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(54) Title: TRANSGENIC CORN SEED WITH ENHANCED AMINO ACID CONTENT

(57) Abstract: Anti-sense-oriented RNA gene suppression agents in the form of a loop of anti-sense-oriented RNA is produced in cells of transgenic organisms, e.g. plants, by transcription from a recombinant DNA construct which comprises in 5' to 3' order a promoter element operably linked to an anti-sense-oriented DNA element and a complementary DNA element.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/04542

A. CLASSIFICATION OF SUBJECT MATTER
IPC: **C12N 15/00**(2006.01),**15/09**(2006.01),**15/82**(2006.01),**15/87**(2006.01);**A01H 5/00**(2006.01)

USPC: 435/320.1;800/285,286,287,295,298
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)
U.S. : 435/320.1; 800/285, 286, 287, 295, 298

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)
WEST, Agricola, CAplus, Biosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ISHIDA et al. High efficiency transformation of maize (Zea mays L.) mediated by Agrobacterium tumefaciens. Nature Biotech. June 1996, Vol. 14, pages 745-750, see whole document.	1, 2, 10, 12-18
Y	WATERHOUSE et al. Virus resistance and gene silencing in plant can be induced by simultaneous expression of sense and antisense RNA. Proc. Natl. Acad. Sci. USA. November 1998, Vol. 95, pages 13959-13964, see pages 13960-13962.	1, 2, 9, 10, 12-18
Y	SMITH et al. Total silencing by intron-spliced hairpin RNAs. Nature. 21 September 2000, Vol. 407, pages 319-320, see whole document.	1, 2, 9, 10, 12-18
Y	WESLEY et al. Construct design for efficient, effective, and high-throughput gene silencing in plants. Plant J. 2001, Vol. 27, No. 6, pages 581-590, see pages 582-586.	1, 2, 9, 10, 12-18

☒ 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

"E" earlier application or patent 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

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"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

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International application No.
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C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 99/53050 (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION. 21 October 1999 (21.10.99), see page 14, lines 71-17; page 21, line 12 to page 23, lie 15; page 57, line 1 to page 67, line 1; Figure 2A.	1, 2, 9, 10, 12-18
Y	ZHU et al. A T-DNA insertion knockout of the bifunctional lysine-ketoglutarate reductase/saccharopine dehydrogenase gene elevates lysine levels in Arabidopsis seeds. Plant Physiol. August 2001, Vol. 126, pages 1539-1545, see pages 1540-1542.	1, 2, 9, 10, 12-18
Y	WU et al. Quantitative nature of the prolamin-box, ACGT and AACA motifs in a rice glutelin gene promoter: minimal cis-element requirements for endosperm-specific gene expression. Plant J. 2000, Vol. 23, No. 3, pages 415-421, see whole document.	13
Y	US 5,545,545 (GENGENBACH et al.) 13 August 1996 (13.08.1996), column 45, line to column 48, line 30.	16-18

BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group I, claim(s) 1, 2, 9, 10, 12-18, drawn seed for producing a transgenic corn with enhanced amino acid content having integrated into its genome a recombinant DNA construct that comprises anti-sense oriented DNA element and a sense-oriented DNA element that is complementary to the 5'-end of anti-sense oriented RNA transcribed by the antisense-oriented DNA element, wherein said transcribed RNA forms a loop of antisense-oriented RNA for suppressing a protein in an amino acid catabolic pathway; and a method of increasing the level of lysine in corn seed by expressing said recombinant DNA construct wherein the gene encoding lysine ketoglutarate reductase is suppressed.

Group II, claim(s) 1, 3-8, 11, drawn to seed for producing a transgenic corn with enhanced amino acid content having integrated into its genome a recombinant DNA construct that transcribes anti-sense oriented RNA.

The inventions listed as Groups I-II 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: the technical feature shared between the groups is the expression of a DNA construct that encodes an RNA that is antisense to a target gene, to cause its suppression. However, Commonwealth Scientific and Industrial Research Organization (WO 99/53050) teaches a reduction of any nucleotide sequence of interest by introduction of a chimeric DNA into cells, encoding an RNA molecule comprising a sense nucleotide sequence that has sequence identity with a nucleotide sequence of interest, and an antisense molecule complementary to the sense sequence, wherein the RNA is capable of forming an artificial hairpin structure with a double-stranded stem (page 8, lines 5-28; claims). The technical feature of the instant claims is not special.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US05/04542

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:
Please See Continuation 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 any 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.: 1,2,9,10 and 12-18

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.