



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification<sup>5</sup> :</b> <b>C12N 15/82, A01H 4/00, 5/00</b> <b>C12N 5/00</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 91/02071</b> <b>(43) International Publication Date:</b> 21 February 1991 (21.02.91)
<b>(21) International Application Number:</b> PCT/US90/04462 <b>(22) International Filing Date:</b> 8 August 1990 (08.08.90) <b>(30) Priority data:</b> 392,176 9 August 1989 (09.08.89) US 513,298 17 April 1990 (17.04.90) US <b>(71) Applicant:</b> DEKALB PLANT GENETICS [US/US]; 3100 Sycamore Road, DeKalb, IL 60115 (US). <b>(72) Inventors:</b> ADAMS, Thomas, R. ; 12 Mystic Road, North Stonington, CT 06359 (US). ADAMS, Whitney, R., Jr. ; 142 Shennecossett Parkway, Groton, CT 06340 (US). CHAMBERS, Sheryl, A. ; 87 Twin Hills Drive, Groton, CT 06340 (US). DAINES, Richard, J. ; 5 Cranwood Road, Ledyard, CT 06339 (US). GORDON-KAMM, William, J. ; RR3, Box 80, Collins Road, Stonington, CT 06378 (US). KAUSCH, Albert, P. ; The Bee House, Briarpatch Road, Stonington, CT 06378 (US). KRUEGER, Roger, W. ; Buckley Road, Salem, CT 06415 (US). LE-MAUX, Peggy, G. ; 11 Ivy Road, Mystic, CT 06355 (US). MACKEY, Catherine, J. ; One Hawthorne Road, Old Lyme, CT 06371 (US). MANGANO, Mary, L. ; 16 Surrey Drive, Westerly, RI 02891 (US). O'BRIEN, James, V. ; 7 1/2 West Mystic Avenue, Mystic, CT 06355 (US). RICE, Thomas, B. ; 64 Twin Lakes Drive, Waterford, CT 06385 (US). SPENCER, T., Michael ; 7 Rossie Street, Mystic, CT 06355 (US). START, William, G. ; 452 Norwich-Westerly Road, North Stonington, CT 06359 (US). WILLETTS, Nancy ; 13 Sunset Avenue, Niantic, CT 06357 (US).		<b>(74) Agent:</b> PARKER, David, L.; Arnold, White & Durkee, P.O. Box 4433, Houston, TX 77210 (US). <b>(81) Designated States:</b> AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CM (OAPI patent), DE*, DE (European patent)*, DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent). <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> <b>(88) Date of publication of the international search report:</b> 14 May 1992 (14.05.92)
<b>(54) Title:</b> METHODS AND COMPOSITIONS FOR THE PRODUCTION OF STABLY TRANSFORMED, FERTILE MONOCOT PLANTS AND CELLS THEREOF		
<b>(57) Abstract</b> <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 embryogenic 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 the 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>		

\* See back of page

• DESIGNATIONS OF "DE"

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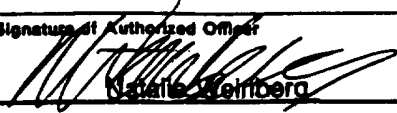
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+ Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/04462

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC <sup>5</sup> : C 12 N 15/82, A 01 H 4/00, A 01 H 5/00, C 12 N 5/00		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
IPC <sup>5</sup>	C 12 N, A 01 H	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	Nature, vol. 338, 16 March 1989, K. Shimamoto et al.: "Fertile trans- genic rice plants regenerated from transformed protoplasts", pages 274- 276, see the whole article	1, 2, 4, 5, 7-9, 13-15, 17, 19, 20-22, 24, 25, 47
Y	--	18, 26
X	Chemical Abstracts, vol. 110, 1989 (Columbus, Ohio, US) M.E. Horn et al.: "Transgenic plants of orchard grass (Dactylis glomerata L.) from proto- plasts", see page 208, abstract no. 89869a, & Plant Cell Rep. 1988, 7(7), 469-72	1, 32
X	EP, A, 0299552 (SOLVAY) 18 January 1989 see example II	23
X	Science, vol. 240, 8 April 1988 C.A. Rhodes et al.: "Genetically trans- formed maze plants from protoplasts",	41 ./.
<p><sup>10</sup> Special categories of cited documents: <sup>16</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document 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</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>"A" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
20th June 1991	09. 08. 91	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	 Natalie Weinberg	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	pages 204-207, see the abstract cited in the application --	
X	EP, A, 0290395 (SANDOZ) 9 November 1988 see example 5 --	31,43,44-46, 48-50
X	EP, A, 0292435 (CIBA-GEIGY) 23 November 1988 see the whole document; particularly page 17 --	30-37, 41-43
Y	WO, A, 89/04371 (LOUISIANA STATE UNIVERSITY) 18 May 1989 see example 11 --	18
Y	Journal of Cellular Biochemistry, Suppl. 13D, 27 March - 7 April 1989 abstract M122, Alan R. Liss, Inc. (New York, US) W.J. Gordon-Kamm et al.: "Stable trans- formation of embryogenic maize cultures by microprojectile bombardment", page 259, see the abstract --	3,6,16,27-31
Y	EP, A, 0270356 (AGRECETUS) 8 June 1988 see page 2, column 2, lines 23-40; page 4, column 6, lines 9-39; page 7, column 11, lines 41-48; page 8, column 13, line 54 - page 10, column 18 --	3,16,28,29
Y	Biological Abstracts, vol. 85, no. 12, 1988, Biological Abstracts, Inc. C.L. Armstrong et al.: "Genetic and cytogenetic variation in plants regenerated from organogenic and friable, embryogenic tissue cultures of maize", see abstract 117662 & CROP. SCI. 28(2), 363-369, 1988 --	6,27-31
Y	Plant Physiol., vol. 90, March 1989 R. Dekeyser et al.: "Evaluation of selectable markers for rice trans- formation", pages 217-223 see the abstract --	26
P,X	EP, A, 0334539 (I.C.I.) 27 September 1989 see page 5, column 7, lines 34-56 --	43-48,50
P,X	The Plant Cell, vol. 2, July 1990, American Society of Plant Physiologists	1,2,4-9,14- 50 ./.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	W.J. Gordon-Kamm et al.: "Transformation of maize cells and regeneration of fertile transgenic plants", pages 603-618 see the whole article --	
P,X	EP, A, 0348348 (CIBA-GEIGY) 27 December 1989 see examples 13-28,32,34,36,40,41,45,46,50 --	1,2,4,5,7-10 13,14,17-25, 30-32,41-50, 33,37
P,X	NL, A, 8801444 (SOLVAY) 2 January 1990 see example 1 --	12,13
A	Proc. Natl. Acad. Sci. USA, vol. 85, June 1988 T.N. Klein et al.: "Transfer of foreign gene into intact maize cells with high-velocity microprojectiles", pages 4305-4309 see "Materials and Methods" --	6,27-31
O,A	UCLA Symp. Mol. Cell. Biol., New Ser., vol. 129, (Plant Gene Transfer), Utah, 1-7 April 1989, A John Wiley & Sons Inc. publication J. Cao et al.: "Transformation of rice and maize using the biolistic process", pages 21-33, see pages 28,30,31 --	3,6,27-31
A	Biotechnology, vol. 7, June 1989 R.D. Shillito et al.: "Regeneration of fertile plants from protoplasts of elite inbred maize", pages 581-587, see page 582, left-hand column, "Cryopreservation of embryogenic suspension cultures" --	10
A	J. Cell. Biochem., Suppl. 13 D, abstract M149 M.C. Ross et al.: "Transient and stable transgenic cells and calli of tobacco and maize following microprojectile bombardment", page 268 see the abstract --	6,27-31
A	Biotechnology, vol. 7, June 1989 L.M. Prioli et al.: "Plant regeneration and recovery of fertile plants from protoplasts of maize (Zea mays L.)", ./.	32,36,37

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	pages 589-594, see page 593, "Experimental protocol" --	
A	EP, A, 0275069 (DNA PLANT TECHNOLOGY) 20 July 1988 see pages 6-12; claims --	3,18,43
A	DE, A, 3738874 (INSTITUT BOTANIKI KIEV) 17 November 1988 see examples 5,6 --	3
X	EP, A, 0202668 (MITSUI) 26 November 1986 see examples --	51-52
Y	EP, A, 0282164 (MITSUI) 14 September 1988 see the whole document --	51-54
Y	US, A, 4370160 (ZIEMELIS) 25 January 1983 see column 8, lines 65-68; column 12, example 6 --	51-54
A	WO, A, 85/02972 (PLANT GENETICS) 18 July 1985 see page 1, lines 4-8; page 2, lines 26-35; page 7, lines 17-28; page 9 --	51-60
A	EP, A, 0280400 (DOW CORNING) 31 August 1988 see column 3, lines 9-12	51-60
A	Chemical Abstracts, vol. 110, 1989, (Columbus, Ohio, US) A.J. Schmidt et al.. " Media and environmental effects on phenolics production from tobacco cell cultures ", see abstract 230156z, & Biotechnol. Bioeng. 1989, 33(11),37-44 --	51-60
A	EP, A, 0262971 (PLANT CELL RESEARCH INSTITUTE) 6 April 1988 see page 8, lines 16-21 --	51-60
E	WO, A, 90/10691 (GRACE SIERRA) 20 September 1990 see the whole document --	51
A	EP, A, 0141373 (PLANT GENETICS INC.) 15 May 1985 see page 3, lines 29-36; page 4, lines 19-27; page 16, lines 30-36 -----	52,53

## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V.  OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>1</sup>

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1.  Claim numbers ..... because they relate to subject matter not required to be searched by this Authority, namely:

2.  Claim numbers ..... 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.  Claim numbers ..... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI.  OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>2</sup>

This International Searching Authority found multiple inventions in this international application as follows:

Claims 1-50, 51-60. Please see Form PCT/ISA/206

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only these claims of the international application for which fees were paid, specifically claims:

3.  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 claim numbers:

4.  As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

The additional search fees were accompanied by applicant's protest.

No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9004462  
SA 39624

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 17/07/91  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

US 9004462  
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