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(54) Title: GENETICALLY TRANSFORMED CASSAVA CELLS AND REGENERATION OF TRANSGENIC CASSAVA PLANTS

(57) Abstract

The invention relates to methods for the stable genetic transformation and regeneration of Cassava.

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INTERNATIONAL SEARCH REPORT

Intern ial Application No
PCT/EP 97/02201

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 A01N63/00 A01H5/00 A01H4/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01N A01H

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHOEPKE C ET AL: "STABLE TRANSFORMATION OF CASSAVA PLANTS (MANIHOT ESCULENIA CRANTZ) BY PARTICLE BOMBARDMENT OF EMBRYOGENIC SUSPENSION CULTURES" IN VITRO CELLULAR AND DEVELOPMENTAL BIOLOGY, vol. 32, no. 3, PART 02, March 1996, page 67A XP002021188	1,2, 5-11, 15-20, 25-30
Y	see abstract P-1003	3,12-14

Y	WO 92 02139 A (AGRICULTURAL GENETICS CO) 20 February 1992 see page 5, line 1 - line 3	3

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

[X] Patent family members are listed in annex.

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

"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)

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

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"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
28 July 1997	18. 11. 97
Name and mailing address of the ISA 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	Authorized officer MADDOX, A

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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/EP 97/02201

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 26634 A (AXIS GENETICS LIMITED ;GEOGHEGAN IRENE (GB); ROBERTSON WALTER (GB)) 12 October 1995 see page 7, paragraph 3	3
P,A	LI, H.-Q., ET AL.: "Genetic transformation of cassava (Manihot esculenta Crantz)" NATURE BIOTECHNOLOGY, vol. 14, no. 6, June 1996, pages 736-740, XP002036203 see the whole document	12-14
Y	& ARTICLES CITED AS REFERENCES 13,15,17,21-23 OF LI ET AL.,	12-14
P,X	SCHÖPKE, C., ET AL.: "Regeneration of transgenic cassava plants (Manihot esculenta Crantz) from microbombarded embryogenic suspension cultures" NATURE BIOTECHNOLOGY, vol. 14, no. 6, June 1996, pages 731-735, XP002036204 see the whole document	1,2, 5-11, 15-20, 25-30
A	KONAN N K ET AL: "OPTIMIZATION OF PLANT REGENERATION FROM EMBRYOGENIC SUSPENSION CULTURES OF CASSAVA (MANIHOT ESCULENTA CRANTZ)" IN VITRO CELLULAR AND DEVELOPMENTAL BIOLOGY, vol. 32, no. 3, PART 02, March 1996, page 89A/90A XP000615296	1-3, 5-20, 25-30
A	LUONG, H.T., ET AL.: "Transient gene expression in cassava somatic embryos by tissue electroporation" PLANT SCIENCE, vol. 107, 1995, pages 105-115, XP002035999 see the whole document	11
A	ARIAS-GARZON, D.I., ET AL.: "Optimization of transient transformation in cassava (Manihot esculenta Crantz)" PLANT PHYSIOLOGY SUPPLEMENT, vol. 108, no. 2, June 1995, page 152 XP002036205 see abstract 802	12-14

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE CAB CAB INTERNATIONAL, WALLINGFORD, OXON, GB dn 941600229, FAUQUET, C.M.: "Status of cassava regeneration and transformation" XP002036208 see abstract & CBN NEWSLETTER, (1993) VOL. 1, NO. 1, PP. 7-9.,	12-14
P,A	TAYLOR, N.J., ET AL.: "Development of friable embryogenic callus and embryogenic suspension culture systems in cassava (Manihot esculenta Crantz)" NATURE BIOTECHNOLOGY, vol. 14, no. 6, June 1996, pages 726-730, XP002036206 see the whole document	1-3, 5-20, 25-30
P,A	CHEMICAL ABSTRACTS, vol. 126, no. 15, 14 April 1997 Columbus, Ohio, US; abstract no. 197455, BELLOTTI, ANTHONY C. ET AL: "Cassava cyanogenic potential and resistance to pests and diseases" XP002036207 see abstract	3
A	& ACTA HORTIC. (1994), 375(INTERNATIONAL WORKSHOP ON CASSAVA SAFETY, 1994), 141-151,	3
P,A	RAEMAKERS C J J M ET AL: "Production of transgenic cassava (Manihot esculenta Crantz) plants by particle bombardment using luciferase activity as selection marker." MOLECULAR BREEDING 2 (4). 1996. 339-349. ISSN: 1380-3743, XP002036000 see reference 28	12-14
A	& SARRIA, R., ET AL.: "Agrobacterium mediated transformation of cassava." ABSTRACTS OF SECOND INTERNATIONAL SCIENTIFIC MEETING OF THE CASSAVA BIOTECHNOLOGY NETWORK- CBNII, 22-26 AUGUST, 1994, BOGOR, INDONESIA, 1995, P47.,	12-14

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INTERNATIONAL SEARCH REPORT

In ational application No.
PCT/EP 97/02201

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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:
Claims searched incompletely: 1-3,5-20,25-30
Reason: The wording pest resistance does not adequately define the subject matter for which protection is sought. The search has been restricted to resistance to invertebrate pests.

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 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:

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.:

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.:

1-3,5-20,25-30 all partially (subject 1.)

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

1: Claims 1-3, 5-20, 25-30 all partially.

Method for the introduction of pest resistance trait in cassava, by stable genetic transformation with DNA whose expression confers said resistance. Cassava cell suspension, tissues and transgenic plants obtained by the method.

2: Claims 1-20,25-30 all partially.

Method for the introduction of disease resistance trait in cassava, by stable genetic transformation with DNA whose expression confers said resistance. Said DNA encoding for example viral coat protein, viral replicases, viral trans-activators, viral movement proteins, viral transmission factors. Cassava cell suspension, tissues and transgenic plants obtained by the method.

3: Claims 1-3, 5-20, 25-30 all partially.

Method for the introduction of environmental stress resistance in cassava, by stable genetic transformation with DNA whose expression confers said resistance. Cassava cell suspension, tissues and transgenic plants obtained by the method.

4: Claims 1-20, 25-30 all partially.

Method for the introduction of improved starch quality/quantity trait in cassava, by stable genetic transformation with DNA whose expression confers said trait. Said DNA encoding for example antisense DNA for cassava GBSS (Granule bound starch synthase), and starch modifying enzymes. Cassava cell suspension, tissues and transgenic plants obtained by the method.

5: Claims 1-3, 5-20, 25-30 all partially.

Method for the introduction of increased tuber protein-content trait in cassava, by stable genetic transformation with DNA whose expression confers said trait. Cassava cell suspension, tissues and transgenic plants obtained by the method.

6: Claims 1-20, 25-30 all partially.

Method for the introduction of foreign secondary metabolite or foreign protein trait in cassava, by stable genetic transformation with DNA whose expression confers said trait. Said DNA encoding for example proteins involved in the production of PHA/PHB's. Cassava cell suspension, tissues and transgenic plants obtained by the method.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

7: Claims 1-20, 25-30 all partially.

Method for the introduction of reduced cyanogenic glucoside trait in cassava by stable genetic transformation with DNA whose expression confers said trait. Said DNA encoding for example, linamarase related enzymes or alpha-hydroxynitrile lyase related enzymes. Cassava cell suspension, tissues and transgenic plants obtained by the method.

8: Claims 1-3, 5-20, 25-30 all partially.

Method for the introduction of extended shelf-life trait in cassava, by stable genetic transformation with DNA whose expression confers said trait. Cassava cell suspension, tissues and transgenic plants obtained by the method.

9: Claims 1-20, 25-30 all partially.

Method for the introduction of traits not covered by any previous group of claims in cassava, by stable genetic transformation with DNA whose expression confers said trait. Cassava cell suspension, tissues and transgenic plants obtained by the method.

10: Claims 21-24 all completely, 29 partially.

Method for regeneration of transformed embryogenic cassava structures using the starting tissues and culture conditions as defined in claims 21-24.

Note that further searches may give rise to further objections concerning lack of unity.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr nal Application No
PCT/EP 97/02201

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9202139 A	20-02-92	AU 654496 B	10-11-94
		AU 8315291 A	02-03-92
		EP 0542833 A	26-05-93
		JP 6502299 T	17-03-94
		US 5545820 A	13-08-96
WO 9526634 A	12-10-95	AU 2079895 A	23-10-95
		CA 2186737 A	12-10-95
		EP 0752816 A	15-01-97