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(54) Title: METHODS FOR SIMULTANEOUS CONTROL OF LIGNIN CONTENT AND COMPOSITION, AND CELLULOSE CONTENT IN PLANTS

(57) Abstract: The present invention relates to a method of concurrently introducing multiple genes into plants and trees is provided. The method includes simultaneous transformation of plants with multiple genes from the phenylpropanoid pathways including 4CL, CAld5H, AldOMT, SAD and CAD genes and combinations thereof to produce various lines of transgenic plants displaying altered agronomic traits. The agronomic traits of the plants are regulated by the orientation of the specific genes and the selected gene combinations, which are incorporated into the plant genome.



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## INTERNATIONAL SEARCH REPORT

International application No. -

PCT/US01/27445

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : C12N 15/82, 15/87, 15/29; A01H 5/00

US CL : 800/278, 285, 286, 287, 289, 290; 435/468

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)

U.S. : 800/278, 285, 286, 287, 289, 290; 435/468

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 practicable, search terms used)  
WEST, STN**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	KAJITA, S. et al. Alterations in the biosynthesis of lignin in transgenic plants with chimeric genes for 4-Coumarate:Coenzyme A ligase. Plant Cell Physiology. 1996, Vol. 37, No. 7, pages 957-965, entire document.	7, 8, 38-41, 47, 50, 54, 57-60, 64 ----- 1-6, 9-37, 42-46, 48- 49, 51-53, 55-56, 61- 63, 65-72
X --- Y	OSAKABE, K. et al. Coniferyl aldehyde 5-hydroxylation and methylation direct syringyl lignin biosynthesis in angiosperms. PNAS. August 1999, Vol. 96, pages 8955-8960, entire document.	57-59 ----- 1-56, 60-72



Further documents are listed in the continuation of Box C.



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	"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
"E" earlier application or patent published on or after the international filing date	"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
"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)	"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
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/27445

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

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

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim 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. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation 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.: for claims not drawn to CAD
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.:

Remark on Protest

☒  
☐

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

No protest accompanied the payment of additional search fees.

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING**

This is a revised Lack of Unity.

- I. Claims 1-6, 9-37, 44-46, 48-49, 51-53, 55-63, and 65-72 are drawn to a method of genetically transforming a plant simultaneously with multiple genes, a plant transformed with multiple genes, and a plurality of DNA constructs.
- II. Claims 7, 8, 38-43, 47, 50, 54, and 64 are drawn to a method of transforming a plant with a 4CL gene, and a plant comprising said isolated gene.
- III. Claims 38-43, and 50 are drawn to a method of transforming a plant with a Cald5H gene, and a plant comprising said isolated gene.
- IV. Claims 38-43, and 50 are drawn to a method of transforming a plant with a AldOMT gene, and a plant comprising said isolated gene.
- V. Claims 38-43, and 50 are drawn to a method of transforming a plant with a SAD gene, and a plant comprising said isolated gene.
- VI. Claims 38-43, and 50 are drawn to a method of transforming a plant with a CAD gene, and a plant comprising said isolated gene.

The species listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

Groups I-VI are drawn to transgenic plants and methods of producing said plants with nucleic acid sequences. The methods of Groups I-VI differ from each other in that they are directed to plant transformation methods and transgenic plants with structurally and functionally distinct nucleic acid sequences which encode structurally and functionally different amino acid sequences.

Furthermore, the claims are not linked by a single special technical feature because they do not constitute an advance over the prior art. At least group VI is taught by Boudet et al (U.S. patent 5,451,514) teach a method of altering lignin composition in a plant by transforming the plant with a nucleotide sequence in antisense orientation, said nucleotide sequence being derived from a CAD gene involved in lignin synthesis (see, e.g., claims 1-2, 6, and 11).

It is acknowledged that a protest has been filed. The protest will be considered and a response will be mailed.