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(54) Title: IMPROVED METHODS FOR THE PRODUCTION OF STABLY TRANSFORMED, FERTILE ZEA MAYS PLANTS

(57) Abstract: The present invention relates to improved methods for the incorporation of DNA into the genome of a *Zea mays* plant by means of *Agrobacterium*-mediated transformation. Preferred is the use of the Zea may lines deposited with American Type Culture Collection under the Patent Deposit Designation PTA-6170 and PTA-6171.

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# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/063448

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82 A01H5/10

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 2004/016030 A1 (LOWE BRENDA A [US] ET AL) 22 January 2004 (2004-01-22) example 5 | 1-15 |
| X | US 6 420 630 B1 (WILSON HERBERT MARTIN [US] ET AL) 16 July 2002 (2002-07-16) example 2 | 1-15 |
| A | WO 98/32326 A2 (PIONEER HI BRED INT [US]) 30 July 1998 (1998-07-30) example 4 | 1-15 |

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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 :

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

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"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

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"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

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"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
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| 24 November 2006 | 09. 01. 2007 |

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | FRAME BRONWYN R ET AL: "Agrobacterium tumefaciens-mediated transformation of maize embryos using a standard binary vector system" PLANT PHYSIOLOGY, AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, US, vol. 129, no. 1, May 2002 (2002-05), pages 13-22, XP002263971 ISSN: 0032-0889 cited in the application page 20; table II | 1-15 |
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# INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2006/063448

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US 5 767 361 A (DIETRICH GABRIELE ELFRIEDE [US]) 16 June 1998 (1998-06-16) the whole document<br>---<br>WO 98/39419 A (ASGROW SEED CO [US]) 11 September 1998 (1998-09-11)<br>--- | |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2006/063448

## 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.:

1-15

**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: 1-15

   A method for generating a transgenic Zea mays plant using immature embryos of Zea mays and a bacterium belonging to genus Rhizobiaceae

   ---

2. claims: 16, 17, 19-24, all partially and as far as applicable

   A maize plant obtained by crossing a (HillA x A188) hybrid with an inbred-line of which representative seed have been deposited with the American Type Culture Collection under the Patent Deposit Designation PTA-6170

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3. claims: 16, 17, 19-24, all partially and as far as applicable

   A maize plant obtained by crossing a (HillA x A188) hybrid with an inbred-line of which representative seed have been deposited with the American Type Culture Collection under the Patent Deposit Designation PTA-6171

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4. claims: 18-24, all partially and as far as applicable

   A transgenic maize plant generated from transforming with a heterologous gene construct a maize plant, cell or tissue originating from a plant of which representative seed have been deposited with the American Type Culture Collection under the Patent Deposit Designation PTA-6170

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5. claims: 18-24, all partially and as far as applicable

   A transgenic maize plant generated from transforming with a heterologous gene construct a maize plant, cell or tissue originating from a plant of which representative seed have been deposited with the American Type Culture Collection under the Patent Deposit Designation PTA-617

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6. claims: 25-29

A method for subsequent transformation of at least two DNA constructs into a plant comprising the steps of:
a) a first transformation with a first construct said construct comprising a first mutated ahas selection marker gene, said first gene conferring resistance to imazethapyr but sensitive to imazaquin, and selecting plants resistant to imazethapyr, and
b) a second transformation with a second construct said construct comprising a second mutated ahas selection marker gene, said second gene conferring resistance to both imazethapyr and imazaquin, and selecting plants resistant to imazaquin;
a plant cell or plant comprising a
i) a first mutated ahas selection marker gene, said first gene conferring resistance to imazethapyr but sensitive to imazaquin, and
ii) a second mutated ahas selection marker gene, said second gene conferring resistance to both imazethapyr and imazaquin

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2006/063448

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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