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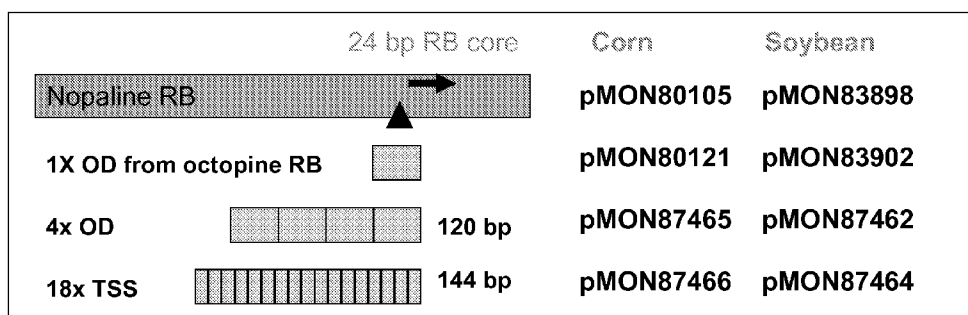
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(54) Title: USE OF MULTIPLE TRANSFORMATION ENHANCER SEQUENCES TO IMPROVE PLANT TRANSFORMATION EFFICIENCY



(57) Abstract: The present invention relates to methods and compositions for improving the efficiency of *Agrobacterium*- and *Rhizobium*-mediated plant cell transformation by use of additional transformation enhancer sequences, such as overdrive or TSS sequences, operably linked to a T-DNA border sequence on a recombinant construct that comprises T-DNA.

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2007/073788

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82

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

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, BIOSIS, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SHURVINTON C E ET AL: "STIMULATION OF AGROBACTERIUM-TUMEFACIENS T DNA TRANSFER BY OVERDRIVE DEPENDS ON A FLANKING SEQUENCE BUT NOT ON HELICAL POSITION WITH RESPECT TO THE BORDER REPEAT" JOURNAL OF BACTERIOLOGY, vol. 173, no. 17, 1991, pages 5558-5563, XP002464019 ISSN: 0021-9193 cited in the application figure 1; table 1 ----- -/--	1-5,7,9, 10, 14-17, 21-25, 27-29



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
- *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.
- *Z* document member of the same patent family

Date of the actual completion of the international search

11 January 2008

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INTERNATIONAL SEARCH REPORT

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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.
X	PERALTA E G ET AL: "OVERDRIVE, A T-DNA TRANSMISSION ENHANCER ON THE A. TUMEFACIENS TUMOUR-INDUCING PLASMID" EMBO JOURNAL, IRL PRESS, EYNSHAM, GB, vol. 5, 1986, pages 1137-1142, XP000910809 ISSN: 0261-4189 cited in the application the whole document	1
X	WO 00/18939 A (PIONEER HI BRED INT [US]; BIDNEY DENNIS L [US]; SCELONGE CHRISTOPHER J) 6 April 2000 (2000-04-06) page 7, line 14 - line 17	1
X	WO 2006/024509 A (BASF PLANT SCIENCE GMBH [DE]; MANKIN LUKE [US]; HILL DWIGHT-STEVEN [US]) 9 March 2006 (2006-03-09) paragraph [0198]	1
A	TORO N ET AL: "ROLE OF THE OVERDRIVE SEQUENCE IN T DNA BORDER CLEAVAGE IN AGROBACTERIUM" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 85, no. 22, 1988, pages 8558-8562, XP002464020 ISSN: 0027-8424 cited in the application the whole document	
A	US 5 608 142 A (BARTON KENNETH A [US] ET AL) 4 March 1997 (1997-03-04) column 9; figure 1	
A	US 2006/070137 A1 (ROMMENS CAIUS [US] ET AL) 30 March 2006 (2006-03-30) paragraph [0075]; claim 11	

INTERNATIONAL SEARCH REPORT

International application No.
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Box No. 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 No. 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 allsearchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
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, 4, 7-18, 21, 22, 23, 24, 25-29 partially; 2, 5, 19 completely

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ 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: 1,3,4,7-18,21,22,23,24,25-29 partially, 2,5,19 completely

Method of increasing the efficiency of bacterially-mediated plant transformation comprising introducing at least one additional *Agrobacterium tumefaciens*-specific transformation enhancer sequence, said additional sequence comprising the sequence selected from SEQID20, SEQID7, SEQID8, SEQID9, SEQID13 or SEQID10 or a complementary sequence thereto; said method wherein said sequence is located proximal of a T-DNA right border (RB) sequence; said method wherein said transformation is mediated by *A. tumefaciens*, *Rhizobia*, *Sinorhizobium*, *Mesorhizobium*, *Bradyrhizobium*; a recombinant DNA construct comprising a T-DNA border sequence operably linked to a sequence selected from SEQID20, SEQID7, SEQID8, SEQID9, SEQID13 and wherein the enhancer sequence comprises at least 4 copies of said sequence; said recombinant construct comprising SEQID10; said construct wherein the RB is originating from either a nopaline or an octopine Ti plasmid; a transgenic cell transformed with said construct, said cell being an *Agrobacterium*, a *Rhizobium* or a plant cell; a transgenic plant transformed with said construct.

2. claims: 1,3,4,7-18,21,22,23,24,25-29 partially, 6,20 completely

Method of increasing the efficiency of bacterially-mediated plant transformation comprising introducing at least one additional *Agrobacterium rhizogenes*-specific transformation enhancer sequence, said additional sequence comprising the sequence selected from SEQID6 or a complementary sequence thereto; said method wherein said sequence is located proximal of a T-DNA right border (RB) sequence and wherein said sequence is from an Ri plasmid of *A. rhizogenes*; said method wherein said transformation is mediated by *A. tumefaciens*, *Rhizobia*, *Sinorhizobium*, *Mesorhizobium*, *Bradyrhizobium*; said method wherein said transformation enhancer sequence comprises SEQID11; a recombinant DNA construct comprising a T-DNA border sequence operably linked to a sequence selected from SEQID6 and wherein the enhancer sequence comprises at least 4 copies of said sequence; said recombinant construct comprising SEQID11; said construct wherein the RB is originating from either a nopaline or an octopine Ti plasmid; a transgenic cell transformed with said construct; said cell being an *Agrobacterium*, a *Rhizobium* or a plant cell; a transgenic plant transformed with said construct.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2007/073788

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