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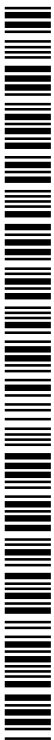
Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

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24 April 2014



WO 2013/184764 A3

(54) Title: CONSTRUCT AND METHOD FOR EXPRESSING TRANSGENES USING A *BRASSICA* BIDIRECTIONAL CONSTITUTIVE PROMOTER

SEQ ID NO: 1 (739 nt core promoter)
acagagagaaaggaatagcttctgtaggaagtgaacgaaaaaagaatggttaagctcaatactgctcggattctaaacca
aattgagagagagttccgaattccgtggtttatctaaaccgaacctcagatcggttgattggttgggtttgtgacatatata
actggaaaaagacatccccgggttgattatgttccaaacttaaacagattgagattatttccgataagcttatacattctctgt
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taaccaaaacctctccactgctctgtacaacgtggcaggttctcattggcttaataaggaaatgctcacaaccatcgttt
gaggtgggttggtaaggattttgatgctacccttctctcaccacaacccc

Figure 1A

(57) Abstract: Provided are constructs and methods for expressing multiple genes in plant cells and/or plant tissues using a disclosed bidirectional promoter from *Brassica napus* or *Brassica* bidirectional constitutive promoter (BBCP). The constructs provided comprise at least one such bi-directional promoter linked to multiple gene expression cassettes, wherein each of the gene expression cassettes comprises at least one transgene. In some embodiments, the constructs and methods provided allow expression of genes between two and twenty.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/44262

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - C12N 15/82, 15/113, 5/14; C07H 21/04 (2013.01)
 USPC - 435/468; 536/24.1; 800/278
 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): C12N 15/82, 15/63, 15/113, 15/11, 15/10, 15/00, 5/14; C12P 19/34; C07H 21/00, 21/04 (2013.01)
 USPC: 435/468, 469, 91.4, 91.1, 89, 85, 84, 72, 41; 536/24.1, 23.1, 22.1, 18.7; 514/44R, 43, 42; 800/278, 294

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)
 MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; ProQuest; ScienceDirect; 'bi-directional,' promoter, vector, cassette, plasmid, enhancer, binary, 'T-DNA,' 'pBIN19,' 'pPVP,' 'pGreen,' intron, '5'-UTR,' '5'-untranslated,' 'IRES,' alternative, splicing, '2A-peptide,' intein, proteasome, cleavage

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6388170 B1 (GAN, S et al.) May 14, 2002; abstract; column 1, lines 6-13; column 2, line 66 to column 3, line 20; column 3, lines 35-47; column 4, lines 38-51	1-5, 7-15, 19-30
A	US 7053265 B2 (KAPRANOV, P et al.) May 30, 2006; abstract; column 16, line 65 to column 17, line 5; column 20, lines 37-51; column 24, lines 1-34	1-5, 7-15, 19-30
A	US 7129343 B2 (LI, Z et al.) October 31, 2006; abstract; figure 13; column 3, lines 1-39; column 4, lines 22-45; column 9, lines 30-37; column 14, lines 30-59	1-5, 7-15, 19-30
A	National Academy of Agricultural Science. Brassica Rapa Subsp. Pekinensis Clone KBrH003H20. 31 May 2010. GenBank Accession No: AC241089.1 Accessed from http://www.ncbi.nlm.nih.gov/nuccore/AC241089 on December 29, 2013.	1-5, 7-15, 19-30

Further documents are listed in the continuation of Box C.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search 03 January 2014 (03.01.2014)	Date of mailing of the international search report 15 JAN 2014
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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: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US13/44262

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:

-Please See Supplemental Page-

- 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.:

Groups I+: Claims 1-5, 7-15, 19-30, SEQ ID NO: 1 (bi-directional promoter nucleotide sequence)

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/US13/44262

---Continuation of Box No. III: Observations Where Unity Of Invention Is Lacking:

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 must be paid.

Groups I+: Claims 1-15, 19-30 and SEQ ID NO: 1 (bi-directional promoter nucleotide sequence) are directed toward a nucleic acid construct for expressing multiple genes in plant cells and/or tissues, comprising, (a) a bi-directional promoter comprising a nucleotide sequence having at least 80% identity to SEQ ID NO: 1; and (b) two gene expression cassettes on opposite ends of the bi-directional promoter; and the use of a bi-directional promoter in the manufacturing of transgenic plants or seeds, wherein the bi-directional promoter comprising a nucleotide sequence having at least 80% identity to SEQ ID NO: 1.

Group II: Claims 16-18, SEQ ID NO: 26 (regulatory element polynucleotide sequence) are directed toward a nucleic acid construct for expressing a transgene in plant cells and/or tissues, comprising a regulator element useful for terminating the expression of a single or multiple genes in plant cells and/or tissues, wherein the regulator element comprises a paralog A 3'untranslated region (UTR) or poly A region.

Groups III+: Claims 31-34, SEQ ID NO: 27 (Brassica intron nucleotide sequence) are directed toward a nucleic acid construct comprising at least one Brassica intron sequence in transgenic plant cells and/or tissues.

The nucleic acid construct for expressing multiple genes in plant cells and/or tissues and the use of a bi-directional promoter in the manufacturing of transgenic plants or seeds will be searched to the extent that they encompass SEQ ID NO: 1 (bi-directional promoter nucleotide sequence). It is believed that Claims 1-5, 7-15 and 19-30 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1. Applicants must indicate, if applicable, the claims which encompass the first named invention if different than what was indicated above for this group. Failure to clearly identify how many paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. Additional SEQ ID NOs can be searched upon the payment of additional fees. An Exemplary Election would be: SEQ ID NO: 2.

The inventions listed as Groups I+-III+ 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 Groups I+ include a nucleic acid construct for expressing multiple genes in plant cells and/or tissues, comprising, (a) a bi-directional promoter comprising a nucleotide sequence having at least 80% identity to SEQ ID NO: 1; and (b) two gene expression cassettes on opposite ends of the bi-directional promoter; and the use of a bi-directional promoter in the manufacturing of transgenic plants or seeds, wherein the bi-directional promoter comprising a nucleotide sequence having at least 80% identity to SEQ ID NO: 1, which are not present in Group II; Group II having special technical features including a nucleic acid construct for expressing a transgene in plant cells and/or tissues, comprising a regulator element useful for terminating the expression of a single or multiple genes in plant cells and/or tissues, wherein the regulator element comprises a paralog A 3'untranslated region (UTR) or poly A region, which are not present in Groups III+; Groups III+ having special technical features including a nucleic acid construct comprising at least one Brassica intron sequence in transgenic plant cells and/or tissues.

Groups I+-III+ share the technical features including nucleic acid constructs and transgenic plants, or plant cells or tissues, and the use of said constructs. Groups I+ and II share the technical features including a regulatory element for regulating the expression of multiple genes in plant cells or tissues. Groups I+ share the technical elements including a nucleic acid construct for expressing multiple genes in plant cells and/or tissues, comprising: (a) a bi-directional promoter comprising a nucleotide sequence; and (b) two gene expression cassettes on opposite ends of the bi-directional promoter; and the use of a bi-directional promoter in the manufacturing of transgenic plants or seeds, the bi-directional promoter comprising a nucleotide sequence. Groups III+ share the technical features including a nucleic acid construct comprising at least one Brassica intron sequence in transgenic plant cells and/or tissues; and the use of at least one Brassica intron sequence in the manufacturing of transgenic plants or seeds.

However, these shared technical features are previously disclosed by US 6,388,170 B1 to Gan, et al (hereinafter 'Gan') in view of US 2002/0184660 A1 to Ulvskov, et al (hereinafter 'Ulvskov'). Gan discloses the use of nucleic acid constructs (nucleic acid constructs; abstract) and transgenic plants (plants comprising the constructs (transgenic plants; column 3, lines 34-35), plant cells or tissues (plant cells or tissues; column 3, lines 48-50) and the use of said constructs (methods to impart agronomically significant characteristics to a plant (use of said constructs); column 3, lines 51-60), a regulatory element (promoter (regulatory element); column 2, lines 59-65) for regulating the expression of multiple genes (for regulating the expression of multiple genes; column 2, lines 59-65) in plant cells or tissues (plant cells or tissues; column 3, lines 48-50), comprising: (a) a bi-directional promoter (bi-directional promoter; column 2, lines 59-65) comprising a nucleotide sequence (specific DNA sequence, called a promoter; column 1, lines 26-31 (it should be noted that the Gan reference discloses a definition)); and (b) two gene expression cassettes on opposite ends of the bi-directional promoter (two gene expression cassettes on opposite ends of the bi-directional promoter; column 2, lines 59-65); and the use (methods to impart agronomically-significant characteristics to a plant (use); column 3, lines 51-60) of a bi-directional promoter (bi-directional promoter; column 2, lines 59-65) in the manufacturing (imparting agronomically-significant characteristics (manufacturing); column 3, lines 51-60) of transgenic plants or seeds (transgenic plants or seeds; column 3, lines 35-50), the bi-directional promoter (bi-directional promoter; column 2, lines 59-65) comprising a nucleotide sequence (specific DNA sequence, called a promoter; column 1, lines 26-31). Gan does not disclose a nucleic acid construct comprising at least one Brassica intron sequence in transgenic plant cells and/or tissues; and the use of at least one Brassica intron sequence in the manufacturing of transgenic plants or seeds. Ulvskov discloses a nucleic acid construct (chimeric gene (nucleic acid construct); abstract) comprising at least one Brassica intron sequence (Brassica intron sequence; Claim 9) in transgenic plant cells and/or tissues (transgenic plant cells and/or tissues; abstract; paragraph [0011]); and the use (use; paragraph [0001]) of at least one Brassica intron sequence (Brassica intron sequence; Claim 9) in the manufacturing of (production of (manufacturing of; paragraph [0052]) transgenic plants or seeds (transgenic plant cells and/or tissues; abstract; paragraph [0011]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the constructs, as previously disclosed by Gan, including a bidirectional promoter, in order to have integrated a Brassica intron-comprising construct, as disclosed by Ulvskov, for enabling the production of transgenic genes in the dehiscence zone of plants.

Since none of the special technical features of the Groups I+-III+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Gan and Ulvskov references, unity of invention is lacking.