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(54) Title: COMPOSITIONS AND METHODS FOR THE MODULATION OF GENE EXPRESSION IN PLANTS

(57) Abstract: Compositions and methods for modulating nucleotide sequence expression, particularly for modulating gene expression in plants, are provided. The compositions comprise precursor RNA constructs for the expression of an RNA precursor. The precursor RNA construct comprises a promoter that is expressed in a plant cell driving the expression of a precursor RNA having a microRNA. The miRNA is complementary or partially complementary to a portion of a target gene or nucleotide sequence and function to modulate expression of the target sequence or gene. In this manner, the RNA precursor construct can be designed to modulate expression of any nucleotide sequence of interest, either an endogenous plant gene or alternatively a transgene. The precursor RNA constructs may be used in combination with modulators to enhance the effect on gene expression. Expression of a modulator in the presence of the precursor RNA alters the accumulation of miRNAs and thus enhances the regulatory capabilities of miRNAs. The invention further comprises the use of a modulator to control gene expression via both siRNA and the miRNA pathway. Transformed plants, tissues, cells and seeds are also provided.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/22718

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>																				
IPC(7) : C12N 15/09, 15/11, 15/63, 15/69, 15/82, 15/86, 15/87, 15/90; A01H 5/00, 5/10																				
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<b>B. FIELDS SEARCHED</b>																				
Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/419, 468; 800/278, 285, 287, 288, 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																				
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>																				
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																		
Y, P, T	MALLORY et al. A Viral Suppressor of RNA Silencing Differentially Regulates the Accumulation of Short Interfering RNAs and Micro-RNAs in Tobacco. PNAS. 12 November 2002, Vol. 99, No. 23, pages 15228-15233, see whole document.	7																		
A, T	PAPP et al. Evidence for Nuclear Processing of Plant Micro RNA and Short Interfering RNA Precursors. Plant Physiol. July 2003, Vol. 132, pages 1382-1390, see whole document.	1-27																		
A, P, T	BARTEL et al. MicroRNAs: At the Root of Plant Development. Plant Physiol. June 2003, Vol. 132, pages 709-717, see whole document.	1-27																		
A, P, T	LEE et al. MicroRNA Maturation: Stepwise Processing and Subcellular Localization. 2002, Vol. 21, pages 4663-4670, see whole document	1-27																		
Y	ANGELL et al. Consistent Gene-Silencing in Transgenic Plants Expressing a Replicating Potato Virus X RNA. EMBO J. 1997, Vol. 16, No. 12, pages 3675-3684, see whole document.	9-19, 22, 25																		
Y	MALLORY et al. The Amplicon-Plus System for High-Level Expression of Transgenes in Plants. Nature Biotech. June 2002, Vol. 20, pages 622-625, see whole document.	9-19, 22, 25																		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.																				
<table style="width: 100%; border: none;"> <tr> <td style="width: 30%; border: none;">* Special categories of cited documents:</td> <td style="width: 30%; border: none;">"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</td> <td style="width: 40%; border: none;"></td> </tr> <tr> <td style="border: none;">"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td style="border: none;">"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</td> <td style="border: none;"></td> </tr> <tr> <td style="border: none;">"E" earlier application or patent published on or after the international filing date</td> <td style="border: none;">"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</td> <td style="border: none;"></td> </tr> <tr> <td style="border: none;">"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)</td> <td style="border: none;">"&amp;" document member of the same patent family</td> <td style="border: none;"></td> </tr> <tr> <td style="border: none;">"O" document referring to an oral disclosure, use, exhibition or other means</td> <td style="border: none;"></td> <td style="border: none;"></td> </tr> <tr> <td style="border: none;">"P" document published prior to the international filing date but later than the priority date claimed</td> <td style="border: none;"></td> <td style="border: none;"></td> </tr> </table>			* Special categories of cited documents:	"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		"A" document defining the general state of the art which is not considered to be of particular relevance	"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		"E" earlier application or patent published on or after the international filing date	"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		"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)	"&" document member of the same patent family		"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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"P" document published prior to the international filing date but later than the priority date claimed																				
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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. (703) 305-3230		Authorized officer <i>Ashwin Mehta</i> Telephone No. 571-272-1600																		

## INTERNATIONAL SEARCH REPORT

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VOINNET, O. RNA Silencing as a Plant Immune System Against Viruses. Trends in Genetics. August 2001, Vol. 17, No. 8, pages 449-459, see pages 456-457.	7
A, T	HUNTER et al. Missing Links: MiRNAs and Plant Development. Curr. Opin. Genet. Devel. 2003, Vol. 13, pages 372-378, see whole document.	1-27