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

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25 June 2015

(54) Title: DIRIGENT GENE EG261 AND ITS ORTHOLOGS AND PARALOGS AND THEIR USES FOR PATHOGEN RESISTANCE IN PLANTS

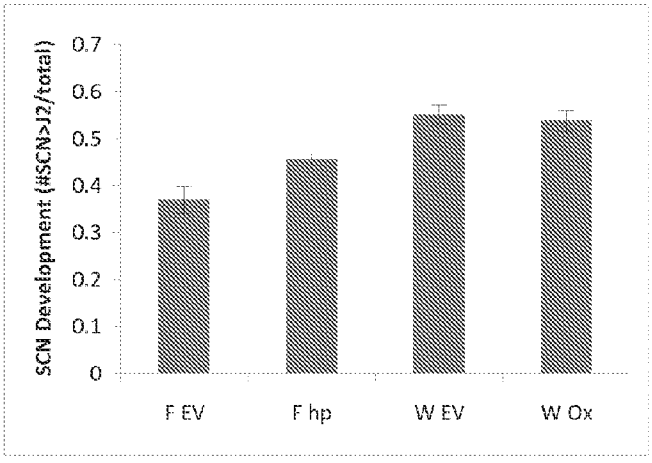

Figure 1.

(57) Abstract: The present invention provides the identification and use of EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof for altering, e.g. increasing, pathogen tolerance and/or resistance in plants.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/42382

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01H 1/00; C12N 15/82, 5/04 (2014.01)
USPC - 800/279, 278; 435/419
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): A01H 1/00; C12N 15/82, 15/87, 5/04, 5/10 (2014.01)
USPC: 800/279, 278; 435/419, 320.1, 410, 6.1, 468; 536/23.6, 23.1; 436/94

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)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Proquest Dialog (Pharmaceutical & Biomedical); Google; Google Scholar; Pubmed; Pubmed central/NCBI Blast; UniProt; 'glycine max,' pescadrensis, tabacina, isolated, recombinant, polynucleotide, resistance, tolerance, 'soybean cyst nematode,' 'heterodera glycines,' 'EG261'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2011/0247096 A1 (MCCAIG, B) October 6, 2011; abstract; paragraphs [0004], [0019]-[0024], [0034]-[0042], [0053]-[0069]; page 6, Table 1; Claims 13-16	1-4, 5/3, 5/4, 6/5/3, 6/5/4, 16, 17, 18/16, 18/17, 30, 32/1, 32/4 ---- 19, 20, 21, 22/20, 22/21, 23-25, 26/24, 26/25, 27, 28, 31, 36-38
Y	US 2012/0060240 A1 (LIGHTFOOT, DA et al.) March 8, 2012; paragraphs [0019]-[0021], [0043]-[0046], [0084], [0093], [00152], [0176], [0242], [0243], [0286]-[0291]	19, 23-25, 26/24, 26/25, 38
Y	US 2009/0100537 A1 (CONCIBIDO, V et al.) April 16, 2009; paragraphs [0119]-[0128]	20, 21, 22/20, 22/21, 37
Y	ZHANG, S et al. Gene Expression Of Soybean During Infection By Phytophthora sojae. Genbank [database online]. 30 May 2011. [retrieved on 2013-11-21] Retrieved from the internet: <URL: http://www.ncbi.nlm.nih.gov/nuccore/323433864?report=genbank&to=567> ACCESSION: HQ993047.	27, 28, 31, 36-38
Y	WO 2009/021153 A2 (CONCIBIDO, V et al.) February 12, 2009; page 25, lines 17-28; page 26, line 18 - page 28, line 16	36-38
Y	XU, P et al. Differentially Expressed Genes Of Soybean During Infection By Phytophthora sojae. Journal of Integrative Agriculture. March 2012, Vol. 11, No. 3; pages 368-377; page 371, first column; page 372, Table 3; DOI: 10.1016/S2095-3119(12)60021-5.	27, 28, 31, 36-38
P,Y	US 2012/0260368 A1 (MITCHUM, MG et al.) October 11, 2012; entire document	1-6, 16-28, 30-32, 36-38

☒ Further documents are listed in the continuation of Box C. ☐

* 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
12 February 2014 (12.02.2014)	03 MAR 2014
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. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/42382

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2012/0131693 A1 (BAI, Y et al.) May 24, 2012; entire document	1-6, 16-28, 30-32, 36-38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/42382

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.: 7-15, 33-35
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.:

Groups I+: Claims 1-6, 16-28, 30-32, 36-38, SEQ ID NO: 1 (nucleic acid sequence coding for EG261)

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/US13/42382

-***-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 1-6, 16-32, 36-38, SEQ ID NO: 1 (nucleic acid sequence coding for EG261) are directed toward an isolated, recombinant, or synthetic polynucleotide comprising a nucleic acid sequence of (a); (b); (c); (d); (e) complements of a nucleic acid sequence of (a), (b), (c) or (d); (f) reverse complements of a nucleic acid sequence of (a), (b), (c) or (d); (g) reverse sequences of a nucleic acid sequence of (a), (b), (c) or (d); (h) mRNA sequence of nucleic acid sequence of (a), (b), (c) or (d); and, (i) fragments and variations of a nucleic acid sequence of (a), (b), (c), (d), (e), (f), (g) and (h); a process of determining the presence or absence of a polynucleotide coding for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof in a plant, wherein the process comprises at least one of: (a) isolating nucleic acid molecules from said plant and amplifying sequences homologous to the polynucleotide; (b) isolating nucleic acid molecules from said plant and performing a Southern hybridization to detect the polynucleotide; (c) isolating proteins from said plant and peforming a Western Blot using antibodies to a protein encoded by the polynucleotide; and/or (d) demonstrating the presence of mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide; a method of producing a plant with conferred or enhanced pathogen tolerance and/or resistance, the process comprising: (b) crossing a first plant containing a polynucleotide coding for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof to a second plant, and harvesting the resultant seed; (b) determining the presence of the polynucleotide in the resultant seed or in cells or tissues of a plant grown from the resultant seed; wherein the determining comprises at least one of: (i) isolating nucleic acid molecules from the resultant seed or in cells or tissues of a plant grown from the resultant seed and amplifying sequences homologous to the polynucleotide; (ii) isolating nucleic acid molecules from the resultant seed or in cells or tissues of a plant grown from the resultant seed and performing a Southern hybridization to detect the polynucleotide; (iii) isolating proteins from the resultant seed or in cells or tissues of a plant grown from the resultant seed and performing a Western Blot using antibodies to a protein encoded by the polynucleotide; and/or (iv) demonstrating the presence in the resultant seed or in cells or tissues of a plant grown from the resultant seed or mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide; an isolated, recombinant, or synthetic polypeptide, wherein the polypeptide comprises an amino acid sequence having at least 90% identity to a polypeptide encoded by an EG261 gene selected from the group consisting of SEQ ID NOs: 1, 2, 16, 17, 18, 19, 20, 21, 22, 23, 24, 34, 35, 36, 40, 41, 42, 46, 47, 48, 49, 50, 56, 58, 60, and 62, wherein the isolated, recombinant, or synthetic polypeptide when expressed in a plant species induces resistance to a plant pathogen.

SEQ ID NO: 1 will be searched without the payment of any additional fees. Additional SEQ ID NOs can be searched upon the payment of additional fees. It is believed that Claims 1-6, 16-28, 30-32 and 36-38 encompass this first named invention and thus these claims will be searched without the payment of additional fees. An Exemplary Election would be: SEQ ID NO: 2. 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.

Groups I+ share the technical features including an isolated, recombinant, or synthetic polynucleotide comprising a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for EG261 selected from the group consisting of SEQ ID NOs: 1, 2, 16, 17,18, 19, 20, 21, 22, 23, 24, 34, 35, 36, 40, 41, 42, 46, 47, 48, 49, 50, 56, 58, 60, and 62: (b) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for a homolog of EG261 selected from the group consisting of SEQ ID NOs: 1,2, 16, 17, 18, 19, 20, 21, 22, 23, 24, 34, 35, 36, 40, 41, 42, 46, 47, 48, 49, 50, 56, 58, 60, and 62; (c) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for an ortholog of EG261 selected from the group consisting of SEQ IDNOs: 1, 2, 16, 17. 18, 19, 20, 21, 22, 23, 24, 34, 35, 36, 40, 41, 42, 46, 47, 48, 49, 50, 56, 58, 60, and 62; (d) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for a paralog of EG261 selected from the group consisting of SEQ ID NOs: 1, 2, 16, 17, 18, 19, 20, 21, 22, 23, 24, 34, 35, 36, 40, 41, 42, 46, 47, 48, 49, 50, 56, 58, 60, and 62; (e) complements of a nucleic acid sequence of (a), (b), (c) or (d); (f) reverse complements of a nucleic acid sequence of (a), (b), (c) or (d); (g) reverse sequences of a nucleic acid sequence of (a), (b), (c) or (d); (h) mRNA sequence of nucleic acid sequence of (a), (b), (c) or (d); and, (i) fragments and variations of a nucleic acid sequence of (a), (b), (c), (d), (e), (f), (g) and (h); a process of determining the presence or absence of a polynucleotide coding for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof in a plant, wherein the process comprises at least one of: (a) isolating nucleic acid molecules from said plant and amplifying sequences homologous to the polynucleotide; (b) isolating nucleic acid molecules from said plant and peforming a Southern hybridization to detect the polynucleotide; (c) isolating proteins from said plant and peforming a Western Blot using antibodies to a protein encoded by the polynucleotide; and/or (d) demonstrating the presence of mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide; a method of producing a plant with conferred or enhanced pathogen tolerance and/or resistance, the process comprising: (b) crossing a first plant containing a polynucleotide coding for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof to a second plant, and harvesting the resultant seed; (b) determining the presence of the polynucleotide in the resultant seed or in cells or tissues of a plant grown from the resultant seed; wherein the determining comprises at least one of: (i) isolating nucleic acid molecules from the resultant seed or in cells or tissues of a plant grown from the resultant seed and amplifying sequences homologous to the polynucleotide; (ii) isolating nucleic acid molecules from the resultant seed or in cells or tissues of a plant grown from the resultant seed and performing a Southern hybridization to detect the polynucleotide; (iii) isolating proteins from the resultant seed or in cells or tissues of a plant grown from the resultant seed and performing a Western Blot using antibodies to a protein encoded by the polynucleotide; and/or (iv) demonstrating the presence in the resultant seed or in cells or tissues of a plant grown from the resultant seed or mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide; an isolated, recombinant, or synthetic polypeptide, wherein the polypeptide comprises an amino acid sequence having at least 90% identity to a polypeptide encoded by an EG261 gene selected from the group consisting of SEQ ID NOs: 1, 2, 16, 17, 18, 19, 20, 21, 22, 23, 24, 34, 35, 36, 40, 41, 42, 46, 47, 48, 49, 50, 56, 58, 60, and 62, wherein the isolated, recombinant, or synthetic polypeptide when expressed in a plant species induces resistance to a plant pathogen.

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from Previous Supplemental Box-***-
However, these shared technical features are previously disclosed by US 2011/0247096 A1 (MCCAIG) in view of US 2012/0060240 A1 to Lightfoot, et al. (hereinafter 'Lightfoot'). McCaig discloses an isolated (isolated; paragraph [0020]), recombinant (expression vector (recombinant); paragraph [0020]) or synthetic polynucleotide comprising a nucleic acid (polynucleotide (nucleic acid); paragraph [0019]) sequence selected from the group consisting of: (a) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for EG261; (b) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for a homolog of EG261; (c) a nucleic acid sequence having at least 95%) identical nucleotides to a nucleic acid sequence coding for an ortholog of EG261; (d) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for a paralog of EG261; (e) complements of a nucleic acid sequence of (a), (b), (c) or (d): (i) reverse complements of a nucleic acid sequence of (a), (b), (c) or (d); (g) reverse sequences of a nucleic acid sequence of (a), (b), (c) or (d): (h) mRNA sequence of nucleic acid sequence of (a), (b), (c) or (d); and, (i) fragments and variations of a nucleic acid sequence of (a), (b), (c), (d), (e), (t), (g) and (h) (nucleotides 1-3 of SEQ ID NO: 49 (a fragment of (b) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for SEQ ID NO: 1) (100% identical to nucleotides 10-12 of SEQ ID NO: 1)) coding for a homolog of EG261 (the N-terminal methionine of a dirigent-like protein conferring SCN resistance (homolog of EG261); Figure 1b/4; paragraph [0019]); a method of producing a plant (a method of plant breeding (a method of producing a plant); paragraph [0060]) with conferred or enhanced pathogen tolerance and/or resistance (nematode-resistant transgenic plant (with conferred or enhanced pathogen tolerance or resistance); paragraph [0059]), the process comprising: (b)(1) crossing a first plant containing a polynucleotide (crossing a first plant containing a polynucleotide; paragraph [0060]) coding for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261 (coding for a Dirigent-like protein that renders plants resistant to SCN infection; (homologs of EG261); paragraph [0019]), and fragments and variations thereof to a second plant, and harvesting the resultant seed (to prepare seed (harvesting the resultant seed); paragraph [0060]); an isolated, recombinant (recombinant; paragraph [0021]), or synthetic polypeptide (polypeptide; paragraph [0033]) encoded by an EG261 gene (encoded by a Dirigent-like protein that renders plants resistant to SCN infection (an EG261 gene); paragraphs [0019], [0053]), wherein the isolated, recombinant (recombinant; paragraph [0021]), or synthetic polypeptide (polypeptide; paragraph [0033]), when expressed in a plant species, induces resistance to a plant pathogen (when expressed in a plant species, induces resistance to SCN infection (when expressed in a plant species, induces resistance to a plant pathogen); paragraph [0019]; isolated nucleic acid molecules (isolated expression vectors (isolated nucleic acid molecules), paragraph [0020]) coding for EG261, homologs of EG261 (a Dirigent-like protein that renders plants resistant to SCN infection; (homologs of EG261); paragraph [0019]), orthologs of EG261, paralogs of EG261, and fragments and variations thereof.

McCaig does not disclose a process of determining the presence or absence of a polynucleotide coding for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof in a plant, wherein the process comprises at least one of: (a) isolating nucleic acid molecules from said plant and amplifying sequences homologous to the polynucleotide; (b) isolating nucleic acid molecules from said plant and performing a Southern hybridization to detect the polynucleotide; (c) isolating proteins from said plant and performing a Western Blot using antibodies to a protein encoded by the polynucleotide; and/or (d) demonstrating the presence of mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide; (b)(2) determining the presence of the polynucleotide in the resultant seed or in cells or tissues of a plant grown from the resultant seed; wherein the determining comprises at least one of: (i) isolating nucleic acid molecules from the resultant seed or in cells or tissues of a plant grown from the resultant seed and amplifying sequences homologous to the polynucleotide; (ii) isolating nucleic acid molecules from the resultant seed or in cells or tissues of a plant grown from the resultant seed and performing a Southern hybridization to detect the polynucleotide; (iii) isolating proteins from the resultant seed or in cells or tissues of a plant grown from the resultant seed and performing a Western Blot using antibodies to a protein encoded by the polynucleotide; and/or (iv) demonstrating the presence in the resultant seed or in cells or tissues of a plant grown from the resultant seed of mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide.

Lightfoot discloses a process of determining the presence or absence of a polynucleotide (method for detecting nucleic acid (process of determining the presence or absence of a polynucleotide); paragraph [0019]) coding for soybean cyst nematode resistance (encodes an SCN resistance polypeptide (coding for soybean cyst nematode resistance); paragraph [0019]), and fragments and variations thereof in a plant (a plant; paragraph [0286]), wherein the process comprises at least one of: (a) isolating nucleic acid molecules from said plant (analyzing genomic DNA from a plant or seed (isolating nucleic acid molecules from said plant); paragraph [0286]) and amplifying sequences homologous to the polynucleotide (amplifying sequences homologous to the polynucleotide; paragraph [0305]); (b) isolating nucleic acid molecules from said plant (analyzing genomic DNA from a plant or seed (isolating nucleic acid molecules from said plant); paragraph [0286]) and performing a Southern hybridization to detect the polynucleotide (performing a Southern hybridization to detect the polynucleotide; paragraph [0176]); (c) isolating proteins from said plant and performing a Western Blot using antibodies to a protein encoded by the polynucleotide (isolating proteins from said plant and performing a Western Blot using antibodies to a protein encoded by the polynucleotide; paragraphs [0242], [0243]) and/or (d) demonstrating the presence of mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide; (b)(2) determining the presence of the polynucleotide in the resultant seed (determining the presence of the polynucleotide in the resultant seed; paragraph [0286]) or in cells or tissues of a plant grown from the resultant seed (or in cells or tissues of a plant grown from the resultant seed; paragraph [0264]); wherein the determining comprises at least one of: (i) isolating nucleic acid molecules from the resultant seed or in cells or tissues of a plant grown from the resultant seed (analyzing genomic DNA from a plant or seed (isolating nucleic acid molecules from said plant); paragraph [0286]) and amplifying sequences homologous to the polynucleotide (amplifying sequences homologous to the polynucleotide; paragraph [0305]); (ii) isolating nucleic acid molecules from the resultant seed or in cells or tissues of a plant grown from the resultant seed (analyzing genomic DNA from a plant or seed (isolating nucleic acid molecules from said plant); paragraph [0286]); and performing a Southern hybridization to detect the polynucleotide (performing a Southern hybridization to detect the polynucleotide; paragraph [0176]); (iii) isolating proteins from the resultant seed or in cells or tissues of a plant grown from the resultant seed and performing a Western Blot using antibodies to a protein encoded by the polynucleotide (isolating proteins from the resultant seed or in cells or tissues of a plant grown from the resultant seed and performing a Western Blot using antibodies to a protein encoded by the polynucleotide; paragraphs [0242], [0243]); and/or (iv) demonstrating the presence in the resultant seed or in cells or tissues of a plant grown from the resultant seed of mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide. 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 McCaig, in order to have incorporated the use of the detection methods previously disclosed by Lightfoot, for ensuring the presence of the desired gene in transgenic plants, seeds, and progeny, without undue experimentation in a laboratory.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the combination of the McCaig and Lightfoot references, unity of invention is lacking.