



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

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>C12N 15/15, 15/18, 15/54, 15/62, 15/82, 5/10, A01H 5/00, 5/10</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 00/03017</b> <b>(43) International Publication Date:</b> 20 January 2000 (20.01.00)
<b>(21) International Application Number:</b> PCT/US99/15713 <b>(22) International Filing Date:</b> 8 July 1999 (08.07.99)  <b>(30) Priority Data:</b> 09/113,690                      10 July 1998 (10.07.98)                      US  <b>(71) Applicant:</b> CALGENE LLC [US/US]; 1920 Fifth Street, Davis, CA 95616 (US).  <b>(71)(72) Applicants and Inventors:</b> MCBRIDE, Kevin, E. [US/US]; 1309 Marina Circle, Davis, CA 95616 (US). STAUB, Jeffrey, M. [US/US]; 819 "J" Foxspring Drive, Chesterfield, MO 63017 (US).  <b>(74) Agent:</b> SCHWEDLER, Carl, J.; Patent Dept. Central, Monsanto/G.D. Searle, P.O. Box 5110, Chicago, IL 60680-5110 (US).		<b>(81) Designated States:</b> CA, JP, MX, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>  <b>(88) Date of publication of the international search report:</b> 6 July 2000 (06.07.00)
<b>(54) Title:</b> ENHANCER ELEMENTS FOR INCREASED TRANSLATION IN PLANT PLASTIDS  <b>(57) Abstract</b> <p>Provided are methods for increasing the production of protein in a plant cell by transforming plastids of plant cells with a construct comprising a promoter functional in a plant plastid, a ribosome binding site, DNA sequence of interest and a transcription termination region, and growing plant cells comprising the transformed plastids under conditions wherein the DNA encoding sequence is transcribed in the plastid. Also provided are methods for increasing protein production by fusing a coding sequence to a gene of interest to a secondary protein for cleavage or targetting of the protein of interest within the plastid, whereby high levels of expression of protein is achieved.</p>		

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Form PCT/ISA/210 (second sheet) (July 1992)

# 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	<p>MCBRIDE ET AL: "Amplification of a chimeric Bacillus gene in chloroplasts leads to an extraordinary level of an insecticidal protein in tobacco"            BIO/TECHNOLOGY,US,NATURE PUBLISHING CO.            NEW YORK,            vol. 13, 13 April 1995 (1995-04-13), page            362-365 XP002090404            ISSN: 0733-222X            the whole document</p>	1-5,19, 20,22
X	<p>WO 95 24493 A (CALGENE INC ;MCBRIDE KEVIN            E (US); STALKER DAVID M (US))            14 September 1995 (1995-09-14)            the whole document</p>	1-5,19, 20,22
X	<p>DANIELL ET AL: "Containment of herbicide resistance through genetic engineering of the chloroplast genome"            NATURE BIOTECHNOLOGY,US,NATURE PUBLISHING,            vol. 16, April 1998 (1998-04), page            345-348 XP002090409            ISSN: 1087-0156            the whole document</p>	1-4,6-8, 19,20, 26,27, 35-39
A	<p>EP 0 293 358 A (MONSANTO CO)            30 November 1988 (1988-11-30)            the whole document</p>	1-8, 19-27, 35-39
A	<p>WO 92 04449 A (MONSANTO CO)            19 March 1992 (1992-03-19)            the whole document</p>	1-8, 19-27, 35-39
A	<p>US 5 693 507 A (DANIELL HENRY ET AL)            2 December 1997 (1997-12-02)            see example 4</p>	1-8, 19-27, 35-39

# INTERNATIONAL SEARCH REPORT

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## 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 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 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.:
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.:

2-5, 7, 8(completely); 1, 6, 19, 20-27, 35-39(partially)

### 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. Claims: 2-5,7,8 completely; 1,6,19,20-27,35-39 partially

A method for increasing the production of a protein in a plant cell, comprising transforming plastids of said plant cell with a construct comprising: a promoter functional in a plant plastid, a ribosome binding site, a DNA of interest and a transcription termination region, and growing said plant cells under appropriate conditions for transcription. The construct further comprising a selectable marker and flanking DNA regions of homology to the plastid genome. Said markers being one out of a selection as claimed, said ribosome binding site being from a leader sequence as claimed, preferably being the gene 10 leader RBS or the rbcLRBS. Said gene of interest encoding a gene conferring tolerance to the herbicide glyphosate, especially being an EPSPS gene out of the selection as claimed, either native or synthetic. The method wherein selection for said herbicide tolerance is made on media containing glyphosate at a concentration from at least about 50- about 200 micromolar, more preferably 1 millimolar.

A plant cell produced according to said method comprising about 1% or about 7%, respectively, or more of total soluble protein as said expressed protein.

A plant comprising said plant cell being tolerant to glyphosate, applied at a rate of at least 16 ounces, preferably 32 ounces, more preferably 64 ounces or greater per acre.

Plant cells, plants, seeds produced according to the said method.

## 2. Claims: 9-16 completely; 1,19,20,26,27 partially

A method for increasing the production of a protein in a plant cell, comprising transforming plastids of said plant cell with a construct comprising: a promoter functional in a plant plastid, a ribosome binding site, a DNA of interest and a transcription termination region, and growing said plant cells under appropriate conditions for transcription. Said gene of interest encoding a peptide derived from a eukaryotic organism other than a peptide of a plant plastid, preferably a mammalian peptide, more preferably selected from the group of interferons, monoclonal antibodies, hematopoietic agents, pituitary hormones, thyroid hormones, hypothalamic hormones, albumins, pancreatic hormones, and proteinase inhibitors, exemplified by bGH, hGH, pBL, and aprotinin. Said gene being a native or a synthetic gene.

A plant cell produced according to said method comprising about 1% or about 7%, respectively, or more of total soluble protein as said expressed protein.

Plant cells, plants, seeds produced according to the said method.

## 3. Claims: 17,18 completely; 1,6,19,20-27,35-39 partially

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method for increasing the production of a protein in a plant cell, comprising transforming plastids of said plant cell with a construct comprising: a promoter functional in a plant plastid, a ribosome binding site, a DNA of interest and a transcription termination region, and growing said plant cells under appropriate conditions for transcription. Said gene of interest encoding a gene conferring tolerance to the herbicide glyphosate, especially encoding a glyphosate-modifying enzyme selected from the group of gox, hph, glpA, and glpB, said gene being a native or a synthetic gene. The method wherein selection for said herbicide tolerance is made on media containing glyphosate at a concentration from at least about 50- about 200 micromolar, more preferably 1 millimolar.

A plant cell produced according to said method comprising about 1% or about 7%, respectively, or more of total soluble protein as said expressed protein.

A plant comprising said plant cell being tolerant to glyphosate, applied at a rate of at least 16 ounces, preferably 32 ounces, more preferably 64 ounces or greater per acre.

Plant cells, plants, seeds produced according to the said method.

#### 4. Claims: 28-34 completely

A method for increasing the production of a protein in a plant cell, comprising transforming plastids of said plant cell with a construct comprising: a promoter functional in a plant plastid, a ribosome binding site, a transcription termination region, a coding sequence to a secondary protein fused to said DNA sequence of interest, and growing said plant cells under appropriate conditions for transcription. Said secondary protein optionally being the thylakoid-targetting terminus of cytochrome f, or the cleavable N-terminal portion of ubiquitin. Said method further comprising cleaving said ubiquitin N-terminus from said eukaryotic peptide by harvesting said plant cells and exposing the contents of said transformed plastid to the cytosol of said plant cell. The method whereby expression from said DNA sequence of interest is enhanced, said sequence preferably encoding bioactive hGH.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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