



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁶ : C12N 15/82, A01H 5/00, C12N 5/04, A01H 4/00</p>	A3	<p>(11) International Publication Number: WO 95/06128 (43) International Publication Date: 2 March 1995 (02.03.95)</p>
<p>(21) International Application Number: PCT/US94/09699 (22) International Filing Date: 24 August 1994 (24.08.94) (30) Priority Data: 08/113,561 25 August 1993 (25.08.93) US (71) Applicant: DEKALB GENETICS CORPORATION [US/US]; 3100 Sycamore Road, Dekalb, IL 60115 (US). (72) Inventors: DAMS, Thomas, R.; 12 Mystic Road, North Stonington, CT 06329 (US). ANDERSON, Paul, C.; 9 Summit Street, Stonington, CT 06378 (US). DAINES, Richard, J.; 5 Cranwood Road, Ledyard, CT 06339 (US). GORDON-KAMM, William, J.; 3916 67th Street, Urbandale, IA 50322 (US). KAUSCH, Albert, P.; R.R. 3 #28, The Bee House, Briarpatch Road, Stonington, CT 06378 (US). MACKEY, Catherine, J.; 14-1 Matson Ridge, Old Lyme, CT 06371 (US). OROZCO, Emil, M., Jr.; 112 Shennecossett Parkway, Groton, CT 06340 (US). ORR, Peter, M.; Sunrise Avenue, Pawcatuck, CT 06379 (US). STEPHENS, Michael, A.; 9 Spring Rock Road, East Lyme, CT 06333 (US). (74) Agent: PARKER, David, L.; Arnold, White & Durkee, P.O. Box 4433, Houston, TX 77210 (US).</p>		<p>(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 14 September 1995 (14.09.95)</p>
<p>(54) Title: FERTILE, TRANSGENIC MAIZE PLANTS AND METHODS FOR THEIR PRODUCTION</p>		
<p>(57) Abstract</p>		
<p>This invention relates to a reproducible system for the production of stable, genetically transformed maize cells, and to methods of selecting cells that have been transformed. One method of selection disclosed employs the <i>Streptomyces bar</i> gene introduced by microprojectile bombardment into embryonic maize cells which were grown in suspension cultures, followed by exposure to the herbicide bialaphos. The methods of achieving stable transformation disclosed herein include tissue culture methods and media, methods for the bombardment of recipient cells with desired transforming DNA, and methods of growing fertile plants from the transformed cells. This invention also relates to the transformed cells and seeds and to the fertile plants grown from the transformed cells and to their pollen.</p>		

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 94/09699

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12N15/82 A01H5/00 C12N5/04 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 A01H C07K

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	WO,A,93 07278 (CIBA-GEIGY AG) 15 April 1993 see Claims. ---	1,12,13, 22-32
X	EP,A,0 485 970 (YEDA RESEARCH AND DEVELOPMENT COMPANY LTD.) 20 May 1992 see column 8, lines 35-38, Examples 7-13 and Claims. ---	1,21-32
Y	WO,A,91 10725 (BIOTECHNICAL INTERNATIONAL, INC.) 25 July 1991 cited in the application see page 17, lines 5-32, Table 1, Examples I-III and Claims. ---	1,2,5,8, 11-13, 21-27, 30-32
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Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

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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

- "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

5 July 1995

Date of mailing of the international search report

16.08.95

Name and mailing address of the ISA

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 Fax: (+31-70) 340-3016

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Yeats, S

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>WO,A,91 02071 (DEKALB PLANT GENETICS) 21 February 1991</p> <p>see page 10, lines 8-20, page 13, lines 7-21, page 17, lines 17-28, Examples 3, 4, 7 and Claims.</p> <p style="text-align: center;">---</p>	<p>1-5,8, 11-13, 21-32</p>
Y	<p>DE,A,40 13 099 (HOECHST AG) 31 October 1991</p> <p>see Claims.</p> <p style="text-align: center;">---</p>	<p>1,2,5,8, 22-26, 29-32</p>
Y	<p>WO,A,92 12250 (DEKALB PLANT GENETICS) 23 July 1992</p> <p>see page 12, line 30 - page 13, line 11, Table 1 and Claims.</p> <p style="text-align: center;">---</p>	<p>1,2,5,8, 11-13, 22,26, 28,30-32</p>
Y	<p>PLANT MOL. BIOL., vol. 18, 1992 pages 201-210, T.M. SPENCER ET AL.; 'Segregation of transgenes in maize' see the whole document.</p> <p style="text-align: center;">---</p>	<p>1,2,5,8, 22-27, 30-32</p>
Y	<p>THE PLANT CELL, vol. 2, 1990 pages 603-618, W.J. GORDON-KAMM ET AL.; 'Transformation of maize cells and regeneration of fertile transgenic plants' see the whole document.</p> <p style="text-align: center;">---</p>	<p>1,2,5,8, 22-27, 30-32</p>
Y	<p>PLASMID, vol. 23, 1990 pages 237-241, M. SUGIYAMA ET AL.; 'Use of the tyrosinase gene from Streptomyces to probe promoter sequences for Escherichia coli' see the whole document.</p> <p style="text-align: center;">---</p>	<p>1-5,8, 22-32</p>
Y	<p>NUCL. ACIDS RES., vol. 19, 1991 page 958 F. BEERMANN ET AL.; 'Tyrosinase as a marker for transgenic mice' see the whole document.</p> <p style="text-align: center;">---</p>	<p>1-5,8, 22-32</p>
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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.
Y	<p>PROC. NATL. ACAD. SCI. USA, vol. 88, 1991 pages 3324-3328, F.J. PERLAK ET AL.; 'Modification of the coding sequence enhances plant expression of insect control protein genes' cited in the application see the whole document.</p> <p style="text-align: center;">---</p>	1-5, 11-13, 22-32
Y	<p>PLANT MOL. BIOL., vol. 20, 1992 pages 81-93, E.Y. WONG ET AL.; 'Arabidopsis thaliana small subunit leader and transit peptide enhance the expression of Bacillus thuringiensis proteins in transgenic plants' see the whole document.</p> <p style="text-align: center;">---</p>	1, 11-13, 22-32
Y	<p>BIO/TECHNOLOGY, vol. 11, 1993 pages 715-718, A. PERL ET AL.; 'Bacterial diglydropicolinate synthase and desensitized aspartate kinase: two novel selectable markers for plant transformation' see the whole document, especially Discussion.</p> <p style="text-align: center;">---</p>	1, 21-32
P,X	<p>EP,A,0 589 110 (PLANT GENETIC SYSTEMS N.V.) 30 March 1994 see Claims.</p> <p style="text-align: center;">---</p>	1, 11-13, 22-32
P,X	<p>WO,A,93 19190 (E.I. DU PONT DE NEMOURS AND COMPANY) 30 September 1993 see Examples 17 and 18 and Claims.</p> <p style="text-align: center;">-----</p>	1, 21-32

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 94/ 09699

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:

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 annex

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.:

1-9, 11-14, 16, 21-32

(all partially)

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.:

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. The application is considered to lack unity of invention since it relates not to a single invention, but rather to a group of forty-eight inventions, all set out in Claim 1. Summarising, Claim 1 relates to a fertile, transgenic maize plant, the genome of which contains:
 - a) a selectable or screenable marker gene comprising one of seven listed possibilities, the first of which is a tyrosinase gene;
 - b) a juvenile hormone esterase gene;
 - c) an MAR region;
 - d) a negatively-selectable marker comprising one of four listed possibilities;
 - e) an exogenous gene encoding a selected trait under the control of one of four listed promoters;
 - f) a modified Bt toxin CryIA(c) gene or one of five other listed genes;
 - g) a disease resistance trait comprising one of three given possibilities;
 - h) a stress resistance trait comprising one of two given alternatives;
 - i) a drought resistance trait comprising one of four listed possibilities; or
 - j) a grain composition trait comprising an aspartokinase gene or one of fifteen other specified genes.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1.1 In view of the word "or" at the end of Claim 1(i), the claim has been interpreted as relating to a fertile transgenic maize plant containing any one of the forty-seven specified genes (or, in the case of Claim 1(c), a MAR region). Fertile, transgenic maize plants containing various foreign genes are widely-known in the art (see the cited documents, for example WO 91/02071). The inventive concept underlying the present application is therefore not the provision of fertile, transgenic maize plants, but the provision of fertile, transgenic maize plants containing a particular gene or DNA region. Since forty-eight separate possibilities are claimed, and there is no visible common inventive link between these, the application is considered to contain forty-eight separate inventions. The requirement for unity of invention is therefore not regarded as fulfilled.

2. The search report has been drawn up for the three inventions in respect of which search fees have been paid, namely a fertile, transgenic maize plant comprising (a) a tyrosinase gene, (b) a modified Bt toxin CryIA(c) gene or (c) an aspartokinase gene.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 94/09699

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9307278	15-04-93	AU-A- 2795292	03-05-93
		BG-A- 98747	28-02-95
		BR-A- 9206578	11-04-95
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