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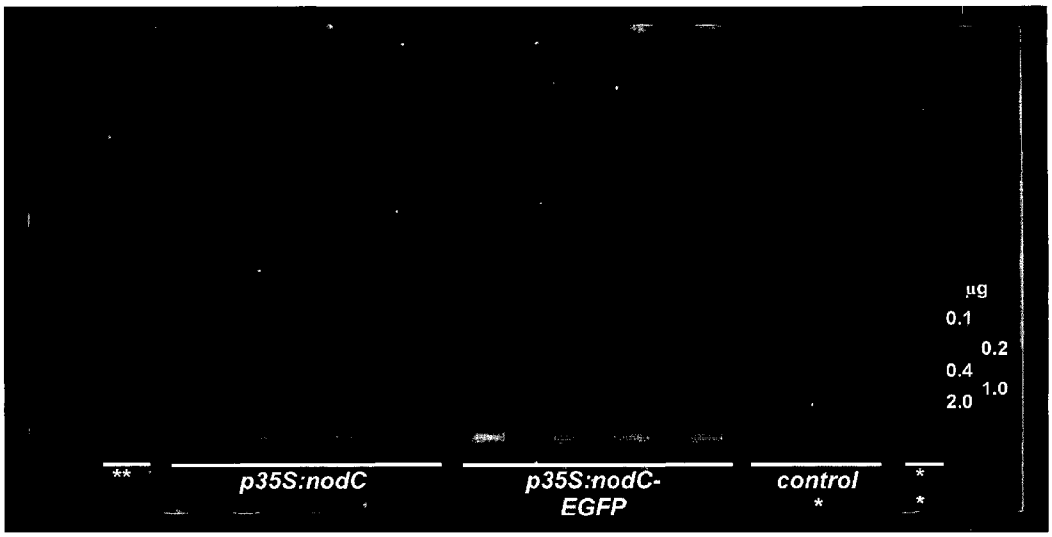
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21 June 2007

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR ALTERING THE REACTIVITY OF PLANT CELL WALLS



(57) Abstract: Methods and means are provided for the modification of the reactivity of plant cell walls, particularly as they can be found in natural fibers of fiber producing plants by inclusion of positively charged oligosaccharides or polysaccharides into the cell wall. This can be conveniently achieved by expressing a chimeric gene encoding an N-acetylglucosamine transferase, particularly an N-acetylglucosamine transferase, capable of being targeted to the membranes of the Golgi apparatus in cells of a plant.

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A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82 C12N15/54 C12N5/04 C12N5/10 A01H5/00
A01H5/10
ADD. D06M101/06
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, Sequence Search, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/09729 A2 (PIONEER HI BRED INT [US]; DHUGGA KANWARPAL S [US]; ANDERSON PAUL C [US) 24 February 2000 (2000-02-24) cited in the application the whole document	1-14,16, 19, 22-30,40
A	WO 99/07830 A (YISSUM RES DEV CO [IL]; SHOSEYOV ODED [IL]; SHANI ZIV [IL]; SHPIEGL ET) 18 February 1999 (1999-02-18) page 70 - page 71 page 76 - page 78	

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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Date of the actual completion of the international search	Date of mailing of the international search report
13 December 2006	16. 02. 2007

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bucka, Alexander

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HAIGLER CANDACE H ET AL: "Trangenic cotton over-expressing sucrose phosphate synthase produces higher quality fibers with increased cellulose content and has enhanced seedcotton yield" INTERNET CITATION, [Online] 2000, XP002365014 Retrieved from the Internet: URL:& ANNUAL MEETING OF THE AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS> [retrieved on 2006-01-30] the whole document	
A	WOJTASZEK PRZEMYSLAW ET AL: "Secondary cell-wall-specific glycoprotein(s) from French bean hypocotyls" PLANT PHYSIOLOGY (ROCKVILLE), vol. 108, no. 3, 1995, pages 1001-1012, XP002411039 ISSN: 0032-0889 figures 1,2	
A	ODA YOSHIHISA ET AL: "Regulation of secondary cell wall development by cortical microtubules during tracheary element differentiation in Arabidopsis cell suspensions" PLANT PHYSIOLOGY (ROCKVILLE), vol. 137, no. 3, March 2005 (2005-03), pages 1027-1036, XP002411040 ISSN: 0032-0889 figure 7	
A	WO 99/29879 A (VON SCHAEWEN ANTJE [DE]) 17 June 1999 (1999-06-17) pages 17,20; examples 2,3	
A	GOMEZ L AND CHRISPEELS M J: "Complementation of an Arabidopsis thaliana mutant that lacks complex asparagine-linked glycans with the human cDNA encoding N-acetylglucosaminyltransferase I" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US, vol. 91, March 1994 (1994-03), pages 1829-1833, XP002100921 ISSN: 0027-8424 page 1831	1-14,16, 19, 22-30,40

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/005853

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GEREMIA ROBERTO A ET AL: "The NodC protein of Azorhizobium caulinodans is an N-acetylglucosaminyltransferase" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 91, no. 7, 1994, pages 2669-2673, XP002411331 ISSN: 0027-8424 the whole document	1-14,16, 19, 22-30,40
A	STRASSER RICHARD ET AL: "Unaltered complex N-glycan profiles in Nicotiana benthamiana despite drastic reduction of beta1,2-N-acetylglucosaminyltransferase I activity" GLYCOCONJUGATE JOURNAL, vol. 21, no. 5, 2004, pages 275-282, XP019207019 ISSN: 0282-0080 figure 1	1-14,16, 19, 22-30,40
A	WO 99/01558 A (UNIV CAMBRIDGE TECH [GB]; DUPREE PAUL [GB]; MOEGELSVANG SOEREN [GB]) 14 January 1999 (1999-01-14) page 17	1-14,16, 19, 22-30,40
A	JOERSBO MORTEN ET AL: "In vivo modification of the cell wall polysaccharide galactomannan of guar transformed with a alpha-galactosidase gene cloned from senna" MOLECULAR BREEDING, vol. 7, no. 3, 2001, pages 211-219, XP002411311 ISSN: 1380-3743 figure 4; table 1	1-14,16, 19, 22-30,40
A	WO 01/17333 A1 (UNIV TEXAS TECH [US]) 15 March 2001 (2001-03-15) tables 2,8	
A	PARK Y W ET AL: "Enhancement of growth and cellulose accumulation by overexpression of xyloglucanase in poplar" FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 564, no. 1-2, 23 April 2004 (2004-04-23), pages 183-187, XP004503613 ISSN: 0014-5793 figures 2,4	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2006/005853

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-14, 16, 19, 23-25, 27-30, completely, 22, 26, 40, partially ...

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-14, 16, 19, 23-25, 27-30, completely, 22, 26, 40, partially and as far as applicable

   methods for increasing the amount of positively charged oligosaccharides or polysaccharides in the cell wall, particularly the secondary cell wall of a plant cell, said method comprising introducing a chimeric gene into the plant cell, said chimeric gene comprising a plant-expressible promoter, a DNA region coding for an N-acetylglucosamine transferase, wherein said N-acetylglucosamine transferase can be targeted to the membranes of the Golgi-apparatus, and a transcription termination and polyadenylation region; products obtainable by said method; uses of a chimeric gene comprising a DNA region coding for an N-acetylglucosamine transferase to modify the amount of positively charged oligosaccharides or polysaccharides

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2. claims: 15, 17, completely, 40, partially and as far as applicable

   a plant cell wall comprising an increased amount of polysaccharides or oligosaccharides, particularly positively charged oligosaccharides, such as oligo-N acetylglucosamines or oligo-glucosamines, preferably oligomers of N-acetylglucosamine or glucosamine with a polymerization degree between 3 and 10, particularly between 3 and 5

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3. claims: 18, 20, 21, completely, 22, 26, 40, partially and as far as applicable

   a secondary plant cell wall capable of binding wheat germ agglutinin

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4. claims: 31-39, completely

   a chimeric gene comprising a plant-expressible promoter, a DNA region coding for an N-acetylglucosamine transferase, said N-acetylglucosamine transferase when expressed in a plant cell, can be targeted to the Golgi apparatus of said plant cell, and a transcription termination and polyadenylation region

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5. claims: 41, completely

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

a method for increasing the amount of positively charged oligosaccharides or polysaccharides in the cell wall, particularly the secondary cell wall of a plant cell, said method comprising introducing a chimeric gene into the plant cell, said chimeric gene comprising a plant-expressible promoter, a DNA region coding for chitin synthase, and a transcription termination and polyadenylation region, and applying to said plant cell an effective amount of N-acetylglucosamine, glucosamine-6-phosphate, N-acetylglucosamine-6-phosphate, N-acetylglucosamine-1-phosphate or UDP-N-acetylglucosamine

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6. claims: 42, 43, completely

use of a DNA region coding for an N-acetylglucosamine transferase to increase the reactivity of plant cell walls for chemical modifications of such plant cell walls

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2006/005853

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0009729 A2	24-02-2000	AU 5561099 A	06-03-2000
WO 9907830 A	18-02-1999	AU 736192 B2	26-07-2001
		AU 8459398 A	01-03-1999
		BR 9810818 A	02-01-2002
		CA 2298068 A1	18-02-1999
		EP 1009805 A1	21-06-2000
		ID 24088 A	06-07-2000
		JP 2001512672 T	28-08-2001
		NZ 502169 A	30-11-2001
WO 9929879 A	17-06-1999	AT 302853 T	15-09-2005
		AU 760394 B2	15-05-2003
		AU 2268899 A	28-06-1999
		CA 2312734 A1	17-06-1999
		DE 19754622 A1	10-06-1999
		EP 1038014 A1	27-09-2000
		JP 2002509696 T	02-04-2002
		US 6653459 B1	25-11-2003
WO 9901558 A	14-01-1999	AU 8226598 A	25-01-1999
WO 0117333 A1	15-03-2001	AT 337701 T	15-09-2006
		AU 784405 B2	30-03-2006
		AU 7119000 A	10-04-2001
		BR 0013903 A	11-06-2002
		CN 1390089 A	08-01-2003
		EP 1220602 A1	10-07-2002
		MX PA02002497 A	30-07-2002
		TR 200201477 T2	21-11-2002
		US 2003070191 A1	10-04-2003
		US 6472588 B1	29-10-2002
		ZA 200201855 A	02-10-2002