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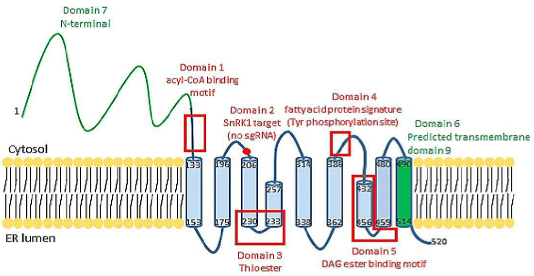
(54) 발명의 명칭 **식물체의 트리아실글리세롤 생합성 증가 방법**

(57) 요 약

본 발명은 대상 식물체의 DGAT1 유전자에서 서열번호 1의 서열에서 1252번 위치에 대응되는 위치의 G를 A로 치환하는 단계를 포함하는 식물체의 트리아실글리세롤 생합성을 증가시키는 방법 및 상기 식물체의 형질전환된 종자에 관한 것으로, 기존보다 높은 효율로 지방을 생산하는 식물을 개발할 수 있을 것으로 기대된다.

대 표 도 - 도1



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기 여 율	1/2
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연구과제명	유용소재 및 농업형질 개선 유전자교정 콩 개발 및 시장경쟁력 확보(1주관)
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명 세 서

청구범위

청구항 1

대상 식물체의 DGAT1 아미노산 서열에서 서열번호 175의 서열에서 418번 위치에 대응되는 발린의 아이소류신으로의 치환을 유도하도록 대상 식물체의 DGAT1 유전자를 염기 교정하는 단계를 포함하는 식물체의 트리아실글리세롤 생합성을 증가시키는 방법.

청구항 2

청구항 1에 있어서, 상기 염기 교정은 유전자 가위를 사용하여 수행되는, 식물체의 트리아실글리세롤 생합성을 증가시키는 방법.

청구항 3

청구항 1의 식물체의 형질전환된 종자.

발명의 설명

기 술 분 야

[0001] 본 발명은 식물체의 트리아실글리세롤 생합성 증가 방법에 관한 것이다.

배 경 기 술

[0003] 트리아실글리세롤(Triacylglycerol, TAG)은 대부분 식물의 대표적인 저장 지질(lipid)이다. TAG는 한 분자의 글리세롤에 세 분자의 지방산이 에스터 결합한 물질로, 디아실글리세롤에 아실기가 전이되는 것에 의해 생성된다. 디아실글리세롤에 아실기를 전이하는 효소는 아실 CoA를 아실 공여체(acyl donor)로 하는 타입의 아실-CoA:디아실글리세롤 아실기 전이 효소(acyl-CoA:diacylglycerol acyltransferase, DGAT)와 인지질을 아실 공여체로 하는 타입의 인지질:디아실글리세롤 아실기 전이 효소(phospholipids:diacylglycerol acyltransferase, PDAT)가 알려져 있다.

[0004] Diacylglycerol acyltransferase 1 (DGAT1)은 식물 종자 내 endoplasmic reticulum membrane 에서 TAG 합성을 담당하는 마지막 효소로, diacylglycerol (DAG)에 Acyl기를 하나 추가하여 TAG를 합성한다. 기존에는 DGAT1를 식물에 과발현시켜 종자 지방함량을 증진시켜 왔다.

[0005] 지구 온난화에 대항하여 대체에너지와 탄소 배출량 감소가 동시에 요구되는 현대 사회의 특성에 따라서 농경지 증가가 아닌 식물 지방생성 효율 증대가 필요하다. 이에 따라 효율이 증대된 DGAT1과 관련된 연구 개발이 요구된다.

선행기술문헌

특허문헌

[0007] (특허문헌 0001) 한국등록특허 제 10-1701129호

발명의 내용

해결하려는 과제

[0008] 본 발명의 목적은 DGAT1은 식물 종자 지방 합성에 관여하는 마지막 단계의 효소라고 할 수 있는 것으로, 식물간에 보존된 영역이 존재하는 DGAT1의 특성상 다른 식물, 특히 유지 작물에서도 동일하게 적용하여 종자 지방의 지방산 조성을 변화시키거나 지방 함량 증진에 활용하기 위함에 있다.

과제의 해결 수단

[0010] 1. 대상 식물체의 DGAT1 유전자에서 서열번호 1의 서열에서 1252번 위치에 대응되는 위치의 G를 A로 치환하는 단계를 포함하는 식물체의 트리아실글리세롤 생합성을 증가시키는 방법.

[0011] 2. 위 1에 있어서, 상기 치환은 유전자 가위를 사용하여 수행되는, 식물체의 트리아실글리세롤 생합성을 증가시키는 방법.

[0012] 3. 위 1의 식물체의 형질전환된 종자.

발명의 효과

[0014] 본 발명의 식물체의 트리아실글리세롤 생합성을 증가시키는 방법을 통해, 기존의 지방 (트리아실글리세롤) 생산보다 더 높은 효율로 지방을 생산하는 식물을 개발할 수 있다.

[0015] 본 발명의 방법을 통해, 트리아실글리세롤 생합성이 증가된 식물체의 형질전환된 종자를 얻을 수 있다.

도면의 간단한 설명

[0017] 도 1은 애기장대 DGAT1의 단백질 서열을 이용하여 구조를 예측하고, 7개의 주요 Domain들을 표시한 것이다. 빨간 박스는 효소의 기능적 측면을 담당하는 것이고, 녹색으로 표시된 부분은 효소의 조절을 담당하는 것이다.

도 2A는 sgRNA insert를 만드는데 사용한 Annealing method와 CBE, ABE 벡터에 Ligation하기까지 과정을 간략하게 나타낸 것이고, 도 2B는 클로닝이 완료된 52개의 콜로니를 PCR로 확인한 결과를 나타낸 것이다.

도 3A는 DGAT1 염기교정(BE) 개체의 선별과정을 그림으로 나타낸 것이고, 도 3B는 세대 진전에 따른 DNA 시퀀싱 결과를 나타낸 것이다. 도 4A의 마지막 단계인 가스 크로마토그래피는 homozygous line이 출현한 마지막 세대에서만 진행되었다.

도 4a는 애기장대 DGAT1과 염기가 치환된 돌연변이 DGAT1의 아미노산 서열을 비교한 것이다. 순서대로 AtDGAT1(야생형 애기장대), D1-1 개체, D1-2 개체, D3-1 개체, D5-1 개체, D5-2 개체, CsDGAT1-A(양구슬냉이), CsDGAT1-B(양구슬냉이), CsDGAT1-C(양구슬냉이), BnDGAT1(유채), GmDGAT1C(대두), AhDGAT1(땅콩), RcDGAT1(피마자) 개체의 DGAT1 아미노산 서열을 나타낸 것이다. 이를 통해, D5-2 개체의 치환부위를 제외하고 모두 보존되어 있음을 알 수 있다. 도 4b는 정성적 분석 결과를 지방산 조성의 비교 그래프로 나타낸 것이고, 도 4c는 정량적 분석 결과를 지방(Fatty acid methyl esters (FAME)) 함량 비교 그래프로 나타낸 것이다.

도 5A는 야생형(WT)과 염기치환 라인 및 D5-1 염기 치환 부위를 비교한 것이고, 도 5B는 야생형(WT)과 염기치환 라인 및 D5-2 염기 치환 부위를 비교한 것이다. 도 5C는 야생형 애기장대 DGAT1의 DAG binding motif를 포함하는 일부 아미노산 서열을 비교한 것이다. D5-1 및 D5-2은 염기치환으로 아미노산 치환된 라인이고, CsDGAT1-A-C은 양구슬냉이 DGAT1, BnDGAT1은 유채 DGAT1, GmDGAT1C은 대두 DGAT1, AhDGAT1은 땅콩 DGAT1, RcDGAT1은 피마자 DGAT1 서열을 나타낸 것이다.

도 6은 각 개체 별 DGAT1의 유전자의 일부 서열을 비교한 것으로, 왼쪽과 오른쪽에 쓰여진 숫자는 각 개체 DGAT1 유전자 서열의 전체 길이에서 표시된 부분 서열의 양 끝을 나타내는 것이다. 또한, CBE6은 해당 부위를 치환하는데 사용한 sgRNA의 이름을 의미한다 (표 1 참고).

발명을 실시하기 위한 구체적인 내용

[0018] 이하 본 발명을 상세히 설명한다.

[0020] 본 발명은 대상 식물체의 DGAT1 유전자에서 서열번호 1의 서열에서 1252번 위치에 대응되는 위치의 G를 A로 치환하는 단계를 포함하는 식물체의 트리아실글리세롤 생합성을 증가시키는 방법에 관한 것이다.

[0021] 본 발명에서 대상 식물체는 DGAT1을 포함하는 식물체면 모두 해당되는 것이고, 예를 들어, 애기장대, 양구슬냉이, 유채, 카멜리나, 대두, 콩, 땅콩, 피마자 등의 오일 작물일 수 있으나, 이에 제한되는 것은 아니다.

[0022] 본 발명에서 서열번호 1의 서열은 애기장대의 DGAT1 효소의 유전자 서열을 나타낸 것이다.

[0023] 본 발명에서 서열번호 2는 AtDGAT1의 CDS sequence를, 서열번호 3은 D5-2의 CDS sequence를, 서열번호 4는 BnDGAT1의 CDS sequence를, 서열번호 5는 GmDGAT1C의 CDS sequence를, 서열번호 6은 RcDGAT1의 CDS sequence를, 서열번호 7은 AhDGAT1의 CDS sequence를, 서열번호 8은 CsDGAT1-A의 CDS sequence를, 서열번호 9는 CsDGAT1-B의 CDS sequence를, 서열번호 10은 CsDGAT1-C의 CDS sequence를 나타낸 것이다.

[0024] 또한, 본 발명에서 서열번호 175는 AtDGAT1의 DGAT1를 align한 서열을 나타낸 것, 서열번호 176은 D1-1의 DGAT1를 align한 서열을 나타낸 것, 서열번호 177은 D1-2의 DGAT1를 align한 서열을 나타낸 것, 서열번호 178은 D3-1의 DGAT1를 align한 서열을 나타낸 것, 서열번호 179는 D5-1의 DGAT1를 align한 서열을 나타낸 것, 서열번호 180은 D5-2의 DGAT1를 align한 서열을 나타낸 것, 서열번호 181은 CsDGAT1-A의 DGAT1를 align한 서열을 나타낸 것, 서열번호 182는 CsDGAT1-B의 DGAT1를 align한 서열을 나타낸 것, 서열번호 183은 CsDGAT1-C의 DGAT1를 align한 서열을 나타낸 것, 서열번호 184는 BnDGAT1의 DGAT1를 align한 서열을 나타낸 것, 서열번호 185는 GmDGAT1C의 DGAT1를 align한 서열을 나타낸 것, 서열번호 186은 AhDGAT1의 DGAT1를 align한 서열을 나타낸 것, 서열번호 187은 RcDGAT1의 DGAT1를 align한 서열을 나타낸 것이다.

[0025] 본 발명은 대상 DGAT1 유전자의 서열을 확인하여 서열번호 1의 서열에 대응되는 대상 DGAT1 서열의 1252번 위치의 G를 A로 치환하는 단계를 포함한다.

[0026] 본 발명에서 대상 DGAT1 유전자와 서열번호 1의 서열을 대응시키는 방법은 유전자를 자동으로 align하여 결과를 확인할 수 있는 컴퓨터 프로그램 등에 의할 수 있으나, 이에 제한되는 것은 아니다.

[0027] 본 발명에서 대상 DGAT1 서열의 1252번 위치의 G를 A로 치환하는 방법은 예를 들어, 염기 교정(Base Editing, BE) 에 의할 수 있으나, 이에 제한되는 것은 아니다. 상기 염기 교정은 타겟 DNA의 염기 하나를 교정하여 결과적으로 단백질 번역 시 Frame-shift 없이 단백질의 아미노산 서열 치환을 유도하는 기술을 의미한다. 염기 교정은 예를 들면 유전자 가위를 사용하여 수행될 수 있고, 유전자 가위는 CRISPR, 구체적으로 CRISPR-Cas9을 사용할 수 있다. 유전자 가위 사용시에는 sgRNA는 대상 식물체의 DGAT1 유전자의 상기 부위를 타겟팅 할 수 있는 서열을 사용할 수 있고, 이는 대상 식물체의 DGAT1 유전자에 맞추어 설계될 수 있다.

[0028] 또한, 본 발명은 대상 식물체의 DGAT1 유전자에서 서열번호 1의 서열에서 1252번 위치에 대응되는 위치의 G를 A로 치환하는 단계를 포함하여 트리아실글리세롤 생합성을 증가시킨 식물체의 형질전환된 종자에 관한 것이다.

[0029] 본 발명에서 형질전환은 DNA를 식물에 전이시키는 임의의 방법을 의미하는 것으로, 형질전환 방법은 반드시 재생 또는 조직 배양 기간을 가질 필요는 없다. 식물 종의 형질전환은 숙주로 선택한 식물의 특성을 고려하여 특정 식물에 적절한 공지의 형질전환 방법을 선택하여 실시할 수 있다. 예를 들어, 아그로박테리아를 이용한 형질전환 방법, 원형질체의 전기천공법, 식물 요소로의 현미주사법, 식물 요소로의 입자충격법 등일 수 있으나, 이에 제한되는 것은 아니다.

[0031] 이하, 본 발명을 구체적으로 설명하기 위해 실시예를 들어 상세하게 설명하기로 한다.

[0033] <u>실시예</u>

[0034] 1. 재료 및 방법

[0035] (1) DGAT1 단백질의 구조와 주요 도메인의 기능 및 위치 분석(도 1)

[0036] DGAT1의 구조의 주요 도메인에 대하여 염기교정을 진행하였다. 해당 부위들은 acyl-CoA binding site, SnRK2 target site, thieosterase, fatty acid signature protein, diacylglycerol (DAG) binding site, 9번째 transmembrane domain의 6가지이다. 마지막으로 N-terminal부분은 folding되지 않은 flexible한 구간으로 regulatory motif를 포함하고 있다 (그림 1). 총 7개의 domain들을 DGAT1에서 중요하게 작용하는 domain이라고 생각했으며 이를 타겟하는 52개의 sgRNA를 디자인 하였다.

[0037] (2) DGAT1 Base Editing 벡터 제작

[0038] 52개의 sgRNA (표 2)는 base editing 벡터에 클로닝할 때 annealing method를 사용하였다. 이는 단일가닥의 형태로 되어있는 sgRNA 타겟 시퀀스 를 이중가닥으로 만들기 위하여 원래 타겟하고자 디자인한 시퀀스와 이에 상보적인 시퀀스를 제작하여 annealing시 dimer를 형성하며 추가적으로 본래의 sgRNA 5’ 말단에 ATTG, 상보적 sgRNA 5'말단에는 AAAC를 더하여 벡터에 삽입할 때 필요한 sticky end를 유도할 수 있다. 벡터의 완성 유무는 PCR로 확인하였으며 벡터에 존재하는 U6 promoter에 결합하는 forward primer (5'-

ACCCTTCAAGAATTTGATTGAATA)와 각 sgRNA의 insert를 만들 때 사용한 complimentary oligo를 reverse로 사용하였다. PCR 결과 밴드 사이즈는 약 200bp로 확인된 벡터는 *E. coli*, *Agrobacterium tumefaciens*를 거쳐 애기장대 식물에 형질전환 시키는데 사용되었다(도 2). 이때 사용된 벡터는 cytosine에서 thymine으로 전환시키는 CBE벡터와 adenine에서 guanine으로 전환시키는 ABE 벡터 두 가지가 사용되었다.

표 1

[0040] 52개 sgRNA의 DNA서열, 변화 부위 및 방향

서열번호	sgRNA sequence	Predicted edition	Direction
11	TCTCTCGCCCTCCGATGAGCTGG	AGA/GAG (Arg118 / Glu119) -> AAA/AAG (Lys118 / Lys119)	Rev
12	CTCCGACGCAATCTTCAAACAGG	TCC (Ser124) -> TTT (Phe124)	For
13	ATCCAGTTTACGTCACCCTAAGG	CCA (Pro224) -> TTA (Leu224)	For
14	TGCTCCTCACTTGCATTGTGTGG	CTC (Lue243) -> TTT (Phe243)	For
15	CTGCCAATATGTTTAACCTTTGG	GCA (Ala382) -> ACA (Thr382)	Rev
16	CGAACCATCCATTTATGAACAGG	GTT (Val418) -> ATT (Ile418)	Rev
17	CTTGCGCAGCAAGATACCAAAGG	CGC (Arg427) -> TGC (Cys427)	Rev
18	TGCGATGCATAGCTGTCAAAAGG	GCA (Ala452) -> ACA (Thr452)	Rev
19	TTGTCGTCTCTTCAAGCTATGGG	CGT (Ala456) -> TGT (Cys456)	For
20	ACCTACTCAAACAAATTGGTTGG	GTG (Val470) -> ATG (Met470)	Rev
21	TCACAAACTATCTACAGGAAAGG	ACA (Thr476) -> ATA (Ile476)	For
22	CTCCGACGCAATCTTCAAACAGG	GAC (Asp125) -> GGC (Gly125)	For
23	TGGATACAAAACCTCTGTCATGG	TAT (Tyr223) -> CAC (His223)	Rev
24	CACCTTAGGGTGACGTAAACTGG	CTA (Leu229) -> CCA (Pro229)	Rev
25	CTGCCAATATGTTTAACCTTTGG	TTG (Leu381) -> CCG (Pro381)	Rev
26	TGGCAGAGCTTCTCTGCTTCGGG	GAG (Glu383) -> GGG (Gly383)	For
27	ATCGTGAATTCTACAAAGATTGG	GAA (Glu391) -> GGG (Gly391)	Rev
28	TGGTGGAATGCAAAAAGTGTGGG	AAT (Asn398) -> GGT (Asp398)	For
29	CGAACCATCCATTTATGAACAGG	ATG (Met417) -> ACG (Thr417)	Rev
30	CATGAAAGACTGCAGAGACTAGG	TTT (Phe446) -> CCC (Pro446)	Rev
31	TGCGATGCATAGCTGTCAAAAGG	ATC (Ile451) -> ACC (Thr451)	Rev
32	AGGCACCTACTCAAACAAATTGG	GTG (Val470) -> GCG (Ala470)	Rev
33	CTTCATCACAAACTATCTACAGG	ATC (Ile475) -> GTC (Val475)	For
34	TCACAAACTATCTACAGGAAAGG	AAC (Asn477) -> GGC(Gly477)	For
35	AACTATCTACAGGAAAGGTTTGG	TAT (Tyr478) -> TGT (Cys478)	For
36	TGCTGGCGTTACTACGGTGACGG	GCT (Ala7) -> GTT (Val7)	For
37	GTTACTACGGTGACGGAGAACGG	ACT (Thr10) -> AGT (Ile10)	For
38	ACTACGGTGACGGAGAACGGTGG	ACT / ACG (Thr10 / Thr11) -> AGT / AGG (Ile10 / Met11)	For
39	ACGGTGACGGAGAACGGTGGCGG	ACG (Thr11) -> AGG (Met11)	For
40	ATCTTGATAGGCTTCGTCGACGG	CTT (Leu23) -> GTT (Phe23)	For
41	TCGAGATCGGATTCTTCTAACGG	TCG (Ser31) -> TGG (Leu31)	For
42	TCTAACGGACTTCTTCTCTCTGG	TCT (Ser36) -> TGT (Phe36)	For
43	TTCCGATAATAATTCTCCTTCGG	TCC (Ser44) -> TGG (Phe44)	For
44	AATTCTCCTTCGGATGATGTTGG	TCT (Ser48) -> TGT (Phe48)	For
45	ATCCGAAGGAGAATTATTATCGG	GAT (Asp51) -> AAT (Asn51)	Rev
46	CTCCCGCCGACGTTAGGGATCGG	CCC (Pro56) -> TTT (Phe56)	For
47	ATCCGATCCCTAACGTCGGCGGG	CGG (Arg62) -> CAA (Gln62)	Rev
48	ATCAATCCGATCCCTAACGTCGG	GAT (Asp64) -> CAT (Asn64)	Rev
49	TTGGCCGGAGATAATAACGGTGG	GCC (Ala78) -> GTT (Val78)	For
50	GCCACCACCGTTATTATCTCCGG	GGC (Gly85) -> AAC (Asn85)	Rev
51	GTTACTACGGTGACGGAGAACGG	ACG (Thr11) -> GCG (Ala11)	For
52	ACGGTGACGGAGAACGGTGGCGG	ACG (Thr13) -> GCG (Ala13)	For
53	ATCTTGATAGGCTTCGTCGACGG	GAT (Asp24) -> GGT (Gly24)	For
54	TCTAACGGACTTCTTCTCTCTGG	AAC (Asn37) -> AGC (Ser37)	For
55	TTCCGATAATAATTCTCCTTCGG	GAT (Asp45) -> GGT (Gly45)	For
56	ATCCGAAGGAGAATTATTATCGG	ACG (Ser50) -> CCG (Pro50)	Rev

57	ATCAATCCGATCCCTAACGTCGG	AGT (Ile63) -> ACT (Thr63)	Rev
58	GTTGTTAACGATGACGCTCAGGG	AAC (Asn68) -> GAC (Asp68)	Rev
59	CGCTCAGGGAACAGCCAATTTGG	CAG (Gln72) -> CGG (Arg72)	For
60	CAGGGAACAGCCAATTTGGCCGG	ACA(Thr74) -> GCA (Ala74)	For
61	GATAATAACGGTGGTGGAAGAGG	AAT / AAC (Asn81 / Asn82) -> AGT / GAC (Ser81 / Asp82)	For
62	AATAACGGTGGTGGAAGAGGCGG	AAC (Asn82) -> AGC (Ser82)	For

표 2

[0041] 52개 sgRNA의 insert 제작을 위한 서열과 각 타겟 도메인

Target group	Name	서열번호	Forward	서열번호	Reverse
Domain 1	AID1	63	ATTGTCTCTCGCCCTCCGATGAGC	115	AAACGCTCATCGGAGGGCGAGAGA
	AID2	64	ATTGCTCCGACGCAATCTTCAAAC	116	AAACGTTTGAAGATTGCGTCGGAG
	ABE1	65	ATTGCTCCGACGCAATCTTCAAAC	117	AAACGTTTGAAGATTGCGTCGGAG
Domain 3	AID3	66	ATTGATCCAGTTTACGTCACCCTA	118	AAACTAGGGTGACGTAAACTGGAT
	AID4	67	ATTGTGCTCCTCACTTGCATTGTG	119	AAACCACAATGCAAGTGAGGAGCA
	ABE2	68	ATTGTGGATACAAAACCTCTGTCA	120	AAACTGACAGAGGTTTTGTATCCA
	ABE3	69	ATTGCACCTTAGGGTGACGTAAAC	121	AAACGTTTACGTCACCCTAAGGTG
Domain 4	AID5	70	ATTGCTGCCAATATGTTTAACCTT	122	AAACAAGGTTAAACATATTGGCAG
	ABE4	71	ATTGCTGCCAATATGTTTAACCTT	123	AAACAAGGTTAAACATATTGGCAG
	ABE5	72	ATTGTGGCAGAGCTTCTCTGCTTC	124	AAACGAAGCAGAGAAGCTCTGCCA
	ABE6	73	ATTGATCGTGAATTCTACAAAGAT	125	AAACATCTTTGTAGAATTCACGAT
Domain 5	AID6	74	ATTGCGAACCATCCATTTATGAAC	126	AAACGTTCATAAATGGATGGTTCG
	AID7	75	ATTGCTTGCGCAGCAAGATACCAA	127	AAACTTGGTATCTTGCTGCGCAAG
	AID8	76	ATTGTGCGATGCATAGCTGTCAAA	128	AAACTTTGACAGCTATGCATCGCA
	AID9	77	ATTGTTGTCGTCTCTTCAAGCTAT	129	AAACATAGCTTGAAGAGACGACAA
	ABE7	78	ATTGTGGTGGAATGCAAAAAGTGT	130	AAACACACTTTTTGCATTCCACCA
	ABE8	79	ATTGCGAACCATCCATTTATGAAC	131	AAACGTTCATAAATGGATGGTTCG
	ABE9	80	ATTGCATGAAAGACTGCAGAGACT	132	AAACAGTCTCTGCAGTCTTTCATG
	ABE10	81	ATTGTGCGATGCATAGCTGTCAAA	133	AAACTTTGACAGCTATGCATCGCA
Domain 6	AID10	82	ATTGACCTACTCAAACAAATTGGT	134	AAACACCAATTTGTTTGAGTAGGT
	AID11	83	ATTGTCACAAACTATCTACAGGAA	135	AAACTTCCTGTAGATAGTTTGTGA
	ABE11	84	ATTGAGGCACCTACTCAAACAAAT	136	AAACATTTGTTTGAGTAGGTGCCT
	ABE12	85	ATTGCTTCATCACAAACTATCTAC	137	AAACGTAGATAGTTTGTGATGAAG
	ABE13	86	ATTGTCACAAACTATCTACAGGAA	138	AAACTTCCTGTAGATAGTTTGTGA
	ABE14	87	ATTGAACTATCTACAGGAAAGGTT	139	AAACAACCTTTCCTGTAGATAGTT

Domain 7	nAID1	88	ATTGTGCTGGCGTTACTACGGTGA	140	AAACTCACCGTAGTAACGCCAGCA
	nAID2	89	AAACTTCTCCGTCACCGTAGTAAC	141	AAACTTCTCCGTCACCGTAGTAAC
	nAID3	90	ATTGACTACGGTGACGGAGAACGG	142	AAACCCGTTCTCCGTCACCGTAGT
	nAID4	91	ATTGACGGTGACGGAGAACGGTGG	143	AAACCCACCGTTCTCCGTCACCGT
	nAID5	92	ATTGATCTTGATAGGCTTCGTCGA	144	AAACTCGACGAAGCCTATCAAGAT
	nAID6	93	ATTGTCGAGATCGGATTCTTCTAA	145	AAACTTAGAAGAATCCGATCTCGA
	nAID7	94	ATTGTCTAACGGACTTCTTCTCTC	146	AAACGAGAGAAGAAGTCCGTTAGA
	nAID8	95	ATTGTTCCGATAATAATTCTCCTT	147	AAACAAGGAGAATTATTATCGGAA
	nAID9	96	ATTGAATTCTCCTTCGGATGATGT	148	AAACACATCATCCGAAGGAGAATT
	nAID10	97	ATTGATCCGAAGGAGAATTATTAT	149	AAACATAATAATTCTCCTTCGGAT
	nAID11	98	ATTGCTCCCGCCGACGTTAGGGAT	150	AAACATCCCTAACGTCGGCGGGAG
	nAID12	99	ATTGATCCGATCCCTAACGTCGGC	151	AAACGCCGACGTTAGGGATCGGAT
	nAID13	100	ATTGATCAATCCGATCCCTAACGT	152	AAACACGTTAGGGATCGGATTGAT
	nAID14	101	ATTGTTGGCCGGAGATAATAACGG	153	AAACCCGTTATTATCTCCGGCCAA
	nAID15	102	ATTGGCCACCACCGTTATTATCTC	154	AAACGAGATAATAACGGTGGTGGC
	nABE1	103	ATTGGTTACTACGGTGACGGAGAA	155	AAACTTCTCCGTCACCGTAGTAAC
	nABE2	104	ATTGACGGTGACGGAGAACGGTGG	156	AAACCCACCGTTCTCCGTCACCGT
	nABE3	105	ATTGATCTTGATAGGCTTCGTCGA	157	AAACTCGACGAAGCCTATCAAGAT
	nABE4	106	ATTGTCTAACGGACTTCTTCTCTC	158	AAACGAGAGAAGAAGTCCGTTAGA
	nABE5	107	ATTGTTCCGATAATAATTCTCCTT	159	AAACAAGGAGAATTATTATCGGAA
	nABE6	108	ATTGATCCGAAGGAGAATTATTAT	160	AAACATAATAATTCTCCTTCGGAT
	nABE7	109	ATTGATCAATCCGATCCCTAACGT	161	AAACACGTTAGGGATCGGATTGAT
	nABE8	110	ATTGGTTGTTAACGATGACGCTCA	162	AAACTGAGCGTCATCGTTAACAAC
	nABE9	111	ATTGCGCTCAGGGAACAGCCAATT	163	AAACAATTGGCTGTTCCCTGAGCG
	nABE10	112	ATTGCAGGGAACAGCCAATTTGGC	164	AAACGCCAAATTGGCTGTTCCCTG
	nABE11	113	ATTGGATAATAACGGTGGTGGAAG	165	AAACCTTCCACCACCGTTATTATC
	nABE12	114	ATTGAATAACGGTGGTGGAAGAGG	166	AAACCCTCTTCCACCACCGTTATT

[0042] (3) DGAT1 유전자의 염기가 편집된 식물체 선발

[0043] DGAT1의 base editing을 위하여 52개의 벡터를 7개의 domain별로 묶어 Agrobacterium 형질전환을 통해 T0 plant를 제작하였다. 이때 ABE, CBE 벡터는 식물에서 hygromycine 저항성을 가지므로 해당 항생제가 포함된 배지에서 T1 종자를 발아시켜 형질전환이 되었다고 판단되는 개체들을 일반 MS배지로 옮겨 회복 후, 일정 크기까지 성장시켜 흙으로 옮겨 심었다. 흙에서 다시 일정크기 이상으로 성장하면, gDNA를 추출하기 위한 sampling을 진행하였으며 추출된 gDNA는 해당 개체의 target domain에 따라 PCR primer set을 적용하였다. 1,7 번 domain은 For1 (CCGACGCTGTTTCGTCAAAC, 서열번호 167), Rev1 (TTCGATGATGAGTCTACTGTTT, 서열번호 168)를 사용했으며, 3번 Domain은 For3 (TGCTCAAGGTTGTCATCTTTC, 서열번호 169), Rev3 (CAGGATTGGCCTAAAGTTCA, 서열번호 170)를 사용하였다. 이어서 4번 Domain은 For4 (CCTTTGGTATGCTGTGATCC, 서열번호 171)와 Rev4 (AAGACAGTGAATACATGAATTTGG, 서열번호 172), 5번과 6번 domain은 For5 (CTGGAGAATGTGGAATATGGT, 서열번호 173), Rev5 (CCCATTCCAAAACAGATCAC, 서열번호 174)를 사용하여 PCR하였다. DNA 시퀀싱 또한 해당 동일한 프라이머를 사용하였다.

[0044] T1세대에서 DNA 시퀀싱 결과를 확인하였을 때 크로마토그래피의 그래프가 깔끔하지 않고 다른 염기와 비교하였을 때 야생형에서 보여준 피크의 높이보다 작게 다른 피크가 동시에 섞여 나온 개체들을 선별하였다. 이는 염기교정이 실제로 일어나긴 했지만 부분적으로만 일어나 아직 기존의 야생형에서 존재하는 염기가 남아있다는 의미이다.

[0045] 이후 세대를 진전해 가면서 동일하게 항생제 저항성 선별과 그 이후에 이어지는 DNA 시퀀싱으로 homozygous한 개체의 출현까지 진행하였다 (도 4). T4세대까지 진전하고 나서야 5개의 개체를 확보하였으며, 각각 domain1에서 2개, domain3에서 1개, domain5에서 2개의 개체를 얻었다.

[0047] 2. 실험 결과

[0048] (1) DGAT1 염기치환 돌연변이체 종자의 지방산 분석

[0049] 1) DGAT1 염기치환 돌연변이체는 총 5개로 target한 domain의 이름을 붙여 각각 D1-1, D1-2, D3-1, D5-1, D5-2로 명명하였다(도 4a).

[0050] 2) D1-1 개체는 124번 serine이 phenylalanine으로 치환되었고 D1-2개체는 123, 124번 serine이 각각 arginine과 leucine으로 치환되었다. 두 치환된 부위 모두 다른 종의 DGAT1 서열분석을 통해 의도한 domain1 내의 보존된 서열에서 돌연변이가 일어난 것을 확인하였다. 이에 따른 지방산 변화는 D1-1개체에서 1% 증가한 18:1 지방산, 2.8% 감소한 18:2 지방산과 1.6% 감소한 20:1 지방산을 보였다. D1-2개체는 1.3% 증가한 18:1 지방산, 4.8% 증가한 18:3 지방산, 3.9% 감소한 20:1 지방산을 보였다. 총량은 D1-1, D1-2개체 모두 야생형 애기장대와 비교하였을 때 1% 미만의 차이를 보였다(도 4a, 4b, 4c).

[0051] 3) D3-1개체는 229번 leucine이 proline으로 치환되었고 다른 DGAT1과의 서열 비교를 통하여 의도한 domain3 내의 보존된 서열에서 돌연변이가 일어난 것을 확인하였다. 해당 부위의 단백질 치환은 애기장대 야생형과 비교하였을 때 각 지방산 별 조성의 측면에서 1.7% 감소한 20:1 지방산을 제외하고 모두 1% 미만의 증감을 보여 큰 변화가 없었다. 하지만 지방산 총량은 애기장대 야생형 대비 12.5% 감소하였다 (도 4a, 4b, 4c).

[0052] 4) D5-1개체는 416번 tryptophan이 cysteine으로 치환되었으며 419번 arginine이 glutamine으로 치환되었다. 다른 종의 DGAT1과 서열 비교를 통해 의도한 domain5 내의 보존된 서열에서 돌연변이가 일어난 것을 확인했다. D5-1개체는 4.5% 감소한 18:1 지방산, 8.4% 증가한 18:3 지방산, 3.7% 감소한 20:1 지방산을 보이며 정량적으로도 13% 감소한 총량을 나타냈다(도 4a, 4b, 4c).

[0053] 5) D5-2개체는 418번 valine이 isoleucine으로 치환되었으며 다른 종의 DGAT1 단백질 서열과 비교하였을 때 의도한 domain5 내의 보존된 서열에서 돌연변이가 발생한 것을 확인하였다. 해당 부위의 치환은 애기장대 야생형과 조성적 측면에서 20:1 지방산이 1.5% 증가한 것 외에 큰 차이를 보이지 않았지만, 정량분석에서 야생형 애기장대보다 8.1% 증가한 지방산 총량을 보였다 (도 4a, 4b, 4c).

[0054] **(2) DGAT1 염기교정 돌연변이체의 아미노산 치환과 종자 지방산 조성의 함량 변화 비교**

표 3

[0055]

DGAT1 아미노산 치환 변이	치환 개수	야생형(wild-type) 대비 아미노산 치환 부위	야생형 대비 종자 지방산 조성 변화	야생형 대비 종자 지방함량 변화
D1-1	1	124번 Serine->Phenylalanine	18:1 지방산 2% 증가 18:3 지방산 1% 감소 20:1 지방산 1.6% 감소	차이 없음
D1-2	2	123번 Serine->Arginine 124번 Serine->Leucine	18:1 지방산 2.2% 감소 18:3 지방산 4.8% 증가 20:1 지방산 3.9% 감소	차이 없음
D3-1	1	229번 Leucine->Proline	차이 없음	총량 12% 감소
D5-1	2	416번 Tryptophan->Cysteine 419번 Arginine->Glutamine	18:1 지방산 4.5% 감소 18:3 지방산 8.4% 증가 20:1 지방산 3.7% 감소	총량 13% 감소
D5-2	1	418번 Valine->Isoleucine	차이 없음	총량 8.1% 증가

[0056] 상기 표 3를 통해 DGAT1 아미노산을 다양하게 치환하여 지방산 함량을 확인한 결과, D5-2 변이체만 종자 지방함량의 총량이 상당히 증가한 것을 알 수 있다.

[0057] 도 4 내지 6과 같이, D5-2 변이체의 치환 부위가 다양한 식물체에서 보존되어 있는 것을 확인할 수 있으므로, 식물 모델로 확인한 애기장대 외에 다른 식물체에서도 치환에 의해 종자 지방 합성의 총량이 증가할 것으로 예상할 수 있다.

도면

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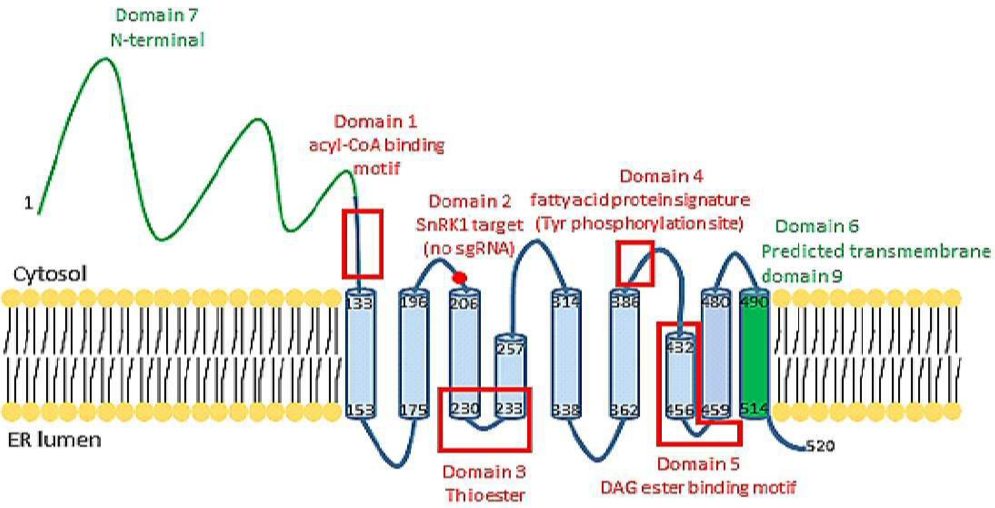
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motif
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Predicted transmembrane
domain 9
Cytosol
ER lumen
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153
198
175
206
230
257
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Domain 3
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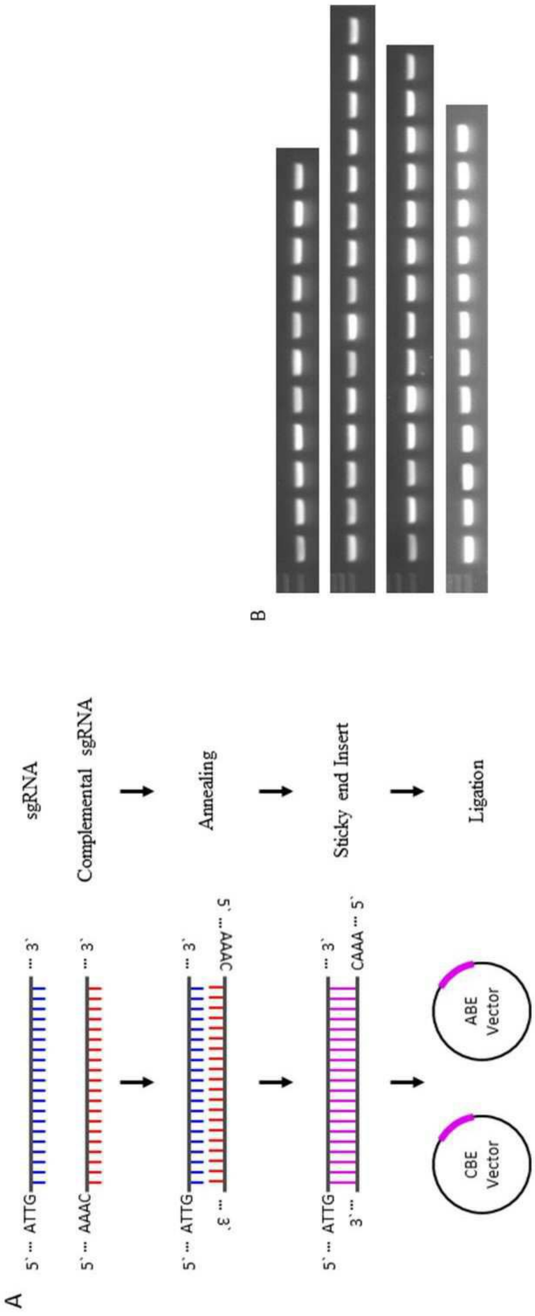
Domain 5
DAG ester binding motif

도면2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… 3'
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5'… AAAC
… 3'
Complemental sgRNA
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… 3'
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AAAC … 5'
Annealing
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… 3'
3'…
CAAA … 5'
Sticky end Insert
CBE Vector
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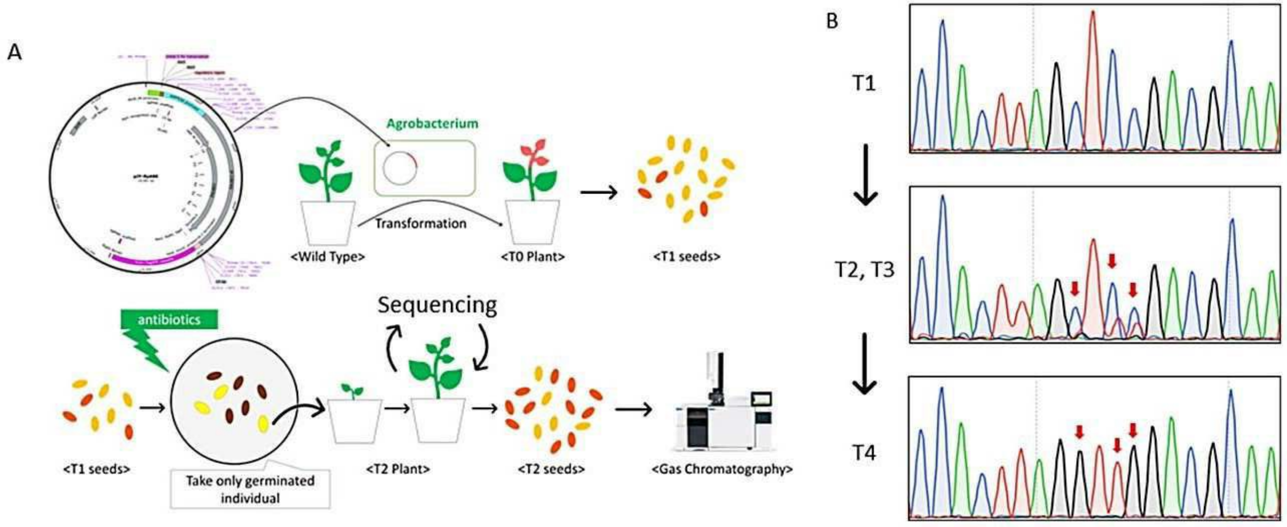
Ligation
B

도면3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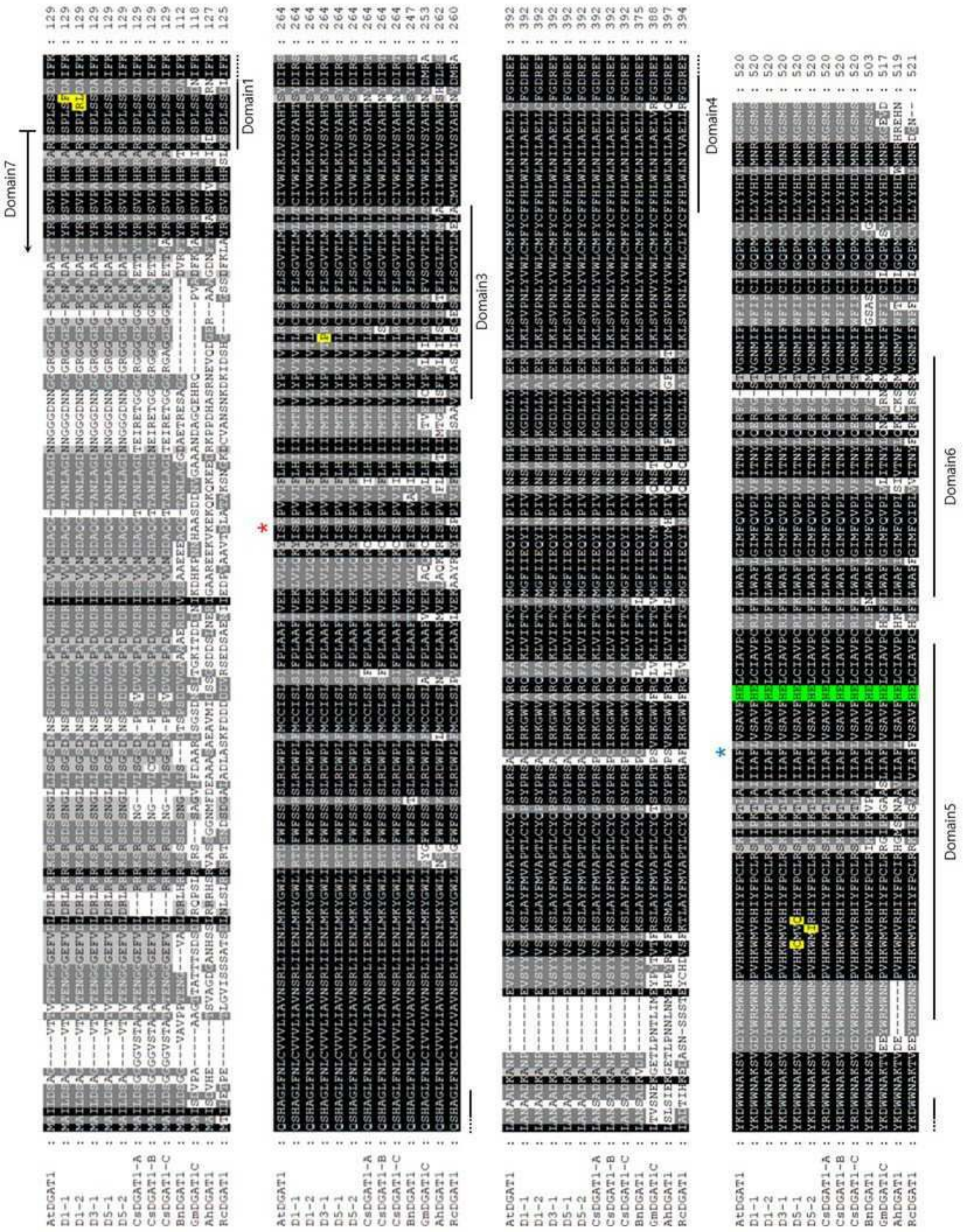
B
T1
T2, T3
T4

도면4a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D5-1
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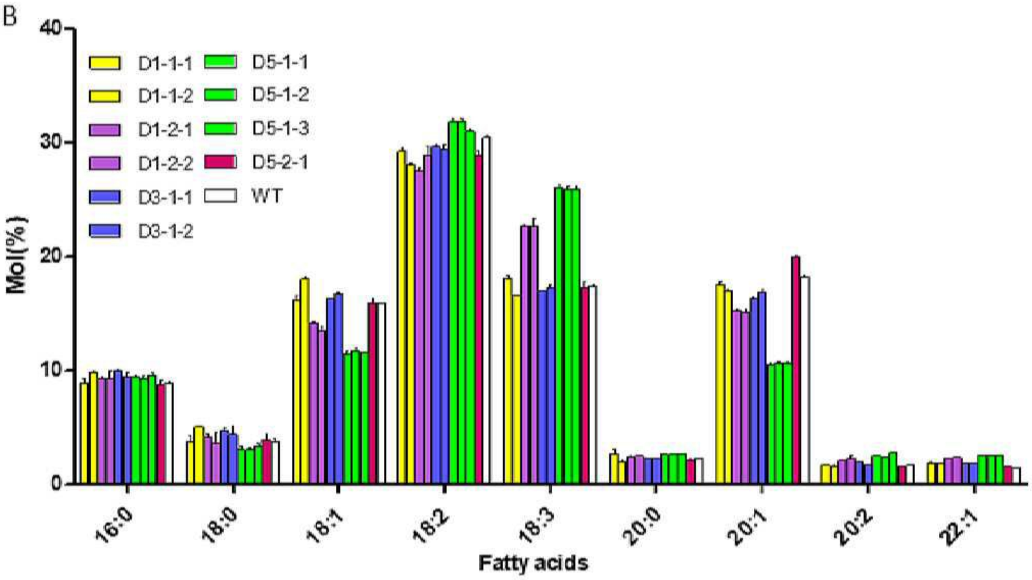
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20
10
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Mol(%)
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D1-1-2
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D1-2-2
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D3-1-2
D5-1-1
D5-1-2
D5-1-3
D5-2-1
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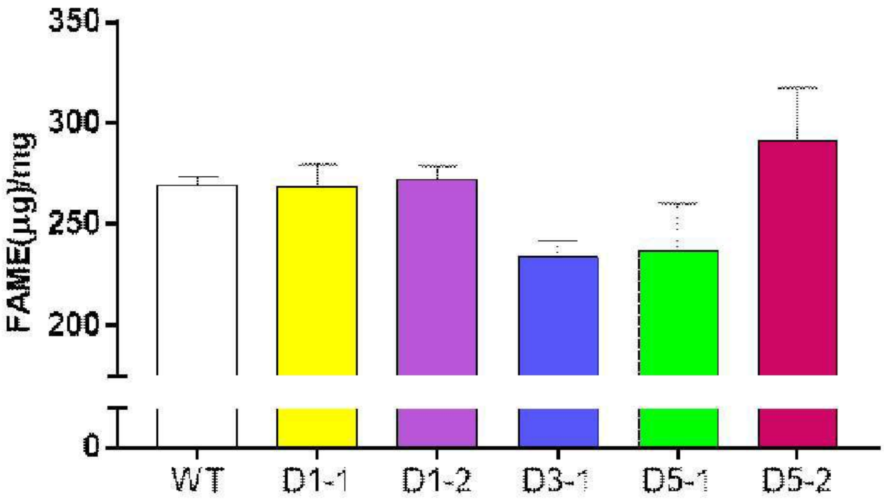
18:0
18:1
18:2
18:3
20:0
20:1
20:2
22:1
Fatty acids

도면4c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200
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FAME(µg)/mg
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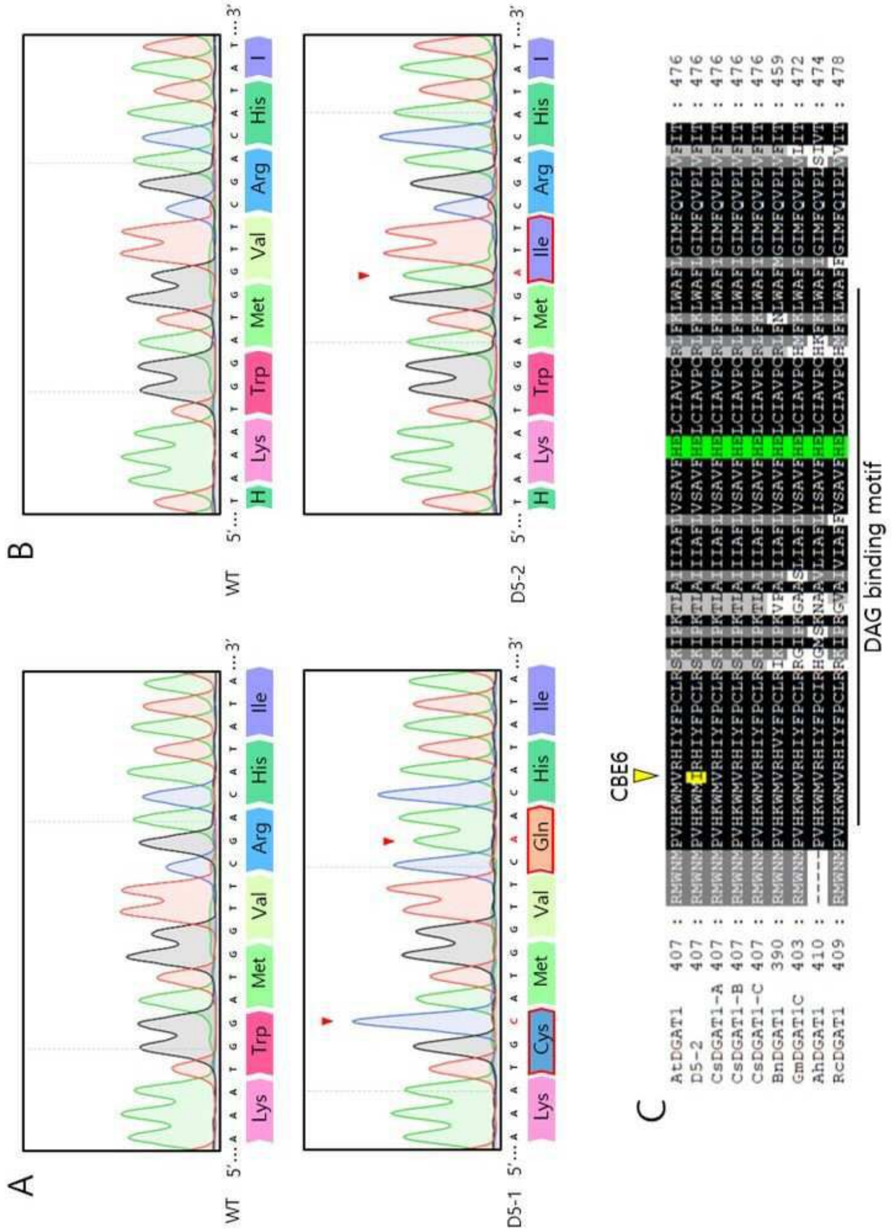
D1-2
D3-1
D5-1
D5-2

도면5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WT
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D5-2
5'...TAAATGGATGATTCGACATAT...3'
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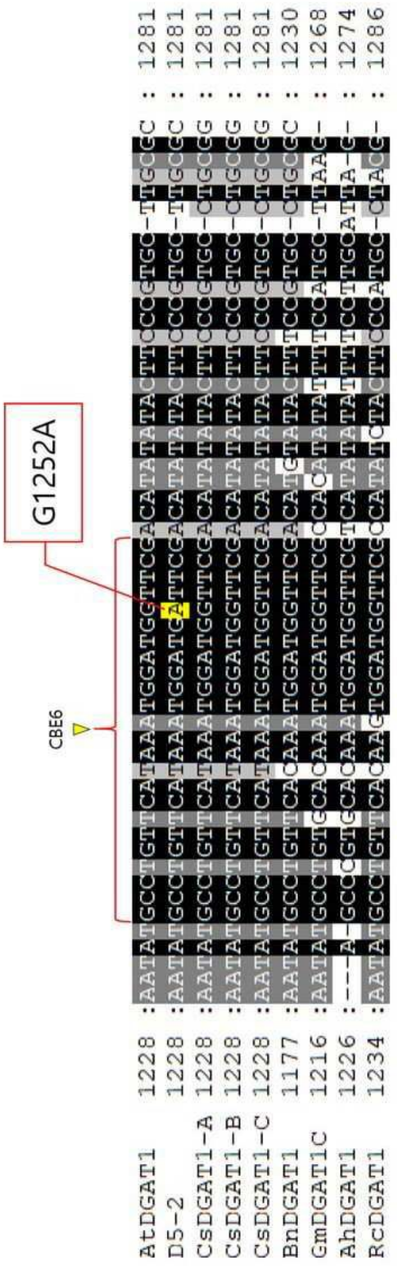
도면6



서 열 목 록

<110> SEJONG UNIVERSITY INDUSTRY ACADEMY COOPERATION FOUNDATION

<120> METHOD FOR INCREASING THE BIOSYNTHESIS OF TRIACYLGLYCEROL IN PLANTS

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<400> 168

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<400> 171

cctttggtat gctgtgatcc 20

<210> 172

<211> 24

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<400> 172

aagacagtga atacatgaat ttgg 24

<210> 173

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<210> 175

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<213> Arabidopsis thaliana

<400> 175

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1 5 10 15

Gly Gly Glu Phe Val Asp Leu Asp Arg Leu Arg Arg Arg Lys Ser Arg

20 25 30

Ser Asp Ser Ser Asn Gly Leu Leu Leu Ser Gly Ser Asp Asn Asn Ser

35 40 45

Pro Ser Asp Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp

50 55 60

Ser Val Val Asn Asp Asp Ala Gln Gly Thr Ala Asn Leu Ala Gly Asp

65 70 75 80

Asn Asn Gly Gly Gly Asp Asn Asn Gly Gly Gly Arg Gly Gly Gly Glu

85 90 95

Gly Arg Gly Asn Ala Asp Ala Thr Phe Thr Tyr Arg Pro Ser Val Pro

100 105 110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe

115 120 125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile

130 135 140

Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp

145 150 155 160

Leu Ile Arg Thr Asp Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp

165 170 175

Pro Leu Phe Met Cys Cys Ile Ser Leu Ser Ile Phe Pro Leu Ala Ala

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            180                 185                 190
Phe Thr Val Glu Lys Leu Val Leu Gln Lys Tyr Ile Ser Glu Pro Val
        195                 200                 205
Val Ile Phe Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro
    210                 215                 220
Val Tyr Val Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr
225                 230                 235                 240

Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala
                245                 250                 255
His Thr Ser Tyr Asp Ile Arg Ser Leu Ala Asn Ala Ala Asp Lys Ala
            260                 265                 270
Asn Pro Glu Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe
        275                 280                 285
Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Ala
    290                 295                 300
Cys Ile Arg Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile

305                 310                 315                 320
Phe Thr Gly Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile
                325                 330                 335
Val Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile
            340                 345                 350
Glu Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys
        355                 360                 365
Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu
    370                 375                 380

Leu Cys Phe Gly Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys
385                 390                 395                 400
Ser Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp
                405                 410                 415
Met Val Arg His Ile Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys
            420                 425                 430
Thr Leu Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu
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435 440 445

Leu Cys Ile Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Leu

450 455 460

Gly Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln

465 470 475 480

Glu Arg Phe Gly Ser Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe

485 490 495

Cys Ile Phe Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu

500 505 510

Met Asn Arg Lys Gly Ser Met Ser

515 520

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<212> PRT

<213> Arabidopsis thaliana

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Gly Gly Glu Phe Val Asp Leu Asp Arg Leu Arg Arg Arg Lys Ser Arg

20 25 30

Ser Asp Ser Ser Asn Gly Leu Leu Leu Ser Gly Ser Asp Asn Asn Ser

35 40 45

Pro Ser Asp Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp

50 55 60

Ser Val Val Asn Asp Asp Ala Gln Gly Thr Ala Asn Leu Ala Gly Asp

65 70 75 80

Asn Asn Gly Gly Gly Asp Asn Asn Gly Gly Gly Arg Gly Gly Gly Glu

85 90 95

Gly Arg Gly Asn Ala Asp Ala Thr Phe Thr Tyr Arg Pro Ser Val Pro

100 105 110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Ser Phe Asp Ala Ile Phe

115 120 125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile

130 135 140

Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp

145 150 155 160

Leu Ile Arg Thr Asp Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp

165 170 175

Pro Leu Phe Met Cys Cys Ile Ser Leu Ser Ile Phe Pro Leu Ala Ala

180 185 190

Phe Thr Val Glu Lys Leu Val Leu Gln Lys Tyr Ile Ser Glu Pro Val

195 200 205

Val Ile Phe Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro

210 215 220

Val Tyr Val Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr

225 230 235 240

Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala

245 250 255

His Thr Ser Tyr Asp Ile Arg Ser Leu Ala Asn Ala Ala Asp Lys Ala

260 265 270

Asn Pro Glu Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe

275 280 285

Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Ala

290 295 300

Cys Ile Arg Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile

305 310 315 320

Phe Thr Gly Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile

325 330 335

Val Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile

340 345 350

Glu Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys

355 360 365

Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu

370 375 380

Leu Cys Phe Gly Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys

385 390 395 400

Ser Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp

405 410 415

Met Val Arg His Ile Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys

420 425 430

Thr Leu Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu

435 440 445

Leu Cys Ile Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Leu

450 455 460

Gly Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln

465 470 475 480

Glu Arg Phe Gly Ser Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe

485 490 495

Cys Ile Phe Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu

500 505 510

Met Asn Arg Lys Gly Ser Met Ser

515 520

<210> 177

<211> 520

<212> PRT

<213> Arabidopsis thaliana

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1 5 10 15

Gly Gly Glu Phe Val Asp Leu Asp Arg Leu Arg Arg Arg Lys Ser Arg

20 25 30

Ser Asp Ser Ser Asn Gly Leu Leu Leu Ser Gly Ser Asp Asn Asn Ser

35 40 45

Pro Ser Asp Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp

50 55 60

Ser Val Val Asn Asp Asp Ala Gln Gly Thr Ala Asn Leu Ala Gly Asp

65 70 75 80

Asn Asn Gly Gly Gly Asp Asn Asn Gly Gly Gly Arg Gly Gly Gly Glu

85 90 95

Gly Arg Gly Asn Ala Asp Ala Thr Phe Thr Tyr Arg Pro Ser Val Pro

100 105 110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Arg Leu Asp Ala Ile Phe

115 120 125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile

130 135 140

Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp

145 150 155 160

Leu Ile Arg Thr Asp Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp

165 170 175

Pro Leu Phe Met Cys Cys Ile Ser Leu Ser Ile Phe Pro Leu Ala Ala

180 185 190

Phe Thr Val Glu Lys Leu Val Leu Gln Lys Tyr Ile Ser Glu Pro Val

195 200 205

Val Ile Phe Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro

210 215 220

Val Tyr Val Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr

225 230 235 240

Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala

245 250 255

His Thr Ser Tyr Asp Ile Arg Ser Leu Ala Asn Ala Ala Asp Lys Ala

260 265 270

Asn Pro Glu Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe

275 280 285

Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Ala

290 295 300

Cys Ile Arg Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile

305 310 315 320

Phe Thr Gly Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile

325 330 335

Val Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile

340 345 350

Glu Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys

355 360 365

Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu

370 375 380

Leu Cys Phe Gly Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys

385 390 395 400

Ser Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp

405 410 415

Met Val Arg His Ile Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys

420 425 430

Thr Leu Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu

435 440 445

Leu Cys Ile Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Leu

450 455 460

Gly Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln

465 470 475 480

Glu Arg Phe Gly Ser Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe

485 490 495

Cys Ile Phe Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu

500 505 510

Met Asn Arg Lys Gly Ser Met Ser

515 520

<210> 178

<211> 520

<212> PRT

<213> Arabidopsis thaliana

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Met Ala Ile Leu Asp Ser Ala Gly Val Thr Thr Val Thr Glu Asn Gly

1 5 10 15

Gly Gly Glu Phe Val Asp Leu Asp Arg Leu Arg Arg Arg Lys Ser Arg

20 25 30

Ser Asp Ser Ser Asn Gly Leu Leu Leu Ser Gly Ser Asp Asn Asn Ser

35 40 45

Pro Ser Asp Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp

50 55 60

Ser Val Val Asn Asp Asp Ala Gln Gly Thr Ala Asn Leu Ala Gly Asp

65 70 75 80

Asn Asn Gly Gly Gly Asp Asn Asn Gly Gly Gly Arg Gly Gly Gly Glu

85 90 95

Gly Arg Gly Asn Ala Asp Ala Thr Phe Thr Tyr Arg Pro Ser Val Pro

100 105 110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe

115 120 125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile

130 135 140

Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp

145 150 155 160

Leu Ile Arg Thr Asp Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp

165 170 175

Pro Leu Phe Met Cys Cys Ile Ser Leu Ser Ile Phe Pro Leu Ala Ala

180 185 190

Phe Thr Val Glu Lys Leu Val Leu Gln Lys Tyr Ile Ser Glu Pro Val

195 200 205

Val Ile Phe Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro

210 215 220

Val Tyr Val Thr Pro Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr

225 230 235 240

Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala

245 250 255

His Thr Ser Tyr Asp Ile Arg Ser Leu Ala Asn Ala Ala Asp Lys Ala

260 265 270

Asn Pro Glu Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe

275 280 285

Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Ala

290 295 300

Cys Ile Arg Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile

305 310 315 320

Phe Thr Gly Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile

325 330 335

Val Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile

340 345 350

Glu Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys

355 360 365

Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu

370 375 380

Leu Cys Phe Gly Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys

385 390 395 400

Ser Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp

405 410 415

Met Val Arg His Ile Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys

420 425 430

Thr Leu Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu

435 440 445

Leu Cys Ile Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Leu

450 455 460

Gly Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln

465 470 475 480

Glu Arg Phe Gly Ser Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe

485 490 495

Cys Ile Phe Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu

500 505 510

Met Asn Arg Lys Gly Ser Met Ser

515 520

<210> 179

<211> 520

<212> PRT

<213> Arabidopsis thaliana

<400> 179

Met Ala Ile Leu Asp Ser Ala Gly Val Thr Thr Val Thr Glu Asn Gly

1 5 10 15

Gly Gly Glu Phe Val Asp Leu Asp Arg Leu Arg Arg Arg Lys Ser Arg

20 25 30

Ser Asp Ser Ser Asn Gly Leu Leu Leu Ser Gly Ser Asp Asn Asn Ser

35 40 45

Pro Ser Asp Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp

50 55 60

Ser Val Val Asn Asp Asp Ala Gln Gly Thr Ala Asn Leu Ala Gly Asp

65 70 75 80

Asn Asn Gly Gly Gly Asp Asn Asn Gly Gly Gly Arg Gly Gly Gly Glu

85 90 95

Gly Arg Gly Asn Ala Asp Ala Thr Phe Thr Tyr Arg Pro Ser Val Pro

100 105 110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe

115 120 125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile

130 135 140

Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp

145 150 155 160

Leu Ile Arg Thr Asp Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp

165 170 175

Pro Leu Phe Met Cys Cys Ile Ser Leu Ser Ile Phe Pro Leu Ala Ala

180 185 190

Phe Thr Val Glu Lys Leu Val Leu Gln Lys Tyr Ile Ser Glu Pro Val

195 200 205

Val Ile Phe Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro

210 215 220

Val Tyr Val Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr

225 230 235 240

Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala

245 250 255

His Thr Ser Tyr Asp Ile Arg Ser Leu Ala Asn Ala Ala Asp Lys Ala

260 265 270

Asn Pro Glu Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe

275 280 285

Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Ala

290 295 300

Cys Ile Arg Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile

305 310 315 320

Phe Thr Gly Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile

325 330 335

Val Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile

340 345 350

Glu Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys

355 360 365

Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu

370 375 380

Leu Cys Phe Gly Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys

385 390 395 400

Ser Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Cys

405 410 415

Met Val Gln His Ile Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys

420 425 430

Thr Leu Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu

435 440 445

Leu Cys Ile Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Leu

450 455 460

Gly Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln

465 470 475 480

Glu Arg Phe Gly Ser Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe

485 490 495

Cys Ile Phe Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu

500 505 510

Met Asn Arg Lys Gly Ser Met Ser

515 520

<210> 180

<211> 520

<212> PRT

<213> Arabidopsis thaliana

<400> 180

Met Ala Ile Leu Asp Ser Ala Gly Val Thr Thr Val Thr Glu Asn Gly

1 5 10 15

Gly Gly Glu Phe Val Asp Leu Asp Arg Leu Arg Arg Arg Lys Ser Arg

20 25 30

Ser Asp Ser Ser Asn Gly Leu Leu Leu Ser Gly Ser Asp Asn Asn Ser

35 40 45

Pro Ser Asp Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp

50 55 60

Ser Val Val Asn Asp Asp Ala Gln Gly Thr Ala Asn Leu Ala Gly Asp

65 70 75 80

Asn Asn Gly Gly Gly Asp Asn Asn Gly Gly Gly Arg Gly Gly Gly Glu

85 90 95

Gly Arg Gly Asn Ala Asp Ala Thr Phe Thr Tyr Arg Pro Ser Val Pro

100 105 110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe

115 120 125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile

130 135 140

Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp

145 150 155 160

Leu Ile Arg Thr Asp Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp

165 170 175

Pro Leu Phe Met Cys Cys Ile Ser Leu Ser Ile Phe Pro Leu Ala Ala

180 185 190

Phe Thr Val Glu Lys Leu Val Leu Gln Lys Tyr Ile Ser Glu Pro Val

195 200 205

Val Ile Phe Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro

210 215 220

Val Tyr Val Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr

225 230 235 240

Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala

245 250 255

His Thr Ser Tyr Asp Ile Arg Ser Leu Ala Asn Ala Ala Asp Lys Ala

260 265 270

Asn Pro Glu Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe

275 280 285

Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Ala

290 295 300

Cys Ile Arg Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile

305 310 315 320

Phe Thr Gly Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile

325 330 335

Val Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile

340 345 350

Glu Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys

355 360 365

Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu

370 375 380

Leu Cys Phe Gly Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys

385 390 395 400

Ser Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp

405 410 415

Met Ile Arg His Ile Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys

420 425 430

Thr Leu Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu

435 440 445

Leu Cys Ile Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Leu

450 455 460

Gly Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln

465 470 475 480

Glu Arg Phe Gly Ser Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe

485 490 495

Cys Ile Phe Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu

500 505 510

Met Asn Arg Lys Gly Ser Met Ser

515 520

<210> 181

<211> 520

<212> PRT

<213> Unknown

<220><223> Camelina sativa

<400> 181

Met Ala Ile Leu Asp Ser Gly Gly Gly Gly Val Ser Thr Ala Thr Ala

1 5 10 15

Thr Glu Asn Gly Gly Gly Glu Phe Val Asp Leu Arg Arg Arg Lys Ser

20 25 30

Arg Ser Asp Ser Asn Gly Val Leu Ser Gly Ser Asp Asn Pro Pro Ser

35 40 45

Val Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp Ser Val

50 55 60

Val Asn Asp Asp Ala Gln Gly Thr Thr Ala Asn Leu Ala Gly Asp Thr

65 70 75 80

Glu Ile Arg Glu Thr Gly Gly Gly Gly Arg Gly Gly Gly Gly Glu Gly

85 90 95

Gly Arg Gly Asn Ala Glu Thr Thr Tyr Thr Tyr Arg Pro Ser Val Pro

100 105 110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe

115 120 125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile

130 135 140

Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp

145 150 155 160

Leu Ile Arg Thr Asp Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp

165 170 175

Pro Leu Phe Met Cys Cys Leu Ser Leu Ser Phe Phe Pro Leu Ala Ala

180 185 190

Phe Thr Val Glu Lys Leu Val Leu Gln Lys Cys Ile Ser Glu Pro Val

195 200 205

Val Ile Ile Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro

210 215 220

Val Tyr Val Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr

225 230 235 240

Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala

245 250 255

His Thr Asn Tyr Asp Ile Arg Thr Leu Ala Asn Ser Ala Asp Lys Ala

260 265 270

Asn Pro Glu Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe

275 280 285

Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Pro

290 295 300

Cys Ile Arg Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile

305 310 315 320

Phe Thr Gly Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile

325 330 335

Val Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile

340 345 350

Glu Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys

355 360 365

Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu

370 375 380

Leu Cys Phe Gly Asp Arg Glu Phe Tyr Arg Asp Trp Trp Asn Ala Lys

385 390 395 400

Ser Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp

405 410 415

Met Val Arg His Ile Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys

420 425 430

Thr Leu Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu

435 440 445

Leu Cys Ile Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Ile

450 455 460

Gly Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln

465 470 475 480

Glu Arg Phe Gly Ser Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe

485 490 495

Cys Ile Phe Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu

500 505 510

Met Asn Arg Lys Gly Ser Met Ser

515 520

<210> 182

<211> 520

<212> PRT

<213>   Unknown

<220><223>   Camelina sativa

<400>   182

Met Ala Ile Leu Asp Ser Gly Gly Gly Gly Val Ser Thr Ala Thr Ala

1   5   10   15

Thr Glu Asn Gly Gly Gly Glu Phe Val Asp Leu Arg Arg Arg Lys Ser

            20   25   30

Arg Ser Asp Ser Asn Gly Val Leu Cys Gly Ser Asp Asn Pro Pro Ser

        35   40   45

Asp Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp Ser Val

    50   55   60

Val Asn Asp Asp Ala Gln Gly Thr Thr Ala Asn Leu Ala Gly Asp Asn

65   70   75   80

Glu Ile Arg Glu Thr Gly Gly Gly Gly Arg Gly Gly Gly Gly Glu Gly

                85   90   95

Gly Arg Gly Asn Ala Glu Thr Thr Tyr Thr Tyr Arg Pro Ser Val Pro

            100   105   110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe

        115   120   125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile

    130   135   140

Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp

145   150   155   160

Leu Ile Arg Thr Asp Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp

                165   170   175

Pro Leu Phe Met Cys Cys Leu Ser Leu Ser Phe Phe Pro Leu Ala Ala

            180   185   190

Phe Thr Val Glu Lys Leu Val Leu Gln Lys Cys Ile Ser Glu Pro Val

        195   200   205

Val Ile Phe Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro

    210   215   220

Val Tyr Val Thr Leu Ser Cys Asp Ser Ala Phe Leu Ser Gly Val Thr

225 230 235 240

Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala

245 250 255

His Thr Asn Tyr Asp Ile Arg Thr Leu Ala Asn Ser Ala Asp Lys Ala

260 265 270

Asn Pro Glu Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe

275 280 285

Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Pro

290 295 300

Cys Ile Arg Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile

305 310 315 320

Phe Thr Gly Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile

325 330 335

Val Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile

340 345 350

Glu Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys

355 360 365

Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu

370 375 380

Leu Cys Phe Gly Asp Arg Glu Phe Tyr Arg Asp Trp Trp Asn Ala Lys

385 390 395 400

Ser Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp

405 410 415

Met Val Arg His Ile Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys

420 425 430

Thr Leu Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu

435 440 445

Leu Cys Ile Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Ile

450 455 460

Gly Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln

465 470 475 480

Glu Arg Phe Gly Ser Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe

485 490 495

Cys Ile Phe Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu

500 505 510

Met Asn Arg Lys Gly Ser Met Ser

515 520

<210> 183

<211> 520

<212> PRT

<213> Unknown

<220><223> Camelina sativa

<400> 183

Met Ala Ile Leu Asp Ser Gly Gly Gly Gly Val Ser Thr Ala Thr Ala

1 5 10 15

Thr Glu Asn Gly Gly Gly Glu Phe Val Asp Leu Arg Arg Arg Lys Ser

20 25 30

Arg Ser Asp Ser Asn Gly Val Leu Ser Gly Ser Asp Asn Pro Pro Ser

35 40 45

Val Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp Ser Val

50 55 60

Val Asn Asp Asp Ala Gln Gly Thr Thr Ala Asn Leu Ala Gly Asp Thr

65 70 75 80

Glu Ile Arg Glu Thr Gly Gly Gly Gly Arg Gly Ala Gly Gly Glu Gly

85 90 95

Gly Arg Gly Asn Ala Glu Thr Thr Tyr Ala Tyr Arg Pro Ser Val Pro

100 105 110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe

115 120 125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile

130 135 140

Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp

145 150 155 160

Leu Ile Arg Thr Asp Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp

165 170 175

Pro Leu Phe Met Cys Cys Leu Ser Leu Ser Ile Phe Pro Leu Ala Ala

180 185 190

Phe Thr Val Glu Lys Leu Val Leu Gln Lys Cys Ile Ser Glu Pro Val

195 200 205

Val Ile Ile Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro

210 215 220

Val Tyr Val Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr

225 230 235 240

Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala

245 250 255

His Thr Asn Tyr Asp Ile Arg Thr Leu Ala Asn Ser Ala Asp Lys Ala

260 265 270

Asn Pro Glu Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe

275 280 285

Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Pro

290 295 300

Cys Ile Arg Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile

305 310 315 320

Phe Thr Gly Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile

325 330 335

Val Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile

340 345 350

Glu Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys

355 360 365

Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu

370 375 380

Leu Cys Phe Gly Asp Arg Glu Phe Tyr Arg Asp Trp Trp Asn Ala Lys

385 390 395 400

Ser Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp

405 410 415

Met Val Arg His Ile Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys

420 425 430

Thr Leu Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu

435 440 445

Leu Cys Ile Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Ile

450 455 460

Gly Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln

465 470 475 480

Glu Arg Phe Gly Ser Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe

485 490 495

Cys Ile Phe Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu

500 505 510

Met Asn Arg Lys Gly Ser Met Ser

515 520

<210> 184

<211> 503

<212> PRT

<213> Brassica napus

<400> 184

Met Ala Ile Leu Asp Ser Gly Gly Val Ala Val Pro Pro Thr Glu Asn

1 5 10 15

Gly Val Ala Asp Leu Asp Arg Leu His Arg Arg Lys Ser Ser Ser Asp

20 25 30

Ser Ser Asn Gly Leu Leu Ser Asp Thr Ser Pro Ser Asp Asp Val Gly

35 40 45

Ala Ala Ala Ala Glu Arg Asp Arg Val Asp Ser Ala Ala Glu Glu Glu

50 55 60

Ala Gln Gly Thr Ala Asn Leu Ala Gly Gly Asp Ala Glu Thr Arg Glu

65 70 75 80

Ser Ala Gly Gly Asp Val Arg Phe Thr Tyr Arg Pro Ser Val Pro Ala

85 90 95

His Arg Arg Thr Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe Lys

100 105 110

Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Val Ala

115 120 125

Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp Leu

130 135 140

Ile Arg Thr Asp Phe Trp Phe Ser Ser Thr Ser Leu Arg Asp Trp Pro

145 150 155 160

Leu Phe Met Cys Cys Leu Ser Leu Ser Val Phe Pro Leu Ala Ala Phe

165 170 175

Thr Val Glu Lys Met Val Leu Gln Lys Phe Ile Ser Glu Pro Val Ala

180 185 190

Ile Ile Leu His Val Ile Ile Thr Met Thr Glu Val Leu Tyr Pro Val

195 200 205

Tyr Val Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr Leu

210 215 220

Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala His

225 230 235 240

Thr Ser Tyr Asp Ile Arg Thr Leu Ala Asn Ser Ala Asp Lys Val Asp

245 250 255

Pro Glu Ile Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe Met

260 265 270

Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Pro Cys

275 280 285

Ile Arg Lys Gly Trp Val Ala Arg Gln Leu Ala Lys Leu Val Ile Phe

290 295 300

Thr Gly Leu Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile Val

305 310 315 320

Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile Glu

325 330 335

Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys Met

340 345 350

Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu Leu
355 360 365

Cys Phe Gly Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys Ser
370 375 380

Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp Met
385 390 395 400

Val Arg His Val Tyr Phe Pro Cys Leu Arg Ile Lys Ile Pro Lys Val
405 410 415

Pro Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu Leu

420 425 430

Cys Ile Ala Val Pro Cys Arg Leu Phe Asn Leu Trp Ala Phe Met Gly
435 440 445

Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Phe Leu Gln Glu
450 455 460

Arg Phe Gly Ser Met Val Gly Asn Met Ile Phe Gly Ser Ala Ser Cys
465 470 475 480

Ile Phe Gly Gln Pro Met Cys Gly Leu Leu Tyr Tyr His Asp Leu Met
485 490 495

Asn Arg Lys Gly Ser Met Ser
500

<210> 185

<211> 517

<212> PRT

<213> Glycine max

<400> 185

Met Ala Ile Ser Asp Val Pro Ala Ala Ala Gly Thr Thr Ala Thr Thr
1 5 10 15

Thr Ser Asp Ser Asp Leu Arg Gln Pro Ser Leu Arg Arg Arg Ser Ser
20 25 30

Ala Gly Val Leu Phe Asp Ala Ala Arg Asp Ser Gly Ser Asp Asn Ser

```
         35                  40                  45

Leu Thr Gly Lys Ile Thr Asp Asp Asp Asn Ile Lys Asp His Lys Pro
     50                  55                  60
Asn Asn His Ala Ala Ser Asp Asp Asn Val Gly Ala Ala Ala Asn Asp
 65                  70                  75                  80
Ala Gly Gln Glu His Arg Gln Pro Val Ala Asp Phe Lys Tyr Ala Tyr
                 85                  90                  95
Arg Pro Ser Val Pro Ala His Arg Arg Ile Lys Glu Ser Pro Leu Ser
            100                 105                 110
Ser Asp Asn Ile Phe Arg Gln Ser His Ala Gly Leu Phe Asn Leu Cys

        115                 120                 125
Ile Val Val Leu Val Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu
    130                 135                 140
Met Lys Tyr Gly Trp Leu Ile Lys Tyr Gly Phe Trp Phe Ser Ser Lys
145                 150                 155                 160
Ser Leu Arg Asp Trp Pro Leu Phe Met Cys Cys Leu Ser Leu Ala Ile
                165                 170                 175
Phe Pro Leu Ala Ala Phe Val Val Glu Arg Leu Ala Gln Gln Lys Cys
            180                 185                 190

Ile Ser Glu Pro Val Val Val Leu Leu His Leu Ile Ile Ser Thr Val
        195                 200                 205
Glu Leu Cys Tyr Pro Val Leu Val Ile Leu Arg Cys Asp Ser Ala Phe
    210                 215                 220
Val Ser Gly Val Thr Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys
225                 230                 235                 240
Leu Val Ser Tyr Ala His Thr Asn Tyr Asp Met Arg Ala Leu Thr Val
                245                 250                 255
Ser Asn Glu Lys Gly Glu Thr Leu Pro Asn Thr Leu Ile Met Glu Tyr

            260                 265                 270
Pro Tyr Thr Val Thr Phe Arg Ser Leu Ala Tyr Phe Met Val Ala Pro
        275                 280                 285
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Thr Leu Cys Tyr Gln Thr Ser Tyr Pro Arg Thr Pro Ser Val Arg Lys

290 295 300

Gly Trp Val Phe Arg Gln Leu Val Lys Leu Ile Ile Phe Thr Gly Val

305 310 315 320

Met Gly Phe Ile Ile Glu Gln Tyr Met Asn Pro Ile Val Gln Asn Ser

325 330 335

Thr His Pro Leu Lys Gly Asn Leu Leu Tyr Ala Ile Glu Arg Ile Leu

340 345 350

Lys Leu Ser Val Pro Asn Val Tyr Val Trp Leu Cys Met Phe Tyr Cys

355 360 365

Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu Val Arg Phe Gly

370 375 380

Asp Arg Glu Phe Tyr Lys Asp Trp Trp Asn Ala Lys Thr Val Glu Glu

385 390 395 400

Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp Met Val Arg His

405 410 415

Ile Tyr Phe Pro Cys Leu Arg Arg Gly Ile Pro Lys Gly Ala Ala Ser

420 425 430

Leu Ile Ala Phe Leu Val Ser Ala Val Phe His Glu Leu Cys Ile Ala

435 440 445

Val Pro Cys His Met Phe Lys Leu Trp Ala Phe Ile Gly Ile Met Phe

450 455 460

Gln Val Pro Leu Val Leu Ile Thr Asn Tyr Leu Gln Asn Lys Tyr Arg

465 470 475 480

Asn Ser Met Val Gly Asn Met Ile Phe Trp Phe Ile Phe Cys Ile Leu

485 490 495

Gly Gln Pro Met Ser Val Leu Leu Tyr Tyr His Asp Leu Met Asn Arg

500 505 510

Lys Gly Glu Val Asp

515

<210> 186

<211> 519

<212> PRT

<213> Arachis hypogaea

<400> 186

Met Ala Ile Ser Asp Val His Glu Thr Ser Val Ala Gly Asp Gly Ala
1 5 10 15

Asn His Ser Ser Leu Arg Arg Arg His Ser Arg Val Ala Ser Ser Gly
20 25 30

Gly Asn Met Phe Asp Glu Ala Ala Ala Ser Ala Glu Ala Val Met Ile
35 40 45

Asp Ser Ser Gly Ser Asp Asp Ser Leu Asn Glu Arg Ile Gly Ala Ala
50 55 60

Arg Glu Glu Lys Val Lys Glu Lys Gln Lys Gln Lys Glu Glu Asp Arg
65 70 75 80

Lys Pro Pro Asp His Ala Ser Arg Asn Glu Val Gln Asp Gly Glu Arg
85 90 95

Ala Ala Ala Gly Asp Asn Phe Thr Tyr Arg Ala Ser Val Pro Val His
100 105 110

Arg Arg Ile Lys Asp Ser Pro Leu Ser Ser Arg Asn Ile Phe Lys Gln
115 120 125

Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile Ala Val
130 135 140

Asn Ser Arg Leu Ile Ile Glu Asn Ile Met Lys Tyr Gly Trp Leu Ile
145 150 155 160

Asn Ser Gly Phe Trp Phe Ser Ser Lys Ser Leu Arg Asp Trp Pro Leu
165 170 175

Leu Met Cys Cys Ile Ser Leu Asn Leu Phe Pro Leu Ala Ala Phe Met
180 185 190

Val Glu Lys Leu Ala Gln Lys Lys Arg Ile Ser Glu Pro Val Ile Phe
195 200 205

Leu Leu His Thr Ile Ile Met Thr Gly Glu Ile Ser Phe Pro Val Leu
210 215 220

Val Ile Leu Ser Cys Asp Ser Thr Phe Leu Ser Gly Leu Thr Leu Met

```
225                 230                 235                 240


Met Val Ala Cys Ile Ile Trp Leu Lys Leu Val Ser Tyr Ala His Thr
                245                 250                 255
Ser His Asp Leu Arg Ser Leu Ser Leu Ser Ile Glu Lys Gly Glu Thr
            260                 265                 270
Leu Pro Asn Asn Leu Asn Met Glu His Pro Tyr Arg Val Ser Phe Arg
        275                 280                 285
Ser Met Ala Tyr Phe Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser
    290                 295                 300
Tyr Pro Arg Thr Pro Ser Val Arg Lys Gly Trp Val Phe Arg Gln Leu


305                 310                 315                 320
Ile Lys Leu Val Ile Phe Thr Gly Leu Met Gly Phe Ile Ile Glu Gln
                325                 330                 335
Tyr Met His Pro Ile Val Gln Asn Ser Gln His Pro Phe Lys Gly Asn
            340                 345                 350
Leu Leu Tyr Gly Phe Glu Arg Thr Leu Lys Leu Ser Val Pro Asn Val
        355                 360                 365
Tyr Val Trp Leu Cys Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn
    370                 375                 380


Ile Leu Ala Glu Leu Val Gln Phe Gly Asp Arg Glu Phe Tyr Lys Asp
385                 390                 395                 400
Trp Trp Asn Ala Lys Thr Val Asp Glu Pro Val His Lys Trp Met Val
                405                 410                 415
Arg His Ile Tyr Phe Pro Cys Ile Arg His Gly Met Ser Lys Asn Ala
            420                 425                 430
Ala Val Leu Ile Ala Phe Leu Ile Ser Ala Val Phe His Glu Leu Cys
        435                 440                 445
Ile Ala Val Pro Cys His Lys Phe Lys Leu Trp Ala Phe Ile Gly Ile


    450                 455                 460
Met Phe Gln Val Pro Leu Ser Ile Val Thr Asn Phe Leu Gln Lys Lys
465                 470                 475                 480
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Cys Lys Ser Ser Met Val Gly Asn Met Val Phe Trp Phe Thr Phe Cys

485 490 495

Ile Leu Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Trp Met

500 505 510

Asn Arg His Arg Glu His Asn

515

<210> 187

<211

> 520

<212> PRT

<213> Ricinus communis

<400> 187

Met Ala Ile Leu Asp Ser Gly Gly Gly Gly Val Ser Thr Ala Thr Ala

1 5 10 15

Thr Glu Asn Gly Gly Gly Glu Phe Val Asp Leu Arg Arg Arg Lys Ser

20 25 30

Arg Ser Asp Ser Asn Gly Val Leu Ser Gly Ser Asp Asn Pro Pro Ser

35 40 45

Val Asp Val Gly Ala Pro Ala Asp Val Arg Asp Arg Ile Asp Ser Val

50 55 60

Val Asn Asp Asp Ala Gln Gly Thr Thr Ala Asn Leu Ala Gly Asp Thr

65 70 75 80

Glu Ile Arg Glu Thr Gly Gly Gly Gly Arg Gly Gly Gly Gly Glu Gly

85 90 95

Gly Arg Gly Asn Ala Glu Thr Thr Tyr Thr Tyr Arg Pro Ser Val Pro

100 105 110

Ala His Arg Arg Ala Arg Glu Ser Pro Leu Ser Ser Asp Ala Ile Phe

115 120 125

Lys Gln Ser His Ala Gly Leu Phe Asn Leu Cys Val Val Val Leu Ile

130 135 140

Ala Val Asn Ser Arg Leu Ile Ile Glu Asn Leu Met Lys Tyr Gly Trp

145 150 155 160

Leu Ile Arg Thr Asp Phe Trp Phe Ser Ser Arg Ser Leu Arg Asp Trp

165 170 175

Pro Leu Phe Met Cys Cys Leu Ser Leu Ser Phe Phe Pro Leu Ala Ala

180 185 190

Phe Thr Val Glu Lys Leu Val Leu Gln Lys Cys Ile Ser Glu Pro Val

195 200 205

Val Ile Ile Leu His Ile Ile Ile Thr Met Thr Glu Val Leu Tyr Pro

210 215 220

Val Tyr Val Thr Leu Arg Cys Asp Ser Ala Phe Leu Ser Gly Val Thr

225 230 235 240

Leu Met Leu Leu Thr Cys Ile Val Trp Leu Lys Leu Val Ser Tyr Ala

245 250 255

His Thr Asn Tyr Asp Ile Arg Thr Leu Ala Asn Ser Ala Asp Lys Ala

260 265 270

Asn Pro Glu Val Ser Tyr Tyr Val Ser Leu Lys Ser Leu Ala Tyr Phe

275 280 285

Met Val Ala Pro Thr Leu Cys Tyr Gln Pro Ser Tyr Pro Arg Ser Pro

290 295 300

Cys Ile Arg Lys Gly Trp Val Ala Arg Gln Phe Ala Lys Leu Val Ile

305 310 315 320

Phe Thr Gly Phe Met Gly Phe Ile Ile Glu Gln Tyr Ile Asn Pro Ile

325 330 335

Val Arg Asn Ser Lys His Pro Leu Lys Gly Asp Leu Leu Tyr Ala Ile

340 345 350

Glu Arg Val Leu Lys Leu Ser Val Pro Asn Leu Tyr Val Trp Leu Cys

355 360 365

Met Phe Tyr Cys Phe Phe His Leu Trp Leu Asn Ile Leu Ala Glu Leu

370 375 380

Leu Cys Phe Gly Asp Arg Glu Phe Tyr Arg Asp Trp Trp Asn Ala Lys

385 390 395 400

Ser Val Gly Asp Tyr Trp Arg Met Trp Asn Met Pro Val His Lys Trp

405 410 415

Met Val Arg His Ile Tyr Phe Pro Cys Leu Arg Ser Lys Ile Pro Lys

420 425 430

Thr Leu Ala Ile Ile Ile Ala Phe Leu Val Ser Ala Val Phe His Glu

435 440 445

Leu Cys Ile Ala Val Pro Cys Arg Leu Phe Lys Leu Trp Ala Phe Ile

450 455 460

Gly Ile Met Phe Gln Val Pro Leu Val Phe Ile Thr Asn Tyr Leu Gln

465 470 475 480

Glu Arg Phe Gly Ser Thr Val Gly Asn Met Ile Phe Trp Phe Ile Phe

485 490 495

Cys Ile Phe Gly Gln Pro Met Cys Val Leu Leu Tyr Tyr His Asp Leu

500 505 510

Met Asn Arg Lys Gly Ser Met Ser

515 520