

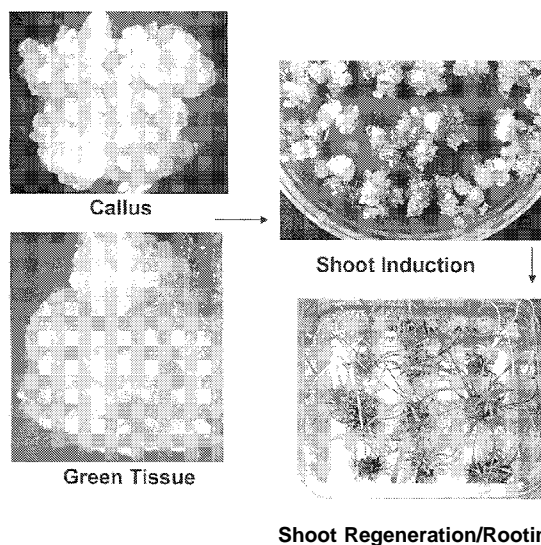


- (51) **International Patent Classification:**  
*CI2N 15/82* (2006.01) *A01H 4/00* (2006.01)
- (21) **International Application Number:**  
PCT/US20 12/053003
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61/529,593 31 August 2011 (31.08.2011) US
- (71) **Applicants (for all designated States except US):** PIONEER HI-BRED INTERNATIONAL, INC. [US/US]; 7100 N.W. 62nd Avenue, Johnston, Iowa 50131-1014 (US). E. I. DUPONT DE NEMOURS & COMPANY [US/US]; 1007 Market Street, Wilmington, Delaware 19898 (US).
- (72) **Inventors; and**
- (75) **Inventors/Applicants (for US only):** CHO, Myeong-Je [US/US]; c/o Pioneer Hi-Bred International, Inc., 7250 N.W. 62nd Avenue, Johnston, Iowa 50131-0552 (US). KLEIN, Theodore M. [US/US]; c/o Pioneer Hi-Bred International, Inc., 7250 N.W. 62nd Avenue, Johnston, Iowa 50131-0552 (US). ZHAO, Zuo-Yu [US/US]; c/o Pioneer Hi-Bred International, Inc., 7250 N.W. 62nd Avenue, Johnston, Iowa 50131-0552 (US).
- (74) **Agent:** BAUER, S. Christopher; Pioneer Hi-Bred International, Inc., 7250 N.W. 62nd Avenue, Johnston, Iowa 50131-0552 (US).
- (81) **Designated States (unless otherwise indicated, for every kind of national protection available):** AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) **Designated States (unless otherwise indicated, for every kind of regional protection available):** ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,

[Continued on nextpage]

(54) **Title:** METHODS FOR TISSUE CULTURE AND TRANSFORMATION OF SUGARCANE

FIGURE 2



(57) **Abstract:** Compositions and methods for the efficient transformation and regeneration of monocot plants are provided. The methods of transformation involve infection with *Agrobacterium*. In this manner, any gene of interest can be introduced into the monocot plant with high transformation efficiency and in low copy number. Transformed and regenerated monocot cells, tissues, plants, and seed are also provided. The invention encompasses regenerating transformed plants, transgenic seeds produced therefrom, and transgenic plants and transgenic seeds from subsequent generations.



WO 2013/033308 A3

UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,  
 TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,  
 EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,  
 LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,  
 SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,  
 GW, ML, MR, NE, SN, TD, TG).

**Published:**

- with international search report (Art. 21(3))
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27 June 2013

**Declarations under Rule 4.17:**

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.1 7(H))

# INTERNATIONAL SEARCH REPORT

International application No <b>PCT/US2012/053003</b>
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A. CLASSIFICATION OF SUBJECT MATTER  
**INV. C12N15/82 A01H4/00**  
 ADD.

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 practicable, search terms used)  
**EPO-Internal , BIOSIS, CHEM ABS Data, Sequence Search , EMBASE, WPI Data**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>Stevens M. Brumbley ET AL: "Sugarcane"            In: "Compendium of Transgenic Crop Plants :            Transgenic Sugar, Tuber and Fiber Crops .",            1 January 2009 (2009-01-01) , Blackwell            Publishing Ltd. , XP055047005 ,            ISBN : 978-1-40-516924-0            pages 1-58, DOI :  <a href="http://dx.doi.org/10.1002/9781405181099.ko701">http://dx.doi.org/10.1002/9781405181099.ko701</a> ,            page 8, column 1, paragraph 1 - page 9,            column 1, paragraph 1; figure 1</p> <p style="text-align: center;">-----            -/- .</p>	1-9

Further documents are listed in the continuation of Box C.       See patent family annex.

\* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"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</p> <p>"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</p> <p>"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</p> <p>"&amp;" document member of the same patent family</p>
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Date of the actual completion of the international search <b>11 December 2012</b>	Date of mailing of the international search report <b>08/03/2013</b>
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  <p style="text-align: center;"><b>Burkhardt, Peter</b></p>
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## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/053003

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>SANDY J SNYMAN ET AL: "Appl i cati ons of cul ture systems for commerci al sugarcane producti on and improvement" ,  IN VITRO CELLULAR &amp; DEVELOPMENTAL BIOLOGY  - PLANT, SPRINGER-VERLAG, NEW YORK,  vol . 47, no. 2,  27 February 2011 (2011-02-27) , pages  234-249 , XP019905660,  ISSN: 1475-2689 , DOI :  10. 1007/S11627-011-9354-7  page 235 , col umn 2, paragraph 2 - page  236, col umn 2, paragraph 1; figures 1,2  page 243 , col umn 1, paragraph 3-4; tabl e 1</p> <p>-----</p>	1-9
Y	<p>LAKSHMANAN PRAKASH ET AL: "Sugarcane  biotechnol ogy: The chal lenges and  opportuni ties" ,  IN VITRO CELLULAR &amp; DEVELOPMENT BIOLOGY.  PLANT, GAITHERSBURG, MD, US,  vol . 41, no. 4, 1 July 2005 (2005-07-01) ,  pages 345-363 , XP009100033 ,  ISSN: 1054-5476, DOI: 10. 1079/ IVP2005643  page 346, col umn 2, paragraph 2 - page  347 , col umn 2, paragraph 2</p> <p>-----</p>	1-9
A	<p>wo 2010/068521 AI (SYNGENTA PARTICI PATIONS  AG [CH] ; ZHONG HENG [US] )  17 June 2010 (2010-06-17)  the whol e document</p> <p>-----</p>	1-9

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2012/053003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010068521 A1	17-06-2010	AR 074605 A1	26-01-2011
		AU 2009324843 A1	23-06-2011
		CA 2746196 A1	17-06-2010
		CN 102307998 A	04-01-2012
		EP 2356244 A1	17-08-2011
		US 2011321199 A1	29-12-2011
		WO 2010068521 A1	17-06-2010
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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2012/053003

## Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
  - a. (means)
    - on paper
    - in electronic form
  - b. (time)
    - in the international application as filed
    - together with the international application in electronic form
    - subsequently to this Authority for the purpose of search
2.  In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2012/053003

## 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 all searchable 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. :

see additional sheet(s)

### 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: 1-9

relating to a method of regenerating a plant

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2. claims: 10-17

relating to a method of producing a transformed plant

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3. claims: 18-27

relating to a method of producing a transformed plant by Agrobacterium-mediated transformation

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