

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference MONS:465WO		FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US 19/52782	International filing date (day/month/year) 24 September 2019 (24.09.2019)	(Earliest) Priority Date (day/month/year) 25 September 2018 (25.09.2018)	
Applicant MONSANTO TECHNOLOGY LLC			

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 5 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

- ☒ the international application in the language in which it was filed.
☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ Certain claims were found unsearchable (see Box No. II).

3. ☒ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

- ☒ the text is approved as submitted by the applicant.
☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

- ☒ the text is approved as submitted by the applicant.
☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

- a. the figure of the **drawings** to be published with the abstract is Figure No. _____
☐ as suggested by the applicant.
☐ as selected by this Authority, because the applicant failed to suggest a figure.
☐ as selected by this Authority, because this figure better characterizes the invention.
- b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

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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:
- a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

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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:

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.

***** See Supplemental Sheet to continue *****

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.:
1-31, restricted to SEQ ID NO: 2

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/US 19/52782

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A01N 63/02, C07K 14/325, C12N 15/82 (2019.01)

CPC - A01N 63/02, C07K 14/325, C12N 15/8285, C12N 15/8286, Y02A 40/164

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)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,174,860 B1 (Kramer, et al.) 16 January 2001 (16.01.2001) pg 77, claim 5, SEQ ID NO: 2, 85.1% identity to SEQ ID NO: 2	1-31
A	US 2012/0278954 A1 (Bowen, et al.) 01 November 2012 (01.11.2012) claims 1-5, 8; para [0061]-[0063]	1-31

☐ 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

"D" document cited by the applicant in the international application

"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 December 2019

Date of mailing of the international search report

31 JAN 2020

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. 571-273-8300

Authorized officer

Lee Young

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US 19/52782

In Continuation of Box III. Observations where unity of invention is lacking:

Group I+, claims 1-31, directed to a recombinant nucleic acid molecule comprising a heterologous promoter operably linked to a polynucleotide segment encoding a pesticidal protein; a plant/seed/insect inhibitory composition comprising thereof; a method of producing said seed; a method for controlling pest infestation; a method of detecting said nucleic acid molecule; a method of detecting said pesticidal protein in a sample. The nucleic acid, a plant/seed/insect inhibitory composition comprising thereof; a method of producing said seed; a method for controlling pest infestation; a method of detecting said nucleic acid molecule and said pesticidal protein will be searched to the extent that the nucleic acid encompasses a polynucleotide encoding the amino acid sequence SEQ ID NO: 2. It is believed that claims 1-31 encompass this first named invention, and thus these claims will be searched without fee to the extent that the nucleic acid encompasses a polynucleotide encoding the amino acid sequence SEQ ID NO: 2. Additional amino acid sequence(s) and/or nucleic acid sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected amino acid sequence(s) and/or nucleic acid sequence(s). 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. An exemplary election would be the nucleic acid of SEQ ID NO: 49, i.e., claims 1-27, 29.

The inventions listed as Group I+ do not relate to a single special technical feature 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 feature of some inventions of Group I+ is a specific amino acid sequence recited therein.

The special technical feature of some inventions of Group I+ is a specific nucleic acid sequence recited therein.

The inventions of Group I+ share the technical feature of a recombinant nucleic acid molecule comprising a heterologous promoter operably linked to a polynucleotide encoding a pesticidal protein (claim 1). Some inventions of Group I+ share the technical feature of a plant/seed/insect inhibitory composition comprising thereof (claims 14, 17, 18, respectively); a method of producing said seed (claim 26); a method for controlling pest infestation (claim 28); a method of detecting the presence of said nucleic acid (claim 29); or a method of detecting the presence of said pesticidal protein (claim 30). However, these shared technical features do not represent an improvement over prior art as being anticipated by US 2012/0278954 A1 to Bowen, et al. (01 November 2012) (hereinafter "Bowen").

Bowen discloses a recombinant nucleic acid molecule comprising a heterologous promoter operably linked to a polynucleotide encoding a pesticidal protein (claim 1, "A polynucleotide molecule encoding a pesticidal polypeptide ... b) wherein said polynucleotide molecule is provided in a recombinant expression cassette; c) wherein said polynucleotide molecule is provided in a transgenic or recombinant host cell...; and e) wherein the polynucleotide molecule is present in a part of a recombinant plant, said plant part being selected from the group consisting of a leaf, ... or a seed; and i) wherein a product is produced from said recombinant plant part and said product comprises a detectable amount of said polynucleotide molecule..."; para [0061], Example 4, "Transformed Plants Nucleotide segments encoding TIC1506 (SEQ ID NO: 11), TIC1503 (SEQ ID NO: 19), ... are codon-optimized for plant expression and operably linked to one or more plant functional promoters and introduced into plant cells. Recombinant plants are regenerated from such transformed plant cells, and the regenerated plants are evaluated for resistance to pest infestation, such as insect tolerance and/or plant pathogenic nematode tolerance").

Bowen discloses a plant and seed comprising said recombinant nucleic acid molecule (claim 5, "A transgenic plant, plant cell, seed, or plant part resistant to nematode and/or lepidopteran insect infestation comprising the polynucleotide of claim 1").

Bowen discloses an insect inhibitory composition (claim 8, "A composition comprising the pesticidal polypeptide of claim 2 in an agriculturally-acceptable carrier...").

Bowen discloses a method of producing said seed (para [0063], "Example 5 Transformation of Soybean This example describes a method of producing transgenic soybean plants and transgenic plant parts such as seeds... Agrobacterium containing a construct of Example 5 are grown in Luria Burtani (LB) media ... The bacterial culture is centrifuged, pellet washed, and resuspended in INO medium for inoculating wet or dry mature embryos explants. The explants are mixed with the Agrobacterium cell suspensions and briefly exposed to sonication energy... The explants are drained of any liquid and transferred to containers containing filter paper moistened with INO media and co-cultured in a lighted chamber ... After co-culture, the explants are placed directly onto regeneration media containing a selective agent such as spectinomycin... The cultures are subsequently transferred to a media suitable for the recovery of transformed plantlets. Spectinomycin resistant shoots that have green buds or leaves are considered transformed and placed in soil or on a soil substitute for rooting in the presence or absence of the selective agent. Progeny transgenic plants and seed are selected that provide pest resistance, especially nematode resistance").

Bowen discloses a method for controlling pest infestation (claim 3, "A method of controlling pesticidal infection of a plant...").

Bowen discloses a method of detecting the presence of said recombinant nucleic acid molecule (claim 4, "A method of: a) detecting in a biological sample a polynucleotide encoding a pesticidal protein... said method comprises the steps of: i) selecting a pair of primers that, when used together in an amplification reaction with said polynucleotide, form an amplicon encoding all or a pesticidally effective part of said pesticidal protein; ii) producing said amplicon from said polynucleotide; iii) detecting said amplicon; and iv) generating DNA sequence information from said amplicon to confirm the amino acid sequence identity of said pesticidal protein").

Bowen discloses a method of detecting the presence of said pesticidal protein in a sample (claim 4, "? b) detecting in a biological sample a pesticidal protein... comprising the steps of: i) contacting said sample with an antibody that specifically binds to said pesticidal protein, or a peptide or epitope derived therefrom; and ii) detecting the binding of said antibody to said pesticidal protein, peptide, or epitope derived therefrom"). As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the inventions.

Therefore, inventions of Group I+ lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: ROBERT E. HANSON
P. O. BOX 061080
WACKER DRIVE STATION, WILLIS TOWER
CHICAGO, IL 60606

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year)

31 JAN 2020

Applicant's or agent's file reference
MONS:465WO

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US 19/52782

International filing date (day/month/year)

24 September 2019 (24.09.2019)

Priority date (day/month/year)

25 September 2018 (25.09.2018)

International Patent Classification (IPC) or both national classification and IPC

IPC - A01N 63/02, C07K 14/325, C12N 15/82 (2019.01)

CPC - A01N 63/02, C07K 14/325, C12N 15/8285, C12N 15/8286, Y02A 40/164

Applicant

MONSANTO TECHNOLOGY LLC

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

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. 571-273-8300

Date of completion of this opinion

18 December 2019

Authorized officer

Lee Young

PCT Help Desk
Telephone No. 571-272-4300

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US 19/52782

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(b)).
3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
4. ☐ 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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US 19/52782

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees the applicant has, within the applicable time limit:
- ☐ paid additional fees.
- ☐ paid additional fees under protest and, where applicable, the protest fee.
- ☐ paid additional fees under protest but the applicable protest fee was not paid.
- ☒ not paid additional fees.
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with.
- ☒ not complied with for the following reasons:

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.

Group I+, claims 1-31, directed to a recombinant nucleic acid molecule comprising a heterologous promoter operably linked to a polynucleotide segment encoding a pesticidal protein; a plant/seed/insect inhibitory composition comprising thereof; a method of producing said seed; a method for controlling pest infestation; a method of detecting said nucleic acid molecule; a method of detecting said pesticidal protein in a sample. The nucleic acid, a plant/seed/insect inhibitory composition comprising thereof; a method of producing said seed; a method for controlling pest infestation; a method of detecting said nucleic acid molecule and said pesticidal protein will be searched to the extent that the nucleic acid encompasses a polynucleotide encoding the amino acid sequence SEQ ID NO: 2. It is believed that claims 1-31 encompass this first named invention, and thus these claims will be searched without fee to the extent that the nucleic acid encompasses a polynucleotide encoding the amino acid sequence SEQ ID NO: 2. Additional amino acid sequence(s) and/or nucleic acid sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected amino acid sequence(s) and/or nucleic acid sequence(s). 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. An exemplary election would be the nucleic acid of SEQ ID NO: 49, i.e., claims 1-27, 29.

The inventions listed as Group I+ do not relate to a single special technical feature 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 feature of some inventions of Group I+ is a specific amino acid sequence recited therein.

The special technical feature of some inventions of Group I+ is a specific nucleic acid sequence recited therein.

***** See Supplemental Sheet to continue *****

4. Consequently, this opinion has been established in respect of the following parts of the international application:
- ☐ all parts.
- ☒ the parts relating to claims Nos. 1-31, restricted to SEQ ID NO: 2

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US 19/52782

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-31	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	1-31	YES
	Claims	NONE	NO
Industrial applicability (IA)	Claims	1-31	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-31 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest the claimed subject matter, specifically, an amino acid sequence having at least 98% identity to SEQ ID NO: 2.

As to claims 1-31, an exemplary prior art on record, US 6,174,860 B1 to Kramer, et al. (hereinafter "Kramer") discloses a *Xenorhabdus nematophilus* insecticidal protein having 85.1% identity to SEQ ID NO: 2 (pg 77, claim 7, SEQ ID NO: 2, 85.1% identity).

There is no prior art on record disclosing an amino acid sequence having at least 98% identity to SEQ ID NO: 2.

Claims 1-31 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US 19/52782

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Box IV. Lack of unity of invention:

The inventions of Group I+ share the technical feature of a recombinant nucleic acid molecule comprising a heterologous promoter operably linked to a polynucleotide encoding a pesticidal protein (claim 1). Some inventions of Group I+ share the technical feature of a plant/seed/insect inhibitory composition comprising thereof (claims 14, 17, 18, respectively); a method of producing said seed (claim 26); a method for controlling pest infestation (claim 28); a method of detecting the presence of said nucleic acid (claim 29); or a method of detecting the presence of said pesticidal protein (claim 30). However, these shared technical features do not represent an improvement over prior art as being anticipated by US 2012/0278954 A1 to Bowen, et al. (01 November 2012) (hereinafter "Bowen").

Bowen discloses a recombinant nucleic acid molecule comprising a heterologous promoter operably linked to a polynucleotide encoding a pesticidal protein (claim 1, "A polynucleotide molecule encoding a pesticidal polypeptide ... b) wherein said polynucleotide molecule is provided in a recombinant expression cassette; c) wherein said polynucleotide molecule is provided in a transgenic or recombinant host cell...; and e) wherein the polynucleotide molecule is present in a part of a recombinant plant, said plant part being selected from the group consisting of a leaf, ... or a seed; and i) wherein a product is produced from said recombinant plant part and said product comprises a detectable amount of said polynucleotide molecule..."; para [0061], Example 4, "Transformed Plants Nucleotide segments encoding TIC1506 (SEQ ID NO:11), TIC1503 (SEQ ID NO: 19), ... are codon-optimized for plant expression and operably linked to one or more plant functional promoters and introduced into plant cells. Recombinant plants are regenerated from such transformed plant cells, and the regenerated plants are evaluated for resistance to pest infestation, such as insect tolerance and/or plant pathogenic nematode tolerance").

Bowen discloses a plant and seed comprising said recombinant nucleic acid molecule (claim 5, "A transgenic plant, plant cell, seed, or plant part resistant to nematode and/or lepidopteran insect infestation comprising the polynucleotide of claim 1").

Bowen discloses an insect inhibitory composition (claim 8, "A composition comprising the pesticidal polypeptide of claim 2 in an agriculturally-acceptable carrier...").

Bowen discloses a method of producing said seed (para [0063], "Example 5 Transformation of Soybean This example describes a method of producing transgenic soybean plants and transgenic plant parts such as seeds... Agrobacterium containing a construct of Example 5 are grown in Luria Burtani (LB) media ... The bacterial culture is centrifuged, pellet washed, and resuspended in INO medium for inoculating wet or dry mature embryos explants. The explants are mixed with the Agrobacterium cell suspensions and briefly exposed to sonication energy... The explants are drained of any liquid and transferred to containers containing filter paper moistened with INO media and co-cultured in a lighted chamber ... After co-culture, the explants are placed directly onto regeneration media containing a selective agent such as spectinomycin... The cultures are subsequently transferred to a media suitable for the recovery of transformed plantlets. Spectinomycin resistant shoots that have green buds or leaves are considered transformed and placed in soil or on a soil substitute for rooting in the presence or absence of the selective agent. Progeny transgenic plants and seed are selected that provide pest resistance, especially nematode resistance").

Bowen discloses a method for controlling pest infestation (claim 3, "A method of controlling pesticidal infection of a plant...").

Bowen discloses a method of detecting the presence of said recombinant nucleic acid molecule (claim 4, "A method of: a) detecting in a biological sample a polynucleotide encoding a pesticidal protein... said method comprises the steps of: i) selecting a pair of primers that, when used together in an amplification reaction with said polynucleotide, form an amplicon encoding all or a pesticidally effective part of said pesticidal protein; ii) producing said amplicon from said polynucleotide; iii) detecting said amplicon; and iv) generating DNA sequence information from said amplicon to confirm the amino acid sequence identity of said pesticidal protein").

Bowen discloses a method of detecting the presence of said pesticidal protein in a sample (claim 4, "? b) detecting in a biological sample a pesticidal protein... comprising the steps of: i) contacting said sample with an antibody that specifically binds to said pesticidal protein, or a peptide or epitope derived therefrom; and ii) detecting the binding of said antibody to said pesticidal protein, peptide, or epitope derived therefrom").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the inventions.

Therefore, inventions of Group I+ lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.