siguientes a ella.

NOTIFÍQUESE Y CUMPLASE
Dada en Bogotá, D.C., a los

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO (E),

GIANCARLO MARCENARO JIMENEZ

Doctor
JOSÉ ANTONIO LLOREDA
Calle 72 N° 5 – 83 Piso 5.
Bogotá, D.C.

202021

Carrera 13 No 27A00 Pisos 5, 7 y 10
Conmutador: 382 0840
Fax: 350 5220
E-mail: info@sic.gov.co
www.sic.gov.co
Bogotá, D.C., Colombia

que circulen en un mismo mercado, han de presentar una similitud real para el consumidor, el que podrá entonces confundirse, que es lo que se trata de evitar mediante el examen de registrabilidad. La naturaleza o la estructura del producto, su composición física o química y aun su misma presentación, tienen sin duda menos influencia que la finalidad, para efecto de establecer similitudes o parecidos. Iguales criterios deberán seguirse para determinar la diversidad real o heterogeneidad entre productos

REPÚBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Resolución no. 5119

Ref. expediente N° 03 14259

Por la cual se resuelve un recurso de apelación
EL SUPERINTENDENTE DELEGADO PARA LA PROPIEDAD INDUSTRIAL
en ejercicio de sus facultades que le confiere el artículo 14 del decreto 2153 de 1992,

CONSIDERANDO

PRIMERO: Que mediante resolución 26366 de 2003 la División de Signos Distintivos decidió declarar infundada la oposición presentada por la sociedad Alta S.A., y negó el registro de la marca TOO CUTE (mixta), para distinguir productos comprendidos en la Clase 25ª de la Clasificación internacional de Niza, al señor Guillermo Marrero González, por considerar que se encuentra incursa en la causal de irregistrabilidad prevista en el artículo 135 literal e) de la Decisión 486 de la Comisión de la Comunidad Andina.

SEGUNDO: Que mediante escrito radicado ante esta Superintendencia dentro del término de ley, la doctora María Victoria Henao Hernández actuando como apoderada del opositor, interpuso recurso de reposición y en subsidio de apelación en contra de la decisión mencionada en el considerando anterior.

El objeto del recurso es que se revoque la decisión, y se fundamenta de la siguiente manera:

"Para apreciar la posibilidad de confusión no son determinantes los diversos detalles, sino las características típicas, la impresión general y los principales elementos del signo. En este plano tenemos en cuenta que la marca de mi mandante, la porción que el consumidor mas retiene en la mente es TOO y por ende, al observar la marca solicitada en su conjunto, encontrará la existencia de dicha palabra, los cuales lo llevará a creer que mi representada está presentando un nuevo producto con el elemento común TOO.

"Concluimos que tanto las sílabas, como las vocales, se encuentran en el mismo orden y disposición, situadas de la misma forma, donde la denominación objetada, impacta en lamente del público usuario y consumidor, al escuchar las marcas..

"Al percibir las marcas en conflicto, es evidente que no difieren en su extensión, ni en la manera como serán pronunciadas, de lo que se deduce, que el número de referentes sonoros son idénticos en las dos marcas."

TERCERO: Que para atender lo señalado en el artículo 59 inciso 2º del Código Contencioso Administrativo es preciso resolver todas las cuestiones que hayan sido planteadas y las que aparezcan con motivo del recurso:

1. Irregistrabilidad por confundibilidad

ES COPIA FIEL DEL ORIGINAL QUE REPOSA EN LOS ARCHIVOS DE ESTA SUPERINTENDENCIA
LA SECRETARIA GENERAL

Resolución No 5119

Ref expediente N° 03 14259

En este sentido, tenemos que la causal contemplada en el artículo 136 literal a) de la Decisión 486 de la Comisión de la Comunidad Andina, indica que hay que negar la solicitud de registro de marca cuando los signos que se presentan: "*Sean idénticos o se asemejen de forma que puedan inducir al público a error, a una marca anteriormente solicitada para registro o registrada por un tercero, para los mismos productos o servicios, o para productos o servicios respecto de los cuales el uso de la marca pueda inducir al público a error;*"

1.1 Concepto

La confundibilidad de la marca es noción estrechamente relacionada con el error, porque inevitablemente la confusión en la oferta de productos ó marcas similares o idénticas genera error en el consumidor, error que desde el punto de vista jurídico vicia el consentimiento. La causal de irregistrabilidad del signo idéntico o similar a otro ya registrado o solicitado en registro evita que se atente contra la distintividad de la marca y contra su identidad con el producto a que ella accede. El signo idéntico o semejante a la marca registrada representa una amenaza de introducir desorden en el mercado de productos y además de inducir a error al consumidor y al productor interesado en que se conozca la calidad y origen de su producto por medio de la identificación marcaría; también introduce desorden en el mercado de bienes y de servicios afectando la libre competencia. Por todo ello las solicitudes de registro de signos marcarios que amenacen con introducir confusión en el mercado, error en el consumidor y fallas en la identificación de los bienes y servicios, con quien los produce, no pueden dar lugar a registro.[1]

1.2. Comparación y examen de registrabilidad

Revisado los registros de la propiedad industrial, advertimos la existencia del certificado 192567 que corresponde a la marca TOOT, registrada para distinguir productos de la clase 25, vigente hasta el 25 de noviembre de 2006.

El presente estudio de confundibilidad, consiste en comparar el signo solicitado TOO CUTE, con la marca registrada TOOT de naturaleza mixta.

Teniendo en cuenta que la comparación debe atender a una visión de conjunto lo que evita el fraccionamiento de los signos y lleva a la extracción de la dimensión característica de los mismos y que ésta debe ser sucesiva, ya que el consumidor al acudir al mercado percibe las marcas de forma individualizada. Teniendo en cuenta las semejanzas y no las diferencias, ya que así actúa el consumidor, se procederá a realizar el cotejo entre estas expresiones.

[1] Proceso 38-IP-2000 de 7 de junio de 2000, marca FRITTY:

EL SECRETARIO GENERAL ADJ

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Resolución No. 5119

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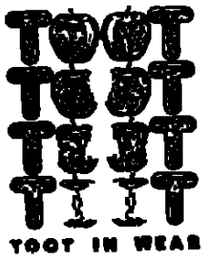

De acuerdo con las pautas antes señaladas y una vez analizadas en conjunto y sucesivamente las marcas TOO CUTE y TOOT, considera esta Delegatura que no existen semejanzas suficientes para generar confusión. En efecto la confundibilidad señalada por el recurrente radica en las semejanzas que existen entre las expresiones TOO y TOOT, sin embargo al realizar un análisis en conjunto, considera este Despacho que los signos cuentan con elementos suficientes como para permitir al consumidor diferenciar uno de otro, sin llegar a asociarlos.

De la comparación entre estas dos marcas observamos que desde el punto de vista ortográfico existen sendas diferencias, por un lado el signo solicitado se compone de dos expresiones TOO CUTE, mientras que la marca fundamento de la negación se compone solo de una TOOT, esta diferencia genera un impacto visual muy diferente, al igual que la pronunciación que se suscita.

Adicionalmente estas dos marcas en su aspecto semántico son muy diferentes y reafirma la distintividad que existe entre ellas. Por una parte la expresión TOO CUTE expresión de habla Inglesa, significa demasiado lindo, mientras que la marca fundamento de la oposición TOOT es una invención de fantasía sin contenido conceptual. Como podemos observar el consumidor retendría el aspecto ideológico de los signos que le permite mantener a salvo su derecho.

Como consideración final, cabe también resaltar que el aspecto gráfico de las marcas es muy predominante en cada signo, y coadyuvan a reafirmar la distinvidad de estas dos marcas.

1.3 Relación de productos

Debido a la ausencia de confusión entre estas dos marcas, no es necesario demostrar la relación que existe entre los productos que distinguen.

Por lo tanto, la marca objeto de la solicitud no está comprendida en la causal de irregistrabilidad establecida en el artículo 136 literal a) de la Decisión 486 de la Comisión de la Comunidad Andina.

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RESUELVE:

ARTÍCULO PRIMERO. Confirmar la decisión contenida en la resolución 26366 de 2003.

ARTICULO SEGUNDO. Notificar personalmente a los doctores Eduardo Marrero González y Maria Victoria Henao Hernández, apoderados del solicitante y del opositor respectivamente, o a quienes hagan sus veces el contenido de la presente resolución, entregándoles copia de la misma, advirtiéndoles que contra ella no procede recurso alguno, por encontrarse agotada la vía gubernativa.

Notifíquese y Cúmplase
Dado en Bogotá D.C., a los 18 MAR. 2004

El Superintendente delegado para la Propiedad Industrial,

GIANCARLO MARCENARO JIMENEZ

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