



(43) International Publication Date
20 December 2012 (20.12.2012)

(51) International Patent Classification:

C12N 15/82 (2006.01) C12N 9/10 (2006.01)
C12N 9/00 (2006.01) C12N 9/88 (2006.01)

(21) International Application Number:

PCT/US2012/042286

(22) International Filing Date:

13 June 2012 (13.06.2012)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/496,864 14 June 2011 (14.06.2011) US

(71) Applicant (for all designated States except US): **SYN-
THON BIOPHARMACEUTICALS B.V.** [NL/NL]; Mi-
croweg 22, P.O. Box 7071, NL-6503 GN Nijmegen (NL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **COX, Kevin, M.**
[US/US]; 1916 Sunset Drive, Raleigh, NC 27608 (US).
NGUYEN, Long [US/US]; Apex, NC 27502 (US).

(74) Agent: **HENRY, Leslie, T.**; Alston & Bird LLP, Bank of
America Plaza, 101 South Tryon Street, Suite 4000, Char-
lotte, NC 28280-4000 (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, 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, 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))
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(88) Date of publication of the international search report:

14 March 2013

(54) Title: COMPOSITIONS AND METHODS FOR MAKING AND B IOC ONT AINING AUXOTROPHIC TRANSGENIC
PLANTS

(57) Abstract: Compositions and methods are described for making and using transgenic plants and plant parts having at least one
auxotrophic requirement for an essential compound such as an amino acid, carbohydrate, fatty acid, nucleic acid, vitamin, plant hor-
mone, or precursor thereof. Transgenic plants and plants parts having at least one auxotrophic requirement can be effectively biocon-
tained by withdrawal of the essential compound.



INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/042286

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/82 C12N9/00 C12N9/10 C12N9/88
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

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, 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.
X	DE 199 14 792 A1 (MAX PLANCK GESELLSCHAFT [DE]) 5 October 2000 (2000-10-05)	86
A	the whole document	1-38, 64-74,98
A	----- MIGGE A ET AL: "Greenhouse-grown conditionally lethal tobacco plants obtained by expression of plastidic glutamine synthetase antisense RNA may contribute to biological safety", PLANT SCIENCE, ELSEVIER IRELAND LTD, IE, vol. 153, no. 2, 25 April 2000 (2000-04-25), pages 107-112, XP027449526, ISSN: 0168-9452, DOI: 10.1016/S0168-9452(99)00232-0 [retrieved on 2000-04-25] ----- -/--	1-38, 64-74, 86,98



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

18 September 2012

Date of mailing of the international search report

04/01/2013

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

Kania, Thomas

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/042286

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.

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/042286

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SMITH D H ET AL: "Production of Auxotrophs in a Duckweed, Spirodela Polyrhiza.", PLANT PHYSIOLOGY NOV 1960 LNKD-PUBMED:16655426, vol. 35, no. 6, November 1960 (1960-11), pages 809-815, XP002682783, ISSN: 0032-0889 page 814, right-hand column, paragraph 3 - page 815, left-hand column, paragraph 4 -----	1-38, 64-74, 86,98
A	PATTON D A ET AL: "An embryo-defective mutant of arabidopsis disrupted in the final step of biotin synthesis", PLANT PHYSIOLOGY, AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, US, vol. 116, no. 3, 1 March 1998 (1998-03-01), pages 935-946, XP002306697, ISSN: 0032-0889 -----	1-38, 64-74, 86,98
A	WO 98/55601 A2 (DU PONT [US]; FALCO SAVERIO CARL [US]; ALLEN STEPHEN M [US]; RAFALSKI) 10 December 1998 (1998-12-10) -----	1-38, 64-74, 86,98
A	BARABAS Z: "HYBRID SEED PRODUCTION USING NUTRITIONAL MUTANTS", EUPHYTICA, KLUWER ACADEMIC PUBLISHERS, NL, vol. 53, no. 1, 1 January 1991 (1991-01-01), pages 67-72, XP008037321, ISSN: 0014-2336, DOI: 10.1007/BF00032035 -----	1-38, 64-74, 86,98
A	WO 2006/005807 A1 (UNICROP LTD [FI]; KUVSHINOV VIKTOR [FI]; KOIVU KIMMO [FI]; KANERVA ANN) 19 January 2006 (2006-01-19) -----	1-38, 64-74, 86,98
A	EP 0 628 635 A1 (NUNHEMS ZADEN BV [NL]) 14 December 1994 (1994-12-14) -----	1-38, 64-74, 86,98
A	COX KEVIN M ET AL: "GLYCAN OPTIMIZATION OF A HUMAN MONOCLONAL ANTIBODY IN THE AQUATIC PLANT LEMNA MINOR", NATURE BIOTECHNOLOGY, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol. 24, no. 12, 1 December 2006 (2006-12-01), pages 1591-1597, XP009084966, ISSN: 1087-0156, DOI: 10.1038/NBT1260 cited in the application ----- -/--	1-38, 64-74, 86,98

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2012/042286

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MOON HONG S ET AL: "Keeping the genie in the bottle: transgene biocontainment by excision in pollen.", TRENDS IN BIOTECHNOLOGY JAN 2010 LNKD-PUBMED:19857909, vol. 28, no. 1, January 2010 (2010-01), pages 3-8, XP002682784, ISSN: 1879-3096 -----	1-38, 64-74, 86,98

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2012/042286

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
DE 19914792	A1	05-10-2000	AU	4113900	A	23-10-2000
			DE	19914792	A1	05-10-2000
			WO	0060101	A1	12-10-2000

WO 9855601	A2	10-12-1998	AR	014351	A1	28-02-2001
			BR	9809967	A	01-08-2000
			CA	2292778	A1	10-12-1998
			CN	1259170	A	05-07-2000
			EP	0979296	A2	16-02-2000
			EP	1959016	A2	20-08-2008
			ID	24686	A	27-07-2000
			US	6664445	B1	16-12-2003
			US	2004209341	A1	21-10-2004
			WO	9855601	A2	10-12-1998

WO 2006005807	A1	19-01-2006	US	2005039229	A1	17-02-2005
			US	2008085996	A1	10-04-2008
			WO	2006005807	A1	19-01-2006

EP 0628635	A1	14-12-1994	AU	701920	B2	11-02-1999
			AU	6972394	A	03-01-1995
			CA	2164732	A1	22-12-1994
			EP	0628635	A1	14-12-1994
			JP	H08510916	A	19-11-1996
			NO	954978	A	26-01-1996
			NZ	267425	A	22-09-1997
			US	6262339	B1	17-07-2001

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-38, 98(completely); 64-74, 86(partially)

A method for biocontaining a transgenic plant, in particular a duckweed plant by employing plants having an auxotrophic requirement, providing an effective amount of the essential component to the plant and subsequently removing said essential component from the plant wherein the growth of the plant is inhibited and the plant is biocontained, wherein the plant comprises a heterologous polynucleotide of interest, as well as subject-matter related thereto.

2. claims: 39-63, 75-85, 87-89, 99-105(completely); 64-74, 86(partially)

A method of making plants having an auxotrophic requirement, in particular duckweed plants, wherein the plants comprise a heterologous polynucleotide of interest, a method of regulating the production of a heterologous polypeptide of interest in an auxotrophic plant, as well as subject-matter related thereto.

3. claims: 90-97

Isolated polynucleotide and polypeptide sequences encoding for threonine deaminase, glutamine synthase I, glutamine synthase II and biotin synthase, respectively, from duckweed according to SEQ ID NO:1-24, as well as subject-matter related thereto.
