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(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, 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, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, 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))
- with sequence listing part of description (Rule 5.2(a))

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(54) Title: METHODS FOR PRODUCING A COMPLEX TRANSGENIC TRAIT LOCUS

(57) Abstract: Methods for producing in a plant a complex transgenic trait locus comprising at least two altered target sequences in a genomic region of interest are disclosed. The methods involve the use of two or more double-strand-break-inducing agents, each of which can cause a double-strand break in a target sequence in the genomic region of interest which results in an alteration in the target sequence. Also disclosed are complex transgenic trait loci in plants. A complex transgenic trait locus comprises at least two altered target sequences that are genetically linked to a polynucleotide of interest. Plants, plant cells, plant parts, and seeds comprising one or more complex transgenic trait loci are also disclosed.



INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2012/030061

A. CLASSIFICATION OF SUBJECT MATTER  
 INV. C12N15/82 A01H1/06 A01H5/10 C12N15/55  
 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, 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	TOWNSEND JEFFREY A ET AL: "High-frequency modification of plant genes using engineered zinc-finger nucleases", NATURE: INTERNATIONAL WEEKLY JOURNAL OF SCIENCE, NATURE PUBLISHING GROUP, UNITED KINGDOM, vol. 459, no. 7245, 21 May 2009 (2009-05-21), pages 442-445, XP002588972, ISSN: 0028-0836, DOI: 10.1038/NATURE07845 the whole document	1-7, 10-22, 25-64, 67-72, 75-79
X	WO 2009/042164 A1 (DOW AGROSCIENCES LLC [US]; SANGAMO BIOSCIENCES INC [US]; GUPTA MANJU []) 2 April 2009 (2009-04-02)  examples 4-6	1-7, 10-22, 25-64, 67-72, 75-79

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 application or patent 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
- "&" document member of the same patent family

Date of the actual completion of the international search  8 June 2012	Date of mailing of the international search report  06/11/2012
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  Maddox, Andrew

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/030061

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	QUE Q., ET AL.: "Trait stacking in transgenic crops: challenges and opportunities", GM CROPS, vol. 1, no. 4, October 2010 (2010-10), pages 220-229, XP002677400, the whole document particularly page 225 -----	1-7, 10-22, 25-79
X	WO 2009/114321 A2 (PREC BIOSCIENCES INC [US]; JANTZ DEREK [US]; SMITH JAMES JEFFERSON [US]) 17 September 2009 (2009-09-17)  paragraph [0024] -----	1-7, 10-22, 25-64, 67-72, 75-79
X	WO 2010/077319 A1 (DOW AGROSCIENCES LLC [US]; SANGAMO BIOSCIENCES INC [US]; BUTLER HOLLY) 8 July 2010 (2010-07-08)  paragraph [0117] -----	1-7, 10-22, 25-64, 67-72, 75-79
X	WO 2008/148559 A1 (BAYER BIOSCIENCE NV [BE]; BAYER CROPSCIENCE SA [FR]; ROLLAND ANNE [FR]) 11 December 2008 (2008-12-11)  claims 1-15 -----	1-7, 10-22, 25-64, 67-72, 75-79
X,P	WO 2011/117249 A1 (PHILIP MORRIS PROD [CH]; OISHI KAREN KEIKO [CH]; FLORACK DIONISIUS ELI) 29 September 2011 (2011-09-29)  examples 1-9 -----	1-7, 10-22, 25-64, 67-72, 75-79
A	WO 2009/006297 A2 (PIONEER HI BRED INT [US]; LYZNIK L ALEKSANDER [US]; TAO YUMIN [US]; GA) 8 January 2009 (2009-01-08) the whole document -----	1-79

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2012/030061

## 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.:

7, 22, 64, 72(completely); 1-6, 10-21, 25-63, 67-71, 75-79(partially)

### 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: 7, 22, 64, 72(completely); 1-6, 10-21, 25-63, 67-71, 75-79(partially)

Transgenic trait locus, plant, methods, etc defined by reference to the target region covered by SEQ ID NOS:1 - 4 and nucleases SEQ ID NOS:9 - 12.

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2. claims: 8, 23, 65, 73(completely); 1-6, 10-21, 25-63, 67-71, 75-79(partially)

Transgenic trait locus, plant, methods, etc defined by reference to the target region covered by SEQ ID NOS:5-8 and nucleases SEQ ID NOS:13 -16.

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3. claims: 9, 24, 66, 74(completely); 1-6, 10-21, 25-63, 67-71, 75-79(partially)

Transgenic trait locus, plant, methods, etc defined by reference to the target region covered by SEQ ID NOS:68 - 77 and nucleases SEQ ID NOS:78 -83

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/US2012/030061
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009042164	A1	02-04-2009	AR 068559 A1 18-11-2009
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			EP 2167666 A2 31-03-2010
			US 2009133152 A1 21-05-2009
			WO 2009006297 A2 08-01-2009
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