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(54) Title: SMALL INTERFERING RNA (SIRNA)-MEDIATED HERITABLE GENE MANIPULATION IN PLANTS

(57) Abstract: The presently disclosed subject matter provides methods and compositions for stably modulating gene expression in plants. Also provided are plants and cells comprising the compositions of the presently disclosed subject matter.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US04/39942

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 5/14, 15/63, 15/82, 15/84, 15/90,
US CL : 435/320.1, 4191, 468; 800/285, 286, 293

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, 4191, 468; 800/285, 286, 293

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, GENEMBL, GENESSEQ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	KLAHRE et al. High molecular weight RNAs and small interfering RNAs induce systemic posttranscriptional gene silencing in plants. PNAS. 03 September 2002, Vol. 99, No. 18, pages 11981-11986, see whole document.	1-3, 9, 12, 14-16, 24-25, 33, 38-44, 46-48, 63-67, 71-72, 74 ----- 4-8, 10, 11, 13, 17-21, 26, 34-37, 45, 49-62, 68-70, 73, 75, 76
A	HAMILTON et al. Two classes of short interfering RNA in RNA silencing. EMBO J. 2002, Vol. 21, No. 17, pages 4671-4679, see whole document.	1-21, 24-26 and 33-76
Y	WATERHOUSE et al. Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA. Proc. Natl. Acad. Sci. USA. November 1998, Vol. 95, pages 13959-13964, see whole document.	1-21, 24-26, 33-76
Y	WO 98/39454 (ZENECA LIMITED) 11 September 1998 (11.09.98), see page 7, line 5 to page 9, line 22.	34, 35, 54, 55, 58, 59

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	"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
"E" earlier application or patent published on or after the international filing date	"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
"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)	"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
"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	"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

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	HEARD et al. An upstream U-snrRNA gene-like promoter is required for transcription of the Arabidopsis thaliana 7SL RNA gene. Nucl. Acids Res. 1995, Vol. 23, No. 11, pages 1970-1976, see page 1972.	6, 7, 19, 20
Y	JACOBS et al. An Arabidopsis callose synthase, GSL5, is required for wound and papillary callose formation. Plant Cell. November 2003, Vol. 15, pages 2503-2513, see pages 2504-2505.	34, 36, 54, 56, 58, 60
Y	CHUANG et al. Specific and heritable genetic interference by double-stranded RNA in Arabidopsis thaliana. PNAS. 25 April 2000, Vol. 97, No. 9, pages 4985-4990, see whole document.	1-21, 24-26, 33-76
Y	ESCOBAR et al. RNAi-mediated oncogene silencing confers resistance to crown gall tumorigenesis. PNAS. 06 November 2001, Vol. 98, No. 23, pages 13437-13442, see pages 13439-13441.	28, 31, 34, 37, 54, 57, 58, 61

INTERNATIONAL SEARCH REPORT

International application No. -

PCT/US04/39942

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.: 22 and 23
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.: 1-26 and 33-76
- 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.

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BOX 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.

Group I, claim(s) 1-26, 33-76, drawn to a first method for stably inhibiting expression of a plant gene comprising transforming a plant cell with a vector encoding a siRNA targeted to the plant gene, and a first product, a vector for stably expressing a siRNA molecule in a plant, and a plant cell, seed, or plant comprising said vector.

Group II, claim(s) 27-31, drawn to a second method for enhancing the expression of a gene in a plant cell, comprising introducing a vector encoding a siRNA corresponding to a subsequence of the gene.

Group III, claim(s) 32, drawn to a third method, for enhancing the expression of a gene in a plant cell, comprising introducing a vector comprising a siRNA molecule that hybridizes to a nucleic acid molecule encoding a repressor of the gene.

The inventions listed as Groups I-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: The invention of Groups I and II share the technical feature of introducing a vector comprising an siRNA of a target gene into plant cells. However, this is not a special technical feature. Klahre et al. (PNAS, 9/03/2002, Vol. 99, No. 18, pages 11981-11986) teach the introduction a vector comprising siRNA targeting a gene present in the host plant genome. Expression of the gene was inhibited upon expression of the siRNA (pages 11982-11983). The instant description indicates that the target gene may be any gene expressed in the plant (page 45, lines 9-10). The method of Group III does not share the technical feature, as the method of Group III is for enhancing the expression of a gene that is not the target of the siRNA.