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(54) Title: INIR6 TRANSGENIC MAIZE

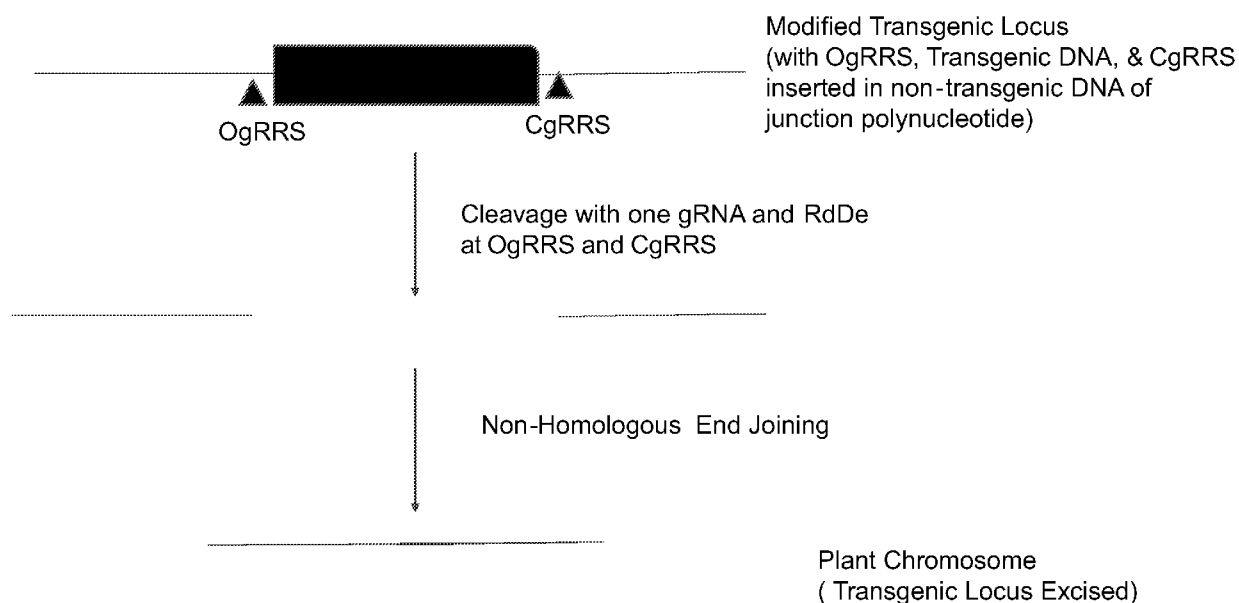


FIG. 3B

(57) Abstract: Transgenic INIR6 maize plants comprising modifications of the DP-4114 maize locus which provide for facile excision of the modified DP-4114 transgenic locus or portions thereof, methods of making such plants, and use of such plants to facilitate breeding are disclosed.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/44198

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/79; C12N 15/82; C12N 15/90 (2021.01)

CPC - C12N 15/79; C12N 15/8213; C12N 15/902; C12N 15/907; C12N 2310/20

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.
Y -- A	US 2019/0136331 A1 (PIONEER HI-BRED INTERNATIONAL, INC. et al.) 9 May 2019 (09.05.2019); especially para [0004], [0009], [0019], [0024], [0030], [0048]; SEQ ID NO: 6	1-2, 5-6 -- 3-4, 9-12
Y -- A	FINNIGAN et al. mCAL: A New Approach for Versatile Multiplex Action of Cas9 Using One sgRNA and Loci Flanked by a Programmed Target Sequence. G3 Genes, Genomics, Genetics. July 2016, Vol. 6, pg 2147-2156; especially abstract; pg 2148, col 1, para 2; Figure 1	1-2, 5-6 -- 3-4
A	US 2011/0154523 A1 (DIEHN et al.) 23 June 2011 (23.06.2011); especially SEQ ID NO: 6	3-4, 9-12
A	US 2006/0070139 A1 (BING et al.) 30 March 2006 (30.03.2006); especially SEQ ID NO: 24	3-4, 9-12

☐ 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

13 December 2021

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/44198

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

International application No.

PCT/US 21/44198

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

Continued on 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.:
1-6, 9-12, limited to CgRRS SEQ ID NO: 31

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 21/44198

Continue from Box No. III Observations where unity of invention is lacking

Group I+, claims 1-6, 9-12, 17-19, directed to a transgenic maize plant cell, a plant, plant part, or seed thereof, comprising an INIR6 transgenic locus comprising an originator guide RNA recognition site (OgRRS) and/or a cognate guide RNA recognition site (CgRRS) in a DNA junction polynucleotide of a DP-4114 transgenic locus, or a DNA/nucleic acid molecule, or a plant product or biological sample containing said molecule. The transgenic plant (cell, part, product, sample) and nucleic acid molecule will be searched to the extent that the INIR6 transgenic locus of the maize plant cell comprises the CgRRS of SEQ ID NO: 31. It is believed that claims 1-6, 9-12 encompass this first named invention, and thus these claims will be searched without fee to the extent that the CgRRS encompasses SEQ ID NO: 31. Additional CgRRS sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected CgRRS 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 CgRRS is SEQ ID NO: 22, (claims 9-12, 17-19).

Group II, claims 7-8, 20-21, directed to a method for obtaining a population of inbred or hybrid seed(s).

Group III, claims 13 and 22, directed to a method of detecting a maize plant cell comprising the INIR6 transgenic locus.

Group IV, claims 14-16, directed to a method of excising the INIR6 transgenic locus from the genome of the maize plant cell.

The inventions listed as Groups I+-IV 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:

Special technical features

Group I+ has the special technical feature of an article comprising or consisting of a transgenic maize plant cell, that is not required by Groups II-IV.

Group II has the special technical feature of a method for obtaining a population of inbred or hybrid seed, that is not required by Groups I+, III, IV.

Group III has the special technical feature of a method of detecting a maize plant cell comprising the INIR6 transgenic locus, that is not required by Groups I+, II, IV.

Group IV has the special technical feature of a method of excising the INIR6 transgenic locus from the genome of the maize plant cell, that is not required by Groups I+, II, III.

The inventions of Group I+ each include the special technical feature of a unique CgRRS nucleic acid sequence, and is considered a distinct technical feature.

Common technical features

The inventions of Groups I+, II, III and IV share the common technical feature of a maize plant cell comprising the INIR6 transgenic locus comprising a cognate guide RNA recognition site (CgRRS).

No technical features are shared between the CgRRS nucleic acid sequences of Group I+ and accordingly, these groups lack unity a priori.

Additionally, even if the inventions listed as Group I+ were considered to further share the technical features of including:

- transgenic maize plant cell comprising an INIR6 transgenic locus comprising an originator guide RNA recognition site (OgRRS) in a first DNA junction polynucleotide of a DP-4114 transgenic locus and a cognate guide RNA recognition site (CgRRS) in a second DNA junction polynucleotide of the DP-4114 transgenic locus;

- a transgenic maize plant cell comprising an INIR6 transgenic locus comprising an insertion and/or substitution of DNA in a DNA junction polynucleotide of a DP-4114 transgenic locus with DNA comprising a cognate guide RNA recognition site (CgRRS);

- a transgenic maize plant or plant part comprising the maize plant cell;

these shared technical features are previously disclosed by the prior art, as further discussed below.

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The features shared by Groups I+, II, III and IV and the features shared by the inventions listed as Group I+ are made obvious by US 2019/0136331 A1 to PIONEER HI-BRED INTERNATIONAL, INC. et al. (hereinafter 'Pioneer'), in view of the publication entitled "mCAL: A New Approach for Versatile Multiplex Action of Cas9 Using One sgRNA and Loci Flanked by a Programmed Target Sequence" by Finnigan et al. (G3: Genes, Genomes, Genetics. 2016 Jul 1;6(7):2147), (hereinafter 'Finnigan').

Pioneer discloses a transgenic maize plant cell comprising a DP-4114 transgenic locus (para [0009] - "Embodiments of this invention relate to methods for producing and selecting an insect resistant monocot crop plant. ... According to one aspect of the invention, a DNA construct, capable of introduction into and replication in a host cell, is provided that when expressed in plant cells and plants confers insect resistance to the plant cells and plants. Maize event DP-004114-3 was produced by Agrobacterium-mediated transformation with plasmid PHP27118. This event contains the cry1F, cry34Ab1, cry35Ab1, and pat gene cassettes, which confer resistance to certain lepidopteran and coleopteran pests, as well as tolerance to phosphinothricin.").

Pioneer does not specifically disclose a transgenic maize plant cell comprising an INIR6 transgenic locus comprising an originator guide RNA recognition site (OgRRS) in a first DNA junction polynucleotide of a DP-4114 transgenic locus and a cognate guide RNA recognition site (CgRRS) in a second DNA junction polynucleotide of the DP-4114 transgenic locus.

Pioneer does disclose the genomic borders of the transgene locus site (para [0030] - "FIG. 5. Schematic representation of the insert and genomic border regions sequenced in 4114 maize...The vertical dash line represents the genomic border/insert junctions. Fragment G and H represent the 5' and 3' genomic border regions, respectively.").

Finnigan discloses creation of artificial transgene loci with flanking gRNA recognition sites for a single gRNA (abstract - "First, any gene(s) of interest are flanked upstream and downstream with a single unique target sequence that does not normally exist in the genome. Thereafter, expression of one sgRNA and cotransformation with appropriate PCR fragments permits concomitant Cas9-mediated alteration of multiple genes (both essential and nonessential). The system we developed also allows for maintenance of the integrated, inducible Cas9- expression cassette or its simultaneous scarless excision."; pg 2148, col 1, para 2 - "Here, we describe a useful alternative strategy-introduction of unique, programmable, artificial target sequences into the genome, thereby permitting multiplex gene manipulation by Cas9 with a single sgRNA."; Figure 1 - depicts gRNA binding sites flanking target gene), and where the system is usable in any species where CRISPR/Cas9 technology is applicable (abstract - "Our scheme-dubbed mCAL for 'Multiplexing of Cas9 at Artificial Loci' - can be applied to any organism in which the CRISPR/Cas9 methodology is currently being utilized.").

Since Pioneer discloses corn comprising a DP-4114 transgenic locus and the surrounding genomic junction, it would have been obvious to one of ordinary skill in the art to modify the corn plant cell, as disclosed by Pioneer, to include or instead use a transgenic locus comprising 5' and 3' flanking gRNA recognition sites recognized by a single gRNA, as disclosed by Finnigan, and to consider mimicking an endogenous gRNA recognition site at the locus, since this would facilitate future modification at the DP-4114 transgenic locus by creating flanking gRNA sites targetable by a single gRNA, enabling facile insertion or elimination of transgenes, thereby increasing the value of the transgenic corn line.

Pioneer discloses a transgenic maize plant cell comprising a DP-4114 transgenic locus (para [0009]).

Pioneer does not specifically disclose a transgenic maize plant cell comprising an INIR6 transgenic locus comprising an insertion and/or substitution of DNA in a DNA junction polynucleotide of a DP-4114 transgenic locus with DNA comprising a cognate guide RNA recognition site (CgRRS).

Finnigan discloses creation of artificial transgene loci with flanking gRNA recognition sites for a single gRNA (abstract; pg 2148, col 1, para 2; pg 2150, Figure 1), and where the system is usable in any species where CRISPR/Cas9 technology is applicable (abstract).

Since Pioneer discloses corn comprising a DP-4114 transgenic locus and the surrounding genomic junction, it would have been obvious to one of ordinary skill in the art to modify the maize plant cell, as disclosed by Pioneer, to insert a 3' flanking recognition site, recognized by a single gRNA, as disclosed by Finnigan, and to consider mimicking an endogenous gRNA recognition site at the locus, since this would facilitate modification at the DP-4114 transgenic locus by creating flanking gRNA sites targetable by a single gRNA, enabling facile insertion or elimination of transgenes, thereby increasing the value of the transgenic maize line.

Pioneer further discloses a transgenic plant or plant part comprising the transgenic plant cell (para [0021]; [0051]).

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.

Therefore, Groups I+, II, III and IV inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.