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(71) Applicant: PURDUE RESEARCH FOUNDATION

[US/US]; 101 Foundry Drive, West Lafayette, Indiana 47906 (US).

(72) Inventors: MA, Jianxin; 610 Ridgewood Drive, West

Lafayette, Indiana 47906 (US). DUAN, Jingbo; 2413 Neil Armstrong Drive #2A, West Lafayette, Indiana 47906 (US).

(74) Agent: DEAN, Natalie J.; REICHEL STORHY DEAN

LLP, 6151 Central Ave, Indianapolis, Indiana 46220 (US).

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Declarations under Rule 4.17:

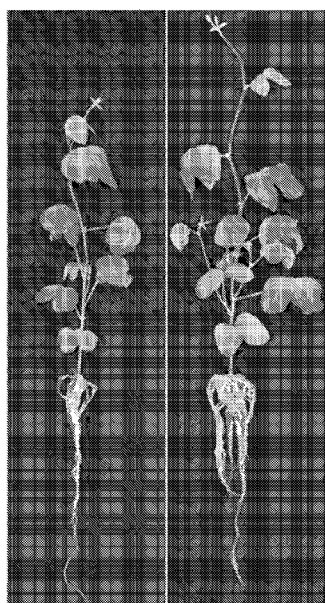
— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

— with international search report (Art. 21(3))

— with sequence listing part of description (Rule 5.2(a))

(54) Title: PLANT GROWTH AND SEED YIELD ENHANCEMENT METHODS AND CONSTRUCTS



Wild type Edited plant

FIG. 16

(57) Abstract: Expression constructs for modulating expression of *Le3* and/or miR4416-5p or miR4416-3p, genome-editing systems for leveraging the same to enhance plant yield, plant growth, and seed composition. Generally improved transgenic plants leveraging such constructs are also provided, as are methods for enhancing plant growth using such expression constructs and/or genome-editing systems.



(88) Date of publication of the international search report:
30 May 2025 (30.05.2025)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 24/33039

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C07K 14/42 (2024.01)

ADD. C07K 14/415 (2024.01)

CPC - INV. C07K 14/42, C12N 15/8241

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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	VAN RHIJN et al. Lotus corniculatus Nodulation Specificity Is Changed by the Presence of a Soybean Lectin Gene. The Plant Cell, August 1998, Vol. 10, pp 1233-1249, abstract, pg 1244, col 2, para 2	1, 3-5, 8-10 ----- 1-12, 16-18
Y	SAEED et al. Promoters of the soybean seed lectin homologues Le2 and Le3 regulate gene expression in vegetative tissues in Arabidopsis., Plant Science, 26 September 2008, vol 175, no 6, pp 868-876, abstract, pg 869, col 1, para 2	1, 3-5, 8-10
Y	CHRAUGH et al. Le4 Is an Epicotyl Preferential Homologue of the Soybean Seed-Specific Le1 Lectin and the Vegetative Le3 Lectin Genes., Plant Mol Biol Rep, 20 March 2015, Vol 33, pp 1779-1789, (pp 1-11), pg 2, col 1, para 2-3, pg 2, col 2, para 3, pg 6, col 1, para 2- col 2, para 2, Figs 3, 5a	1, 3-5, 8-10
Y	ZENG et al. Comparative transcriptome analysis of soybean response to bean pyralid larvae., BMC Genomics, 13 November 2017, vol 18, article No 871, pp 1-28, pg 18, col 1, para 1, pg 21, col 2, para 2	1, 3-5, 8-10
Y	US 2020/0216917 A1 (MONSANTO TECHNOLOGY LLC) 9 July 2020 (09.07.2020) para [0004], [0006]	4, 5, 10

☒ 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

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Facsimile No. 571-273-8300

Authorized officer

Kari Rodríguez

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 24/33039

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YAN et al. Identification and functional characterization of soybean root hair microRNAs expressed in response to Bradyrhizobium japonicum infection, Plant Biotechnol J, 14 May 2015, vol 14, pp 332-341, abstract, pg 332, col 2, para 2, pg 333, col 2, para 1	1-12, 16-18
A	GenBank submission LN041931, 7 October 2014 [online], [Retrieved on 17 October 2024]. Retrieved from the internet <URL:https://www.ncbi.nlm.nih.gov/nuccore/LN041931> entire document	1-12, 16-18
A	US 20030233675 A1 (CAO et al.) 18 December 2003 (18.12.2003) abstract, SEQ ID NO: 46173	1-12, 16-18

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 24/33039

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.: 39-42, 51-53, 56-57
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:

-----See extra sheet-----

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.:
1, 3-5 and 8-10 (first named invention), 1-12 and 16-18 (elected invention, limited to SEQ ID NO: 3)
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.:

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 24/33039

Continuation of Box No. III, Observations where unity of invention is lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-20, directed to a leguminous plant comprising at least one mutation, comprising a first mutation that upregulates expression of a Le3 gene in the leguminous plant as compared to a wild-type leguminous plant, and/or a second mutation that downregulates or silences expression of a miR4416 microRNA. The leguminous plant composition will be searched to the extent that the first mutation that upregulates expression of a Le3 gene comprises integrating an exogenous Le3 gene operably linked to a promoter that upregulates expression of the Le3 gene into a genomic locus. The first named invention was determined based on the first named alternative first mutation that upregulates expression of a Le3 gene in the leguminous plant in claims 1, 4-5 and 8. 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, 3-5 and 8-10 limited to said mutant leguminous plant encompass this first named invention, and thus these claims will be searched without fee to the extent that the leguminous plant encompass an exogenous Le3 gene operably linked to a promoter that upregulates expression of the Le3 gene. Additional mutant leguminous plants will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected mutant leguminous plants. 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 a leguminous plant comprising first and second mutations, wherein the first mutation is a Le3 gene comprises integrating an exogenous Le3 gene operably linked to a promoter that upregulates expression of the Le3 gene into a genomic locus, and the second mutation results in Le3 upregulation by downregulating or silencing expression of miR4416-5p with the mutant sequence of SEQ ID NO: 3, a miR4416-5p short tandem target mimic (STTM) sequence (claims 1-12).

Group II+: Claims 21-36, directed to an expression construct comprising: a first nucleic acid sequence that upregulates expression of a Le3 gene in a leguminous plant as compared to a corresponding wild-type leguminous plant; and/or a second nucleic acid sequence that downregulates or silences expression of microRNA (miRNA) miR4416-5p or miR4416-3p. Group II+ will be searched upon payment of additional fees. The leguminous plant expression construct may be searched, for example, to the extent that the first nucleic acid sequence that upregulates expression of a Le3 gene in a leguminous plant encodes two or more Le3 genes at a single genomic locus, wherein the first nucleic acid sequence comprises gene regulatory elements with at least a promoter for upregulating expression of the Le3 gene as compared to wild-type expression. It is believed that claims 21, 24-25 and 27-28 limited to said Le3 gene expression construct, read on this exemplary invention. Additional Le3 gene expression constructs will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected Le3 gene expression constructs. 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 a Le3 gene expression construct comprising first and second nucleic acids, wherein (i) the first nucleic acid comprises two or more Le3 genes, wherein the two or more Le3 genes are encoded at multiple genomic loci, and wherein the first nucleic acid sequence comprises gene regulatory elements with at least a promoter for upregulating expression of the Le3 gene as compared to wild-type expression; and (ii) the second nucleic acid sequence comprises SEQ ID NO: 1 (see applicant Fig. 6A-B), that downregulates or silences expression of microRNA (miRNA) miR4416-5p (Claims 21-24 and 26-28). Another exemplary election would be a Le3 gene expression construct comprising first and second nucleic acids, wherein (i) the first nucleic acid comprises two or more Le3 genes, wherein the two or more Le3 genes are encoded at a single genomic loci, and wherein the first nucleic acid sequence comprises gene regulatory elements with at least a promoter for upregulating expression of the Le3 gene as compared to wild-type expression; and (ii) the second nucleic acid sequence comprises a modified miR4416-5p that downregulates or silences miR4416-5p, comprising SEQ ID NO: 3, which comprises a STTM structure having mismatched nucleotides (CTA) causing a bulge when hybridized to wild-type miR4416-5p (see applicant Fig. 7A-C) (claims 21-25, 27-31 and 33).

Group III+: Claims 37-38, 43-50 and 54-55, directed to a method for enhancing leguminous plant growth, upregulating expression of a Le3 gene in a plant as compared to a wild-type leguminous plant; and/or downregulating or silencing expression of microRNA (miRNA) miR4416-5p, miR4416-3p, or both miR4416-5p and miR4416-3p in the plant as compared to a wild-type leguminous plant, wherein the plant yield is enhanced by at or more than about 20% and seed protein is increased as compared to an unmodified, wild-type plant. Group III+ will be searched upon payment of additional fees. The plant growth enhancement method may be searched, for example, to the extent that upregulating expression of a Le3 gene in a plant includes providing an Le3 gene to a plant cell that is operably linked to a promoter that upregulates expression of the Le3 gene, i.e., by incorporating one or more gene regulatory elements into a genome of the plant. It is believed that claims 37, 43-45 and 54-55, limited to said leguminous plant growth enhancement method, read on this exemplary invention. Additional plant growth enhancement methods will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected plant growth enhancement methods. 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 a plant growth enhancement method wherein the method comprises both (i) upregulating expression of a Le3 gene by providing an Le3 gene to a plant cell by incorporating one or more gene regulatory elements into a genome of the plant, i.e. wherein the gene is operably linked to a promoter that upregulates expression of the Le3 gene, and (ii) downregulating or silencing expression of miR4416-5p, wherein the downregulating or silencing expression of miR4416-5p comprises transforming the plant with a construct comprising SEQ ID NO: 3 (Claims 37-38, 43-46 and 54-55).

The inventions listed as Groups I+, II+ and III+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The technical feature of each of the inventions listed as Group I+ is the leguminous plant mutations recited therein. Each invention of Group II+ requires recombinant Le3 genes and/or miR4416 suppressing sequences not required by any of the other inventions.

***** See Next Extra Sheet to continue *****

INTERNATIONAL SEARCH REPORT

International application No.

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continuation of previous extra sheet:

Group II+ requires an isolated expression construct comprising Le3 gene and/or miR4416 sequences, not required by groups I +and III+.

Group III+ requires a method of enhancing leguminous plant growth, not required by groups I+ and II+.

Common Technical Features

The inventions of Groups I+, II+ and III+ share the technical feature of recombinant Le3 genes in a leguminous plant having upregulated expression.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is made obvious by the article entitled "Lotus corniculatus Nodulation Specificity Is Changed by the Presence of a Soybean Lectin Gene" by van Rhijn et al (hereinafter 'van Rhijn') (The Plant Cell, , August 1998, Vol. 10, pp 1233-1249). van Rhijn teaches that soybean lectin genes, when overexpressed, improve nodulation in plants (abstract "Plant lectins have been implicated as playing an important role in mediating recognition and specificity in the Rhizobium- legume nitrogen-fixing symbiosis. To test this hypothesis, we introduced the soybean lectin gene Le1 either behind its own promoter or behind the cauliflower mosaic virus 35S promoter into Lotus corniculatus, which is nodulated by R. loti. We found that nodulelike outgrowths developed on transgenic L. corniculatus plant roots in response to Bradyrhizobium japonicum, which nodulates soybean and not Lotus spp", pg 1244, col 2, para 2 "Lectin secretion and targeting to the root hair tips may result in the aggregation and attachment of a sufficient number of rhizobia so that a threshold level of Nod factor can interact with a receptor in a signal transduction pathway leading to nodule formation."). Since van Rhijn teaches that recombinant expression of a soybean lectin, including expression with a heterologous promoter, induces nodule formation in a heterologous plant, it would have been obvious to an artisan of ordinary skill in the art to experiment with over-expressing soybean lectin genes in a leguminous plat to encourage nodule formation, thus allowing increased nitrogen-fixing symbiosis and improved growth.

The inventions of Groups I+, II+ and III+ share the technical feature of miR4416 suppressing sequences in leguminous plants, plant cells or constructs to improve growth of plants. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is made obvious by the article entitled "Identification and functional characterization of soybean root hair microRNAs expressed in response to Bradyrhizobium japonicum infection", by Yan et al. (hereinafter 'Yan') (Plant Biotechnol J, 14 May 2015, vol 14, pp 332-341).

Yan teaches that soybean miR4416 expression reduces root nodule formation (abstract "The roles of gma-miR4416 and gma-miR2606b during nodulation were further analysed. Ectopic expression of these two miRNAs in soybean roots resulted in significant changes in nodule numbers", pg 333, col 2, para 1 "The data indicate that gma-miR2606b and gma-miR4416 positively and negatively regulate soybean nodulation, respectively", i.e., expression of miR4416 suppresses nodulation) and that root nodules support growth in leguminous plants (pg 332, col 2, para 2 "Soybean [Glycine max (L) Merr] is a legume crop that is a major source of vegetable oil and feed ...A key feature of soybean is the ability to establish a nitrogen-fixing symbiosis with the soil bacterium Bradyrhizobium japonicum, thereby alleviating the need for nitrogen fertilizer application. Under conditions of nitrogen deficiency, B. japonicum infects soybean roots through root hair cells, induces the formation of symbiotic root nodules and establishes a persistent, intracellular infection. Within the nodule, the bacteria convert atmospheric nitrogen into ammonia, which is provided to the plant to support growth"). Since Yan teaches that overexpression of miR4416 reduces root nodule formation in soybeans, and that nodules support plant growth, it would have been obvious to an artisan of ordinary skill in the art to experiment with suppressing miR4416 in leguminous plants to improve plant growth, yield or oil content.

As the technical features were known in the art, they cannot be considered special technical features that would otherwise unify the groups.

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