



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> : C12N 15/29, 15/52, 15/82, 9/02, 5/10, A23D 9/00, A23K 1/14		A3	(11) International Publication Number: <b>WO 00/11176</b>  (43) International Publication Date: 2 March 2000 (02.03.00)
(21) International Application Number: PCT/US99/18562 (22) International Filing Date: 16 August 1999 (16.08.99) (30) Priority Data: 60/097,186      20 August 1998 (20.08.98)      US 60/142,756      8 July 1999 (08.07.99)      US (71) Applicant (for all designated States except US): E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): CAHOON, Edgar, Benjamin [US/US]; 2331 West 18th Street, Wilmington, Delaware 19806 (US). CARLSON, Thomas, J. [US/US]; 2316 Orchard Road, Ardentown, DE 19810 (US). HITZ, William, Dean [US/US]; 404 Hillside Road, Wilmington, DE 19807 (US). RIPP, Kevin, G. [US/US]; 2310 West 18th Street, Wilmington, DE 19806 (US). (74) Agent: CHRISTENBURY, Lynne, M.; E.I. du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).		(81) Designated States: AE, AL, AU, BA, BB, BG, BR, CA, CN, CR, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report. (88) Date of publication of the international search report: 5 October 2000 (05.10.00)	

(54) Title: GENES FOR PLANT FATTY ACID MODIFYING ENZYMES ASSOCIATED WITH CONJUGATED DOUBLE BOND FORMATION

		1		60
	Hydroxylase	MGGGGRMSTVITSNNSEKKG--GSS--HLKRAPHTKPPFTLGDLCRAIPPHCFERSFVRS		
	ImpFad2H8	MGEVGPNTNR--TKTKLDKQSESEN-----RVPHEPPPFTLSDLKKAIPPHCFERSLVKS		
5	MomFad2	MGGGGAIGVLRNGGGPKKKMGPGQGLPGERITHARPPFSISQIKKAIPPHCFQRSLLRS		
	ChrFad2	MGAGGQKTFPRLEEEKQQQAAAAGF---KRIPTTKPPFTLSDLKKAIPPHCFQRSLLRS		
	soy omega-6	MGLA-KETTMGGRGRVAKVEVQKK--PLSRVPNTKPPFTVGQLKKAIPPHCFQRSLLTS		
		61		120
10	Hydroxylase	FSYVAYDVCLSFIFYSIATNFFPYISSPL-SYVAWLWVWLFQGCILTGLWVIG	H	ECG
	ImpFad2H8	FYHVIHDIILSFFYYVAANYIPLMPQNL-RYVAWPIYWAIQGCQVQLGILVLG	H	ECG
	MomFad2	FSYLLSDIALVSAFYVADTYFHRLLPHLLHYLAWPVYVFCQGAULTGMWGIA	H	DCG
	ChrFad2	FSYVFIDLTIIISILGYIGATYICLLPPPS-KYLAWLLYWAVQGCFFTGAWALA	H	DCG
	soy omega-6	FSYVVYDLSFAFIFY-IATTYFHLPPQPF-SLIAWPIYWVLQGCCLLTGVWVIA	H	ECG
15				
		121		180
	Hydroxylase	FSEYQLADDIVGLIVHSALLVPYFSWKYS	H	RR
	ImpFad2H8	FSDYQWVDDMVGFVLHSSQLIPYFSWKHS	H	RR
	MomFad2	FSDYQLVDDVVGFLLHSLVFPYFSFKIS	H	RR
20	ChrFad2	FSDYQWIDDAVGMVLHSTLMVPYFSFKYS	H	RR
	soy omega-6	FSKYQWVDDVGLTLHSTLLVPYFSWKIS	H	RR
		181		240
25	Hydroxylase	SNNPPGRVLTLAATLLLGWPLYLAFNVSGRPYDRFACHYDPYGPIFSEERLQIYIADLG		
	ImpFad2H8	LRNPVGRFLMIFGALLFGWPSYLLFNANGRLYDRFASHYDPQSPIFNNRERLQVIASDVG		
	MomFad2	LTNPAPRVFIIFITLLGWPMYLTFNISGRYGRFTSHFDPNSPIFSPKERVLVHISNAG		
	ChrFad2	LNNPLGRVLTAVTLILGWPMYLLALNASGRDYDRFVSHFYPIYNDREERLQIYISDAG		
	soy omega-6	LNNPLGRAVSLVTLTIGWPMYLLAFNVSGRPYDSFASHYHPYAPIYSNRERLLIYVSDVA		

## (57) Abstract

The preparation and use of nucleic acid fragments encoding plant fatty acid modifying enzymes associated with conjugated double bond formation or functionally equivalent subfragments thereof are disclosed. Chimeric genes incorporating such nucleic acid fragments or functionally equivalent subfragments thereof or complement thereof and suitable regulatory sequences can be used to create transgenic plants having altered lipid profiles.

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# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/18562

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/29 C12N15/52 C12N15/82 C12N9/02 C12N5/10  
A23D9/00 A23K1/14

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 7 C12N

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 practical, search terms used)

STRAND, EPO-Internal, WPI Data, PAJ, MEDLINE, EMBASE, BIOSIS, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	<p>WO 94 11516 A (DU PONT ;LIGHTNER JONATHAN EDWARD (US); OKULEY JOHN JOSEPH (US)) 26 May 1994 (1994-05-26) cited in the application</p> <p>abstract; claims page 4, line 26 - line 35 page 7, line 5 - line 22 page 10, line 15 -page 12, line 20 --- -/--</p>	<p>1,3,7, 11,15, 19,23, 27,31, 37,41, 48,50-52</p>
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

21 June 2000

Date of mailing of the international search report

06.07.00

Name and mailing address of the ISA

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/18562

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

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 97 21340 A (CARGILL INC ;DEBONTE R LORIN (US); FAN ZHEGONG (US); LOH H T WILLI) 19 June 1997 (1997-06-19) abstract  page 4, line 11 - line 16 page 8, line 3 - line 19 page 11, line 3 - line 24 ----	1,3,7, 48,50,51  1,15,23, 27,31, 37,43
A	US 5 554 646 A (COOK MARK E ET AL) 10 September 1996 (1996-09-10) cited in the application abstract ----	53-61
A	US 5 428 072 A (COOK MARK E ET AL) 27 June 1995 (1995-06-27) cited in the application abstract ----	53-61
A	OHLROGGE J B: "DESIGN OF NEW PLANT PRODUCTS: ENGINEERING OF FATTY ACID METABOLISM" PLANT PHYSIOLOGY,US,AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 104, page 821-826 XP002068787 ISSN: 0032-0889 cited in the application ----	
X	EP 0 794 250 A (SOREMARTEC ET AL.) 10 September 1997 (1997-09-10) Seq Id Nos 1,2 abstract ----	1-3
X	US 5 668 292 A (SOMMERVILLE ET AL.) 16 September 1997 (1997-09-16) Seq Id No 43 abstract ----	1-3
X	OKULEY ET AL.: "Omega-6 fatty acid desaturase ...." EMBL SEQUENCE DATABASE, 1 November 1995 (1995-11-01), XP002140709 HEIDELBERG DE Ac P46313 the whole document -----	1-3

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 99/18562

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 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 II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

As a result of the prior review under R. 40.2(e) PCT,  
all additional fees are to be refunded.

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 an additional fee, this Authority did not invite payment of any additional fee.
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.:

#### Remark on Protest

- ☒ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-62 all partly

Isolated nucleic acid fragment encoding a plant fatty acid modifying enzyme associated with conjugate double bond formation and its uses, where the sequence hybridizes to SEQ ID NO 1 or is at least 45% identical to SEQ ID NO 1 or encodes a protein that is at least 45% identical to SEQ ID NO 2.

2. Claims: 1-62 all partly

Isolated nucleic acid fragment encoding a plant fatty acid modifying enzyme associated with conjugate double bond formation and its uses, where the sequence hybridizes to SEQ ID NO 3 or is at least 45% identical to SEQ ID NO 3 or encodes a protein that is at least 45% identical to SEQ ID NO 4.

3. Claims: 1-62 all partly

Isolated nucleic acid fragment encoding a plant fatty acid modifying enzyme associated with conjugate double bond formation and its uses, where the sequence hybridizes to SEQ ID NO 19 or is at least 45% identical to SEQ ID NO 19 or encodes a protein that is at least 45% identical to SEQ ID NO 20.

4. Claims: 1-62 all partly

Isolated nucleic acid fragment encoding a plant fatty acid modifying enzyme associated with conjugate double bond formation and its uses, where the sequence hybridizes to SEQ ID NO 23 or is at least 45% identical to SEQ ID NO 23 or encodes a protein that is at least 45% identical to SEQ ID NO 24.

5. Claims: 1-62 all partly

Isolated nucleic acid fragment encoding a plant fatty acid modifying enzyme associated with conjugate double bond formation and its uses, where the sequence hybridizes to SEQ ID NO 29 or is at least 45% identical to SEQ ID NO 29 or encodes a protein that is at least 45% identical to SEQ ID NO 30.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/18562

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9411516 A	26-05-1994	AU 5407594 A AU 6984198 A CA 2149223 A EP 0668919 A JP 8503364 T	08-06-1994 01-10-1998 26-05-1994 30-08-1995 16-04-1996
WO 9721340 A	19-06-1997	AU 1293297 A CA 2238964 A EP 0880312 A	03-07-1997 19-06-1997 02-12-1998
US 5554646 A	10-09-1996	US 5430066 A US 5428072 A EP 0731699 A JP 2992836 B JP 10508189 T WO 9606605 A US 5760082 A US 5814663 A DE 69301693 D DE 69301693 T EP 0579901 A US 5504114 A US 5827885 A DE 69328497 D EP 0680318 A JP 2745245 B JP 8505775 T WO 9416690 A	04-07-1995 27-06-1995 18-09-1996 20-12-1999 18-08-1998 07-03-1996 02-06-1998 29-09-1998 11-04-1996 25-07-1996 26-01-1994 02-04-1996 27-10-1998 31-05-2000 08-11-1995 28-04-1998 25-06-1996 04-08-1994
US 5428072 A	27-06-1995	US 5430066 A DE 69328497 D EP 0680318 A JP 2745245 B JP 8505775 T WO 9416690 A US 5504114 A US 5554646 A DE 69301693 D DE 69301693 T EP 0579901 A US 5827885 A	04-07-1995 31-05-2000 08-11-1995 28-04-1998 25-06-1996 04-08-1994 02-04-1996 10-09-1996 11-04-1996 25-07-1996 26-01-1994 27-10-1998
EP 794250 A	10-09-1997	AU 718602 B AU 1498497 A TR 970130 A US 6025172 A	20-04-2000 11-09-1997 21-03-1997 15-02-2000
US 5668292 A	16-09-1997	AU 718512 B AU 3677895 A CA 2200202 A EP 0781327 A JP 10506783 T WO 9610075 A US 5801026 A US 6028248 A	13-04-2000 19-04-1996 04-04-1996 02-07-1997 07-07-1998 04-04-1996 01-09-1998 22-02-2000