



- (51) **International Patent Classification:**
C10L 5/44 (2006.01)
- (21) **International Application Number:**
PCT/US2012/028132
- (22) **International Filing Date:**
7 March 2012 (07.03.2012)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/449,769 7 March 2011 (07.03.2011) US
- (71) **Applicant (for all designated States except US):** **AG-RIVIDA, INC.** [US/US]; 200 Boston Avenue, Suite 3100, Medford, Massachusetts 02155 (US).
- (72) **Inventors; and**
- (75) **Inventors/Applicants (for US only):** **RAAB, R. Michael** [US/US]; 35 Wall Street, Arlington, Massachusetts 02476 (US). **ZHANG, Dongcheng** [CN/US]; 41 Myerson Lane, Newton, Massachusetts 02459 (US). **BOUGRI, Oleg** [US/US]; 2017 Mortimer Drive, Boise, Idaho 83712 (US).
- (74) **Agent:** **O'Donnell, Ryan W.**; Volpe and Koenig, P.C., United Plaza, 30 S. 17th Street, Philadelphia, Pennsylvania 19103 (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, 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, GE, 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:**
8 November 2012

(54) **Title:** CONSOLIDATED PRETREATMENT AND HYDROLYSIS OF PLANT BIOMASS EXPRESSING CELL WALL DEGRADING ENZYMES

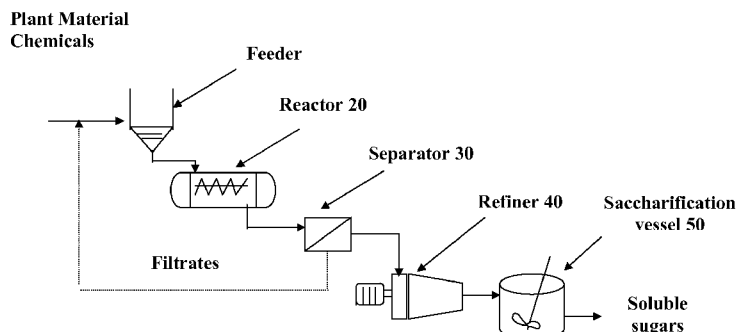


FIG. 1

(57) **Abstract:** Methods for consolidated pretreatment and hydrolysis of genetically engineered plants expressing cell wall degrading enzymes are provided. Expression cassettes and vectors for making transgenic plants are described. Plants engineered to express one or more cell wall degrading enzymes using expression cassettes and vectors of the invention are also provided.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/028132

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C10L 5/44 (2012.01)

USPC - 44/307

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(8) - C10L 5/00, 40, 44; C12N 9/00, 02, 14, 26, 36, 42; C12P 7/00, 06, 08, 10 (2012.01)

USPC - 44/307, 308, 589, 605; 435/183, 189, 195, 201, 206, 207, 209, 210

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)

PatBase, ProQuest, Google Patents

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/0159510 A1 (RAAB) 24 June 2010 (24.06.2010) entire document	1-14, 16-43
Y	US 7,049,485 B2 (STICKLEN et al) 23 May 2006 (23.05.2006) entire document	1-14, 16-42
Y	WO 2010/060056 A2 (MCBRIDE et al) 27 May 2010 (27.05.2010) entire document	43
Y	US 2009/0193541 A1 (MILES) 30 July 2009 (30.07.2009) entire document	4, 7, 10
Y	WO 2008/064314 A2 (MCBRIDE et al) 29 May 2008 (29.05.2008) entire document	9, 39
Y	LINDH et al. Production of the p24 capsid protein from HIV-1 subtype C in Arabidopsis thaliana and Daucus carota using an endoplasmic reticulum-directing SEKDEL sequence in protein expression constructs. Protein Expression and Purification. 66:46-51. 07 January 2009. entire document	14
Y	HOOD et al. Subcellular targeting is a key condition for high-level accumulation of cellulase protein in transgenic maize seed. Plant Biotechnology Journal. 5:1-11. 2007. doi:10.1111/j.1467-7652.2007.00275.x entire document	12, 41
Y	US 2009/0298149 A1 (WANG et al) 03 December 2009 (03.12.2009) entire document	16-32
Y	MORRIS et al. Cloning of the xynB Gene from Dictyoglomus thermophilum Rt46B.1 and Action of the Gene Product on Kraft Pulp. Applied and Environmental Microbiology. 64(5):1759-1765	13

☒ Further documents are listed in the continuation of Box C.

* 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

24 August 2012

Date of mailing of the international search report

17 SEP 2012

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/028132

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/0143967 A1 (MCFARLAND) 10 June 2010 (10.06.2010) entire document	32
Y	US 5,432,074 A (EVANS et al) 11 July 1995 (11.07.1995) entire document	34, 35
Y	US 2010/0159494 A1 (SWEENEY et al) 24 June 2010 (24.06.2010) entire document	36

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/028132

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 Extra 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 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.:
1-43

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
Information on patent family members

International application No.
PCT/US2012/028132

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: claims 1-43 are drawn to engineered plant materials.

Group II: claims 44-49 are drawn to an expression cassette.

The inventions listed in Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I, a method of producing an engineered plant material including a first polynucleotide sequence encoding an amino acid or protein sequence selected from one of SEQ ID NOs 1-11, are not present in Group II; and the special technical features of Group II, an expression cassette comprising a first polynucleotide sequence capable of hybridizing under conditions of moderate stringency to a nucleic acid selected from one of SEQ ID NOs 32-39 and a second polynucleotide sequence capable of hybridizing under conditions of moderate stringency to a nucleic acid selected from one of SEQ ID NOs 32-43, are not present in Group I.

Groups I and II share the technical feature of a polynucleotide sequence for expression. However, these shared technical features do not represent a contribution over the prior art. Specifically, US 2010/0159510 A1 to Raab discloses transgenic plants that express controllable intervening protein sequence (CIVPS) or intein modified proteins (Abstract) where recombinant plants are produced by providing an expression construct that encode(s) at least one modified protein comprising a target protein, or protein segment(s), which is(are) fused, either internally or terminally, to a CIVPS or intein sequence(s) or segment(s) thereof, or to an amino terminus(i) or a carboxyl terminus(i) thereof (Paras. [0020] through [0025]; see also Paras. [0068] through [0076]).

Groups I and II therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/028132

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☐

in the international application as filed

☒

together with the international application in electronic form

☐

subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments: