

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

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Date of mailing
(day/month/year)

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Applicant's or agent's file reference

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FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US 23/65445

International filing date (day/month/year)

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Priority date (day/month/year)

12 April 2022 (12.04.2022)

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

IPC - INV. C12N 15/82, C12N 9/12, A01H 1/04, C12Q 1/6895 (2023.01)

ADD.

CPC - INV. C12N 15/82, C12N 9/1241, C12N 15/8213, A01H 1/045, C12N 15/8205, C12Q 1/6895

ADD. C12Q 2600/158

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
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Date of completion of this opinion

21 August 2023

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WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 23/65445

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.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
4. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established to the extent that a meaningful opinion could be formed without a WIPO Standard ST.26 compliant sequence listing.
5. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 23/65445

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. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-9, 14-19, directed to a recombinant DNA construct comprising a DNA sequence, or a transgenic plant, plant part or plant cell, or bacterial cell comprising a recombinant DNA construct comprising a DNA sequence. The recombinant DNA construct will be searched to the extent that the DNA sequence encompasses SEQ ID NO: 1. The first named invention was determined based on the first SEQ ID NO listed in claim 1. This first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. It is believed that claims 1-9 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a DNA regulatory sequence comprising SEQ ID NO: 1. Additional DNA regulatory sequences will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected DNA regulatory 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. An exemplary election would be where the DNA regulatory sequence comprises SEQ ID NO: 2 (claims 1-9).

Group II: Claims 10-13, directed to a method for producing a transgenic plant or plant part or a method for excising an expression cassette from the genome of a transgenic plant.

Group III: Claims 20-21, directed to a method of producing a commodity product comprising obtaining a transgenic plant or part.

Group IV: Claim 22, directed to a method of expressing a transcribable DNA construct comprising obtaining a transgenic plant and cultivating said plant, wherein the transcribable DNA is expressed.

Group V: Claim 23, directed to a Cre-recombinase coding sequence.

The inventions listed as Groups I+, II, III, IV, and V 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:

Special Technical Features

Group I+ requires composition of matter consisting of a recombinant DNA construct or plant or cell comprising a recombinant DNA construct, not required by Groups II-V.

No technical features are shared between the DNA sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share technical features, these shared technical features are previously disclosed by the prior art, as further discussed below.

Continued on Supplemental Page

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. 1-9, limited to SEQ ID NO: 1

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US 23/65445

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

1. Statement

Novelty (N)	Claims	1-9	YES
	Claims	NONE	NO
Inventive step (IS)	Claims	1-9	YES
	Claims	NONE	NO
Industrial applicability (IA)	Claims	1-9	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1-9 have been analyzed subject to the restriction that the claims read on SEQ ID NO: 1.

Claims 1-9 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest a DNA regulatory sequence comprising SEQ ID NO:1, wherein said DNA regulatory sequence is operably linked to a heterologous transcribable DNA sequence encoding a site-specific recombinase.

Regarding claim 1, US 2018/105820 A1 to Monsanto Technology LLC (hereinafter 'Monsanto'820') discloses a recombinant DNA construct comprising a DNA regulatory sequence operably linked to a heterologous transcribable DNA sequence encoding a site-specific recombinase (para [0007]-[0008] - "In aspects of the invention the recombinant DNA constructs further comprise DNA for one or more other elements, such as DNA encoding a selectable marker, DNA encoding a recombinase and/or DNA for a second pair of site-specific recombination sites.... One important aspect of the invention that a promoter operably linked to DNA encoding the recombinase is not constitutive... In one aspect transformed plant contains DNA for a recombinase operably linked to a constitutive promoter between recognition sites, e.g. between a second pair of recognition sites, expressing the recombinase either of the pairs of the recombination sites can be involved in recombination, recombination at the second pair of recombination sites will remove the DNA for the recombinase allowing suppression of the protein with selectable marker."; Claim 1 - "A recombinant DNA construct comprising a promoter operably linked to DNA encoding a protein in the sense orientation..."; Claim 3 - "The recombinant DNA construct of claim 1 further comprising DNA for expressing a selectable marker, DNA for expressing a site specific recombinase or both."; claim 7 - "The recombinant DNA construct of claim 3 wherein DNA for expressing a selectable marker and DNA for expressing a site specific recombinase are operably linked to a constitutive promoter."; Note, the term "construct", as defined by the instant application, includes any DNA molecule including a chromosome, see instant para [0084].

Monsanto'820 does not disclose a recombinant DNA construct comprising a DNA regulatory sequence comprising SEQ ID NO:1; wherein said DNA regulatory sequence is operably linked to a heterologous transcribable DNA sequence encoding a site-specific recombinase.

US 2015/0247154 A1 to Monsanto Technology, LLC (hereinafter 'Monsanto'154') discloses a recombinant DNA construct comprising a DNA regulatory sequence operably linked to a heterologous transcribable DNA sequence encoding a site-specific recombinase (para [0013]-[0014] - "Embodiments of this invention include methods to increase or improve yield of crop plants by expressing in such plants recombinant DNA constructs of this invention, for example, recombinant DNA constructs encoding a native miRNA precursor sequence or an artificial precursor sequence, or recombinant DNA constructs encoding a cleavage blocker or translational inhibitor or decoy. Further aspects of this invention provide non-natural transgenic plant cells having in their genome a recombinant DNA construct of this invention"; para [0028] - "In many embodiments, the recombinant DNA construct of this invention includes other DNA elements in addition to the DNA that undergoes processing to an RNA... These additional DNA elements include at least one element selected from the group consisting of: (a) a promoter functional in a eukaryotic (plant, animal, fungus, or protist) cell, such as any of the promoters described under the heading 'Promoters'... (g) DNA encoding a site-specific recombinase recognition site (as described under the heading 'Recombinases')"; para [0082] - "the recombinant DNA construct of this invention includes DNA encoding one or more site-specific recombinase recognition sites. In one embodiment, the recombinant DNA construct includes at least a pair of loxP sites, wherein site-specific recombination of DNA between the loxP sites is mediated by a Cre recombinase. The position and relative orientation of the loxP sites is selected to achieve the desired recombination").

Monsanto'154 does not disclose a recombinant DNA construct comprising a DNA regulatory sequence comprising SEQ ID NO:1; wherein said DNA regulatory sequence is operably linked to a heterologous transcribable DNA sequence encoding a site-specific recombinase.

GenBank Accession No. AC155419 "Zea mays strain B73 clone ZMMBBb0216G14, *** SEQUENCING IN PROGRESS ***", 26 unordered pieces" (hereinafter 'AC155419') discloses the sequence of SEQ ID NO: 1 (Sequence of AC155419, residues 111091-108871, exhibits 100% identity with SEQ ID NO: 1 of the instant application; Note, Sequence of AC155419 is a 142 kbp chromosome assembly). AC155419 does not disclose a recombinant DNA construct comprising a DNA regulatory sequence comprising SEQ ID NO:1; wherein said DNA regulatory sequence is operably linked to a heterologous transcribable DNA sequence encoding a site-specific recombinase.

Continued on Supplemental Page

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.

PCT/US 23/65445

Supplemental Box

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

Continuation of:

Box No. IV Lack of unity of invention

Group II requires a method comprising transforming a plant cell with a DNA molecule or vector comprising the recombinant DNA construct to produce one or more transformed plant cells comprising a recombinant DNA construct stably transformed into the genome of the one or more transformed plant cells; regenerating or developing a transgenic plant at least in part from the one or more stably transformed plant cells; crossing the transgenic plant to itself or another plant; and selecting one or more progeny plants in which one or both of the transcribable DNA sequence encoding the site-specific recombinase and/or the selectable marker transgene between the pair of site-specific recombination site sequences of the recombinant DNA construct are excised and no longer present in the genome of the progeny plants, not required by Groups I+ or III-V.

Group III requires a method of producing a commodity product comprising obtaining a transgenic plant or part thereof, not required by Groups I+, II, or IV-V.

Group IV requires a method of expressing a transcribable DNA construct comprising obtaining a transgenic plant and cultivating said plant, wherein the transcribable DNA is expressed, not required by Groups I+, II, III, or V.

Group V requires a Cre-recombinase coding sequence, not required by Groups I+ or II-IV.

Common Technical Features

The feature shared by Groups I+, II, III, IV and V is a nucleic acid sequence.

Additionally,

The features shared by Groups I+, II, III, and IV is a recombinant DNA construct and a transgenic plant or plant part.

The feature shared by Groups I+ is a recombinant DNA construct comprising a DNA sequence, or a transgenic plant, plant part or plant cell, or bacterial cell comprising a recombinant DNA construct comprising a DNA sequence, wherein said DNA sequence is operably linked to a heterologous transcribable DNA sequence encoding a site-specific recombinase.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught, by US 2015/0247154 A1 to Monsanto Technology, LLC (hereinafter 'Monsanto').

Monsanto discloses a transgenic plant comprising a recombinant DNA construct a coding sequence (para [0013]-[0014] - "Embodiments of this invention include methods to increase or improve yield of crop plants by expressing in such plants recombinant DNA constructs of this invention, for example, recombinant DNA constructs encoding a native miRNA precursor sequence or an artificial precursor sequence, or recombinant DNA constructs encoding a cleavage blocker or translational inhibitor or decoy. Further aspects of this invention provide non-natural transgenic plant cells having in their genome a recombinant DNA construct of this invention"), wherein said DNA sequence is operably linked to a heterologous transcribable DNA sequence encoding a site-specific recombinase (para [0082] - "the recombinant DNA construct of this invention includes DNA encoding one or more site-specific recombinase recognition sites. In one embodiment, the recombinant DNA construct includes at least a pair of loxP sites, wherein site-specific recombination of DNA between the loxP sites is mediated by a Cre recombinase. The position and relative orientation of the loxP sites is selected to achieve the desired recombination").

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 groups.

Groups I+, II, III, IV, and V therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 23/65445

Supplemental Box

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

Continuation of:

Box No. V(2) Citations and explanations:

GenBank Accession No. AC190647 "Zea mays cultivar B73 chromosome 5 clone ZMMBBb-216G14, ***SEQUENCING IN PROGRESS ****" (hereinafter 'AC190647') discloses the sequence of SEQ ID NO: 1 (Sequence of AC190647, residues 13384-11164, exhibits 100% identity with SEQ ID NO: 1 of the instant application; Note, Sequence of AC190647 is a 148 kbp chromosome assembly). AC190647 does not disclose a recombinant DNA construct comprising a DNA regulatory sequence comprising SEQ ID NO:1; wherein said DNA regulatory sequence is operably linked to a heterologous transcribable DNA sequence encoding a site-specific recombinase.

The prior art, either alone or in combination, do not specifically disclose a DNA regulatory sequence comprising SEQ ID NO:1, wherein said DNA regulatory sequence is operably linked to a heterologous transcribable DNA sequence encoding a site-specific recombinase, nor does sufficient motivation exist to select and use the sequence according SEQ ID NO: 1 in a recombinant construct comprising a linked heterologous transcribable DNA sequence encoding a site-specific recombinase. Thus, claim 1 meets the criteria set out in PCT Article 33(2)-(3).

Regarding claims 2-9, the claims are dependent upon claim 1 and therefore meet the criteria for substantially the same reasons.

Thus, claims 1-9 meet the criteria set out in PCT Article 33(2)-(3).

Claims 1-9 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.