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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 January 2016 (14.01.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/007606 A3

(51) International Patent Classification:
A61K 48/00 (2006.01) *C12N 1/15* (2006.01)
A61K 38/00 (2006.01)

(21) International Application Number:
PCT/US2015/039520

(22) International Filing Date:
8 July 2015 (08.07.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/021,995 8 July 2014 (08.07.2014) US

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(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, BN, 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, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, 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, ST, 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, KM, 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))*

(88) Date of publication of the international search report:
3 March 2016

(54) Title: FLAGELLIN-SENSING 3 ('FLS3') PROTEIN AND METHODS OF USE

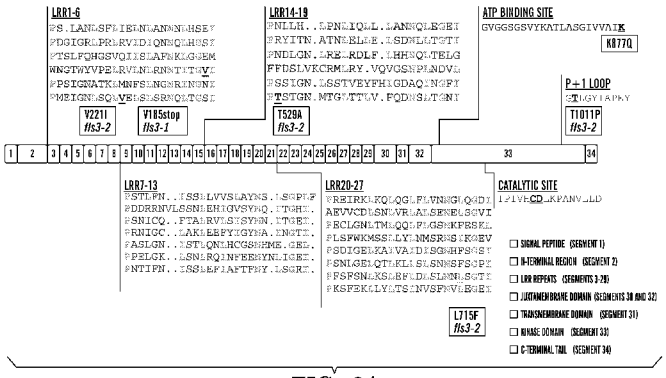

FIG. 2A

(57) Abstract: One aspect of the present invention relates to a nucleic acid construct that includes a nucleic acid molecule that encodes FLAGELLIN-SENSING 3 ("FLS3") protein; a 5' heterologous DNA promoter sequence; and a 3 ' terminator sequence, where the nucleic acid molecule, the DNA promoter sequence, and the terminator sequence are operatively coupled to permit transcription of the nucleic acid molecule. The present invention also relates to a method of imparting disease resistance to a plant. This method involves transforming a plant or a plant seed with a nucleic acid molecule that increases expression of an FLS3 protein, where said transforming is effective in imparting disease resistance to the transformed plant or to a transgenic plant produced from the transformed plant seed. The present invention also relates to methods of expressing a nucleic acid molecule in a plant, identifying a candidate plant suitable for breeding that displays enhanced disease resistance, and enhancing efficiency of transformation of a plant by *Agrobacterium*.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/39520

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K48/00, A61K38/00, C12N1/15 (2015.01)
CPC - C07K14/415, C07K14/4711, A61K38/00
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) Classifications: A61K48/00, A61K38/00, C12N1/15, C12N1/19 , A61K39/00 OR C07K14/47, C12N1/21, C12N15/82, C12N5/10, C12N15/09 (2015.01); CPC Classifications: A61K2039/6093, C07K14/415, C07K14/4711, A61K38/00, A61K2039/53, A61K2039/6037

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)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); PubMED/ NCBI, EBSCO, Google Scholar; Search Terms: MARTIN, HIND, STRICKLER, flagellin, 'flagellin sensing protein 3', flagellin sensing protein 2, PAMP, 'transgenic plants', transformation, transformed plants, Agrobacterium, A. tumefaciens, progeny

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2008/0307539 A1 (ZIPFEL, C, et al.) December 11, 2008; [0004], [0037], [0074], [0121], [[0143]-0146], [0180]-[0182][0199]- [0200],[0209]-[0210], [0213]-[0214]	1-9, 11, 30-42
Y	CLARKE, CR et al. Allelic Variation In Two Distinct Pseudomonas Syringae Flagellin Epitopes Modulates The Strength Of Plant Immune Responses But Not Bacterial Motility; New Phytologist. Epub 19 July 2013; Vol., 200: pages 847-860. DOI: 10.1111/nph.12408; page 858, first column, first paragraph, last paragraph.	1-42
Y	US 2012/0005785 A1 (FRANKARD, V et al.) January 5, 2012; abstract, paragraphs [0006], 0011], [0053], [0074]-[0078], [0080], [0083], [0089], [0091], [0096], [0099], [0122]; Claim 1	10, 12-29, 34
Y	BUDIMAN, MA et al. Solanum lycopersicum (Lycopersicon esculentrum): Predicted: Solanum lycoperiscum Probable LRR Receptor-Like Serine/Threonine-Protein Kinase At3g47570-Like (LOC101248095), mRNA: NCBI Reference Sequence: XM_004237116.1' by NCBI, in combination with BLAST XM_004237116.1 with SEQ ID NO: 1 &2). 14 May 2012; pages 1-9.	2, 14, 22, 27, 31, 32

☐ 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	Date of mailing of the international search report
11 December 2015 (11.12.2015)	06 JAN 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/39520

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.:
Claims 1, 2 (in-part), 3-13, 14 (in-part), 15-21, 22 (in-part), 23-26, 27 (in-part), 28-30, 31 (in-part), 32 (in-part) and 33-42, SEQ ID NO: 1, 2, 7, and 8

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/US15/39520

-***-Continued from 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 1-42 and 46-48 are directed toward a nucleic acid construct comprising: a nucleic acid molecule that encodes a FLAGELLIN-SENSING 3 ("FLS3") protein a method of expressing said nucleic acid molecule in a plant; a method of imparting disease resistance to a plant comprising transforming a plant with the nucleic acid; a method of identifying a candidate plant suitable for breeding that displays enhanced disease resistance, and a method for enhancing efficiency of transformation of a plant by Agrobacterium, comprising silencing expression of said nucleic acid.

The nucleic acid construct and methods will be searched to the extent that they encompass SEQ ID NOs: 1 (Solanum lycopersicum DNA sequence), 2 (Solanum lycopersicum amino acid sequence), 7 (Capsicum annum nucleic acid sequence) and 8 (Capsicum annum amino acid sequence). It is believed that Claims 1, 2 (in-part), 3-13, 14 (in-part), 15-21, 22 (in-part), 23-26, 27 (in-part), 28-30, 31 (in-part), 32 (in-part) and 33-42 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 1 (Solanum lycopersicum DNA sequence), 2 (Solanum lycopersicum amino acid sequence), 7 (Capsicum annum nucleic acid sequence), 8 (Capsicum annum amino acid sequence). Applicants must specify the claims that encompass any additionally elected amino acid and nucleic acid sequences. Applicants must further 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 any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An Exemplary Election would be: SEQ ID NOs: 3 (Solanum pimpinellifolium DNA sequence), 4 (Solanum pimpinellifolium amino acid sequence), 9 (Solanum tuberosum DNA sequence), 10 (Solanum tuberosum amino acid seuqence).

Group II: Claims 43-45 are directed toward a method of imparting disease resistance to a plant, said method comprising: providing a plant comprising a gene encoding an FLS3 protein, and applying to the plant and/or area of cultivation of the plant a flgII-28 peptide or an FLS3-binding portion thereof, thereby imparting disease resistance to the plant.

The inventions listed as Groups I+-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 Groups I + include SEQ ID NO: 1, not present in Group II; the special technical features of Group II include a flgII-28 peptide, not present in any of Groups I+.

Groups I+ and II share the technical features including a method of imparting disease resistance to a plant, comprising providing a plant comprising an FLS3 protein. Groups I+ share the technical features including: a nucleic acid construct comprising: a nucleic acid molecule that encodes a FLAGELLIN-SENSING 3 ("FLS3") protein; a 5' heterologous DNA promoter sequence; and a 3' terminator sequence, wherein the nucleic acid molecule, the DNA promoter sequence, and the terminator sequence are operatively coupled to permit transcription of the nucleic acid molecule; an expression vector comprising the nucleic acid; a host cell transformed with the nucleic acid; a plant transformed with the nucleic acid; a component part of the plant; a seed of the plant, a fruit of the plant; a method of expressing a nucleic acid molecule in a plant, said method comprising: providing a transgenic plant or plant seed transformed with a nucleic acid construct comprising: a nucleic acid molecule that encodes a FLAGELLIN-SENSING 3 ("FLS3") protein; a 5' heterologous DNA promoter sequence; and a 3' terminator sequence, wherein the nucleic acid molecule, the DNA promoter sequence, and the terminator sequence are operatively coupled to permit transcription of the nucleic acid molecule, and growing the transgenic plant or a plant grown from the transgenic plant seed under conditions effective to express the nucleic acid molecule in said transgenic plant or said plant grown from the transgenic plant seed; a method of imparting disease resistance to a plant comprising: transforming a plant or a plant seed with a nucleic acid molecule that increases expression of an FLS3 protein, wherein said transforming is effective in imparting disease resistance to the transformed plant or to a transgenic plant produced from the transformed plant seed; a plant produced by the method; a method of identifying a candidate plant suitable for breeding that displays enhanced disease resistance, said method comprising: providing a candidate plant; analyzing the candidate plant for the presence, in its genome, of a gene encoding an FLS3 protein; identifying, based on said analyzing, a candidate plant suitable for breeding that includes in its genome, a gene encoding an FLS3 protein; and breeding the identified plant with at least one other plant; a method for enhancing efficiency of transformation of a plant by Agrobacterium, said method comprising: transforming a plant or a plant seed with a nucleic acid construct effective to silence expression of a nucleic acid molecule that encodes an FLS3 protein, wherein said transforming is effective to reduce or eliminate expression of FLS3 protein in the plant and said nucleic acid construct comprising: a nucleic acid molecule configured to silence FLS3 protein expression; a 5' DNA promoter sequence; and a 3' terminator sequence, wherein the nucleic acid molecule, the promoter, and the terminator are operatively coupled to permit expression of the nucleic acid molecule.

-***-Continued on Next Supplemental Page-***-

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However, these shared technical features are previously disclosed by US 2008/0307539 A1 to Zipfel, et al. (hereinafter 'Zipfel') and further in view of US 2012/0005785 A1 to Frankard, et al. (hereinafter 'Frankard') and the publication entitled 'Solanum lycopersicum (Lycopersicon esculentrum): Predicted: Solanum lycopersicum Probable LRR Receptor-Like Serine/Threonine-Protein Kinase At3g47570-Like (LOC101248095), mRNA: NCBI Reference Sequence: XM_004237116.1' by NCBI (hereinafter 'NCBI').

Zipfel discloses a method comprising providing a plant (providing N. benthamiana transformed with plasmid encoding an LRR protein; paragraphs [0037], [0041]) comprising an LRR protein (comprising EFR (an LRR protein); paragraphs [0037], [0041]); a nucleic acid construct (a plasmid; paragraph [0121]) comprising: a nucleic acid molecule that encodes a LRR protein (a nucleic acid molecule that encodes EFR (a LRR protein); paragraphs [0037], [0121]); a 5' heterologous DNA promoter sequence (a 5' lacZ promoter in pGREENII/T-0229 (a 5' heterologous DNA promoter sequence); paragraph [0121]; pGreen II 0229 plasmid map); and a 3' terminator sequence (and a 3' NOS terminator in pGREENII/T-0229 (a 3' terminator sequence); paragraph [0121]; pGreenII 0229 plasmid map), wherein the nucleic acid molecule, the DNA promoter sequence, and the terminator sequence are operatively coupled to permit transcription of the nucleic acid molecule (wherein the coding sequence is inserted into the plasmid between the promoter and terminator to enable expression thereof; paragraph [0121]); an expression vector comprising the nucleic acid (an expression plasmid (vector) comprising the nucleic acid; paragraph [0121]); a host cell transformed with the nucleic acid (N. benthamiana leaves (a host cell) transformed with the nucleic acid; paragraph [0041]); a plant transformed with the nucleic acid (N. benthamiana (a plant) transformed with the nucleic acid; paragraph [0040]); a component part of the plant (a leaf (a component part) of the plant; paragraph [0041]); a seed of the plant (paragraph [0215]), a fruit of the plant (N. benthamiana leaves (a fruit of the plant); paragraph [0041]); a method of expressing a nucleic acid molecule in a plant (paragraphs [0040], [0121]), said method comprising: providing a transgenic plant transformed with a nucleic acid construct (paragraphs [0040], [0041], [0121]) comprising: a nucleic acid molecule that encodes a LRR protein (a nucleic acid molecule that encodes EFR (a LRR protein); paragraphs [0037], [0121]); a 5' heterologous DNA promoter sequence (a 5' lacZ promoter in pGREENII/T-0229 (a 5' heterologous DNA promoter sequence); paragraph [0121]; pGreen II 0229 plasmid map); and a 3' terminator sequence (and a 3' NOS terminator in pGREENII/T-0229 (a 3' terminator sequence); paragraph [0121]; pGreenII 0229 plasmid map), wherein the nucleic acid molecule, the DNA promoter sequence, and the terminator sequence are operatively coupled to permit transcription of the nucleic acid molecule (wherein the coding sequence is inserted into the plasmid between the promoter and terminator to enable expression thereof; paragraph [0121]), and growing the transgenic plant under conditions effective to express the nucleic acid molecule in said transgenic plant (and culturing the transformed plants or plant parts for 4 days to express the nucleic acid molecule in the transgenic plant or part thereof (growing the transgenic plant under conditions effective to express the nucleic acid molecule in said transgenic plant); paragraphs [0040], [0041], [0121], [0122]); a method for enhancing efficiency of transformation of a plant by Agrobacterium (paragraph [0060], [0069]), said method comprising: transforming a plant with a nucleic acid construct (paragraphs [0181], [0182]) effective to silence expression of a nucleic acid molecule (effective to down-regulate expression of EFR; paragraphs [0180]-[0182], [0191]) that encodes an LRR protein (that encodes EFR (an LRR protein); paragraphs [0037], [0180], [0191]), wherein said transforming is effective to reduce or eliminate expression of LRR protein in the plant (wherein said transforming is effective to reduce or eliminate expression of EFR (a LRR protein) in the plant; paragraphs [0180]-[0182], [0191]) and said nucleic acid construct comprising: a nucleic acid molecule configured to silence LRR protein expression (said nucleic acid construct comprising: a nucleic acid molecule configured to silence EFR (LRR) protein expression; paragraphs [0180]-[0182], [0191]); a 5' DNA promoter sequence (a 5' DNA promoter sequence; paragraph [0182]); wherein the nucleic acid molecule, and the promoter are operatively coupled to permit expression of the nucleic acid molecule (paragraph [0182]); and wherein FLS2 is a membrane bound receptor kinase with an LRR domain (wherein FLS2 is a membrane bound receptor kinase with an LRR domain; paragraph [0004]).

Zipfel does not disclose: a method of imparting disease resistance to a plant, comprising providing a plant comprising an FLS3 protein; a nucleic acid molecule that encodes a FLAGELLIN-SENSING 3 ("FLS3") protein; a method of imparting disease resistance to a plant comprising: transforming a plant or a plant seed with a nucleic acid molecule that increases expression of an FLS3 protein, wherein said transforming is effective in imparting disease resistance to the transformed plant or to a transgenic plant produced from the transformed plant seed (a plant produced by the method); a method of identifying a candidate plant suitable for breeding that displays enhanced disease resistance, said method comprising: providing a candidate plant; analyzing the candidate plant for the presence, in its genome, of a gene encoding an FLS3 protein; identifying, based on said analyzing, a candidate plant suitable for breeding that includes in its genome, a gene encoding an FLS3 protein; and breeding the identified plant with at least one other plant; effective to reduce or eliminate expression of FLS3 protein; a nucleic acid molecule configured to silence FLS3 protein expression; and a 3' terminator sequence. ... Continued on Next Page ...

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... Continued from Previous Page ... Frankard discloses a method of imparting disease resistance to a plant (a method of improving growth characteristics of plants, including disease resistance (a method of imparting disease resistance to a plant); abstract; paragraph [0006]), comprising providing a plant comprising an LRR protein (comprising providing a plant comprising increased expression of an LRR receptor kinase (comprising providing a plant comprising an LRR protein); abstract); a nucleic acid molecule that encodes a LRR protein (abstract); a method of imparting disease resistance to a plant (a method of improving growth characteristics of plants, including disease resistance (a method of imparting disease resistance to a plant); abstract; paragraph [0006])) comprising: transforming a plant or a plant seed with a nucleic acid molecule that increases expression of an LRR protein (transforming a plant an RLK827 polypeptide or homologue thereof (transforming a plant or a plant seed with a nucleic acid molecule that increases expression of an LRR protein); abstract; paragraphs [0059], [0060]), wherein said transforming is effective in imparting disease resistance to the transformed plant (paragraphs [0006], [0059]); a plant produced by the method (transgenic plants produced by introducing an LRR receptor kinase nucleic acid molecule (a plant produced by the method); abstract); a method of identifying a candidate plant suitable for breeding (identifying plants having desirable characteristics for breeding; paragraph [0004]); plants displaying enhanced disease resistance (paragraph [0006]), said method comprising: providing a candidate plant (selecting candidate plants having an identified marker; paragraph [0102]); analyzing the candidate plant for the presence, in its genome, of a gene encoding an LRR protein (identifying an RLK827 (LRR) protein gene or genetically linked marker in the plant (analyzing the candidate plant for the presence, in its genome, of a gene encoding an LRR protein); paragraph [0102]); identifying, based on said analyzing, a candidate plant suitable for breeding that includes in its genome, a gene encoding an LRR protein (identifying a plant having said marker or RLK827 (LRR) protein gene and altered growth characteristics (identifying, based on said analyzing, a candidate plant suitable for breeding that includes in its genome, a gene encoding an LRR protein); paragraph [0102]); and breeding the identified plant with at least one other plant (breeding the identified plant with at least one other plant; paragraph [0097]).

NCBI discloses an LRR receptor like kinase (an LRR receptor like kinase; page 1) including an amino acid sequence identical to SEQ ID NO: 2 of the instant PCT application (having an amino acid sequence (including an amino acid sequence identical to SEQ ID NO: 2 of the instant PCT application); page 1 to page 2; wherein the amino acid sequence disclosed is 100% identical to SEQ ID NO: 2 of the instant PCT application), and a nucleic acid sequence identical to SEQ ID NO: 1 of the instant PCT application (and a nucleic acid sequence (a nucleic acid sequence identical to SEQ ID NO: 1 of the instant PCT application); page 2).

It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Zipfel, for providing a termination signal 3' to the antisense sequence, as previously disclosed by Zipfel, for providing a more effective generation of the antisense nucleic acids by a host cell for downregulation of the LRR polypeptide. Furthermore, it would have been obvious to a person of ordinary skill in the art at, the time of the invention, to have modified the previous disclosure of Zipfel, for providing alternative LRR polypeptides, such as the LRR receptor kinase, as previously disclosed by NCBI, heterologously, and to have downregulated the expression of said polypeptides in host or natural cells expressing the polypeptide, for verifying the effects of the alternative proteins on the efficiency of transformation using agrobacterium, and modulating the transformation efficiency. Moreover, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Zipfel, for assessing the effects of expression of an LRR polypeptide, such as the polypeptide previously disclosed by NCBI, on the disease resistance of a host plant expressing the polypeptide, provided the previous disclosure of Frankard, for selecting plants or plant cells for breeding having the enhanced disease resistance, as previously disclosed by Fankard, utilizing the LRR protein as a selection marker, for ensuring its passage to progeny.

Since none of the special technical features of the Groups I+-II 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 Zipfel, Frankard and NCBI references, unity of invention is lacking.