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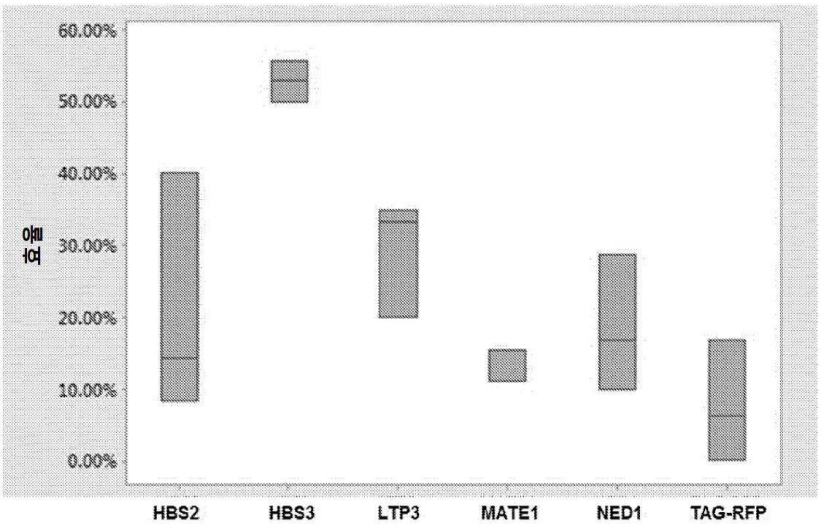
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(54) 발명의 명칭 **조직-선호적 프로모터 및 이용 방법**

(57) 요 약

식물에서 이종 뉴클레오티드 서열의 발현을 조절하기 위한 조성물 및 방법이 제공된다. 조성물은 형태형성 유전자에 작동 가능하게 연결되는 조직-선호적 프로모터를 포함한다. 형태형성 유전자에 작동 가능하게 연결되는 본원에 개시된 프로모터 서열을 이용해 식물에서 이종 뉴클레오티드 서열을 발현시키는 방법이 또한 제공된다. 형태형성 유전자에 작동 가능하게 연결되는 프로모터를 포함하고 이종의 관심 뉴클레오티드 서열을 추가로 포함하는 DNA 구축물이 또한 제공된다.

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청구범위

청구항 1

(a) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열;

(b) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열;

(c) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열;

(d) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 뉴클레오티드 서열의 단편 또는 변이체이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열의 단편 또는 변이체; 또는

(e) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 뉴클레오티드 서열의 100개 이상의 연속 뉴클레오티드이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열의 100개 이상의 연속 뉴클레오티드

로 이루어진 군으로부터 선택되는 뉴클레오티드 서열을 갖는 조직-선호적 프로모터를 포함하는 형태형성 유전자 카세트를 포함하는 핵산 분자로서, (a) 내지 (e)의 조직-선호적 프로모터는 형태형성 유전자에 작동 가능하게 연결되는, 핵산 분자.

청구항 2

제1항의 형태형성 유전자 카세트를 포함하는 발현 카세트.

청구항 3

제2항의 발현 카세트를 포함하는 벡터.

청구항 4

제2항의 발현 카세트를 포함하는 식물 세포.

청구항 5

제4항에 있어서, 식물 세포는 외떡잎식물, 쌍떡잎식물, 또는 겉씨식물에서 유래된, 식물 세포.

청구항 6

제5항에 있어서, 외떡잎식물, 쌍떡잎식물, 또는 겉씨식물은 옥수수, 알팔파, 수수, 벼, 밀렛, 대두, 밀, 목화, 해바라기, 보리, 귀리, 호밀, 아마, 사탕수수, 바나나, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 소나무, 미송, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 대나무, 라이밀, 멜론, 또는 배추속으로 이루어진 군으로부터 선택되는, 식물 세포.

청구항 7

제4항에 있어서, 형태형성 유전자는 WUS/WOX 호메오박스 폴리펩티드를 암호화하고,

WUS/WOX 호메오박스 폴리펩티드는 서열 번호 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 또는 148 중 어느 하나의 아미노산 서열을 포함하거나, 서열 번호 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 또는 147 중 어느 하나의 뉴클레오티드 서열에 의해 암호화되는, 식물 세포.

청구항 8

제4항에 있어서, 형태형성 유전자는 식물 대사, 기관 발육, 줄기 세포 발육, 세포 성장 자극, 기관형성, 재생, 체세포 배발생 개시, 체세포배 성숙 촉진, 정단 분열조직의 발생 및/또는 발육, 싹 분열조직의 발생 및/또는 발육, 싹의 발생 및/또는 발육, 또는 이들의 조합에 관여하는 유전자 산물을 암호화하는, 식물 세포.

청구항 9

제4항에 있어서, 상기 발현 카세트는 영양분 강화, 수확량 증가, 무생물 스트레스 내성, 가뭄 내성, 내한성, 제초제 내성, 해충 저항성, 병원체 저항성, 곤충 저항성, 질소 이용 효율(NUE), 병해 저항성, 또는 대사 경로 변경 능력을 부여하는 유전자 산물을 암호화하는 이종 폴리뉴클레오티드를 포함하는 형질 유전자 카세트를 추가로 포함하는, 식물 세포.

청구항 10

제9항에 있어서, 상기 발현 카세트는 FLP, FLPe, KD, Cre, SSV1, 람다 Int, 파이 C31 Int, HK022, R, B2, B3, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, 또는 U153으로 이루어진 군으로부터 선택되는 부위-특이적 재조합효소를 암호화하는 뉴클레오티드 서열을 포함하는 부위-특이적 재조합효소 카세트를 추가로 포함하고, 부위-특이적 재조합효소는 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터에 작동 가능하게 연결되는, 식물 세포.

청구항 11

제10항에 있어서, 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터는 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, 또는 LEA-D34로 이루어진 군으로부터 선택되는, 식물 세포.

청구항 12

제11항에 있어서, 상기 발현 카세트의 형태형성 유전자 카세트 및 부위-특이적 재조합효소 카세트는 식물 세포에서 일시적으로 발현되고, 상기 발현 카세트의 형질 유전자 카세트는 식물 세포의 게놈에 안정적으로 통합되는, 식물 세포.

청구항 13

제11항에 있어서, 상기 발현 카세트의 형태형성 유전자 카세트 및 부위-특이적 재조합효소 카세트는 식물 세포로부터 절제되고, 상기 발현 카세트의 형질 유전자 카세트는 식물 세포의 게놈에 안정적으로 통합되는, 식물 세포.

청구항 14

제2항의 발현 카세트를 포함하는 식물.

청구항 15

제14항에 있어서, 식물은 외떡잎식물, 쌍떡잎식물, 또는 겉씨식물인, 식물.

청구항 16

제15항에 있어서, 외떡잎식물, 쌍떡잎식물, 또는 겉씨식물은 옥수수, 알팔파, 수수, 벼, 밀렛, 대두, 밀, 목화, 해바라기, 보리, 귀리, 호밀, 아마, 사탕수수, 바나나, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 소나무, 미송, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 대나무, 라이밀, 멜론, 또는 배추속으로 이루어진 군으로부터 선택되는, 식물.

청구항 17

제14항에 있어서, 형태형성 유전자는 WUS/WOX 호메오박스 폴리펩티드를 암호화하고,

WUS/WOX 호메오박스 폴리펩티드는 서열 번호 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 또는 148 중 어느 하나의 아미노산 서열을 포함하거나, 서열 번호 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 또는 147 중 어느 하나의 뉴클레오티드 서열에 의해 암호화되는, 식물.

청구항 18

제14항에 있어서, 형태형성 유전자는 식물 대사, 기관 발육, 줄기 세포 발육, 세포 성장 자극, 기관형성, 재생, 체세포 배발생, 체세포배 성숙 촉진, 정단 분열조직의 발생 및/또는 발육, 싹 분열조직의 발생 및/또는 발육, 싹의 발생 및/또는 발육, 또는 이들의 조합에 관여하는 유전자 산물을 암호화하는, 식물.

청구항 19

제14항에 있어서, 상기 발현 카세트는 영양분 강화, 수확량 증가, 무생물 스트레스 내성, 가뭄 내성, 내한성, 제초제 내성, 해충 저항성, 병원체 저항성, 곤충 저항성, 질소 이용 효율(NUE), 병해 저항성, 또는 대사 경로 변경 능력을 부여하는 유전자 산물을 암호화하는 이종 폴리뉴클레오티드를 포함하는 형질 유전자 카세트를 추가로 포함하는, 식물.

청구항 20

제19항에 있어서, 상기 발현 카세트는 FLP, FLPe, KD, Cre, SSV1, 람다 Int, 파이 C31 Int, HK022, R, B2, B3, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, 또는 U153으로 이루어진 군으로부터 선택되는 부위-특이적 재조합효소를 암호화하는 뉴클레오티드 서열을 포함하는 부위-특이적 재조합효소 카세트를 추가로 포함하고, 부위-특이적 재조합효소는 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터에 작동 가능하게 연결되는, 식물.

청구항 21

제20항에 있어서, 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터는 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, 또는 LEA-D34로 이루어진 군으로부터 선택되는, 식물.

청구항 22

제21항에 있어서, 상기 발현 카세트의 형태형성 유전자 카세트 및 부위-특이적 재조합효소 카세트는 식물에서 일시적으로 발현되고, 상기 발현 카세트의 형질 유전자 카세트는 식물의 게놈에 안정적으로 통합되는, 식물.

청구항 23

제22항의 식물의 종자로서, 상기 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 24

제21항에 있어서, 상기 발현 카세트의 형태형성 유전자 카세트 및 부위-특이적 재조합효소 카세트는 식물로부터 절제되고, 상기 발현 카세트의 형질 유전자 카세트는 식물의 게놈에 안정적으로 통합되는, 식물.

청구항 25

제24항의 식물의 종자로서, 상기 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 26

식물 또는 식물 세포에서 전사를 개시할 수 있는 뉴클레오티드 서열을 포함하는 재조합 폴리뉴클레오티드를 포함하는 발현 카세트로서, 뉴클레오티드 서열은 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나로 이루어진 군으로부터 선택되는 뉴클레오티드 서열의 100개 이상의 연속 뉴클레오티드를 가지며, 전사를

개시할 수 있는 뉴클레오티드 서열은 형태형성 유전자에 작동 가능하게 연결되는, 발현 카세트.

청구항 27

식물 또는 식물 세포에서 전사를 개시할 수 있는 기능적 단편 또는 변이체를 포함하는 재조합 폴리뉴클레오티드를 포함하는 발현 카세트로서, 기능적 단편 또는 변이체는 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나로 이루어진 군으로부터 선택되는 뉴클레오티드 서열로부터 유도되고, 전사를 개시할 수 있는 기능적 단편 또는 변이체는 형태형성 유전자에 작동 가능하게 연결되는, 발현 카세트.

청구항 28

식물 또는 식물 세포에서 전사를 개시할 수 있는 뉴클레오티드 서열을 포함하는 재조합 폴리뉴클레오티드를 포함하는 발현 카세트로서, 뉴클레오티드 서열은 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나로 이루어진 군으로부터 선택되는 뉴클레오티드 서열과 적어도 70%의 동일성을 가지며, 전사를 개시할 수 있는 뉴클레오티드 서열은 형태형성 유전자에 작동 가능하게 연결되는, 발현 카세트.

청구항 29

식물 또는 식물 세포에서 전사를 개시할 수 있는 뉴클레오티드 서열을 포함하는 재조합 폴리뉴클레오티드를 포함하는 발현 카세트로서, 뉴클레오티드 서열은 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나로 이루어진 군으로부터 선택되는 뉴클레오티드 서열과 적어도 95%의 동일성을 가지며, 전사를 개시할 수 있는 뉴클레오티드 서열은 형태형성 유전자에 작동 가능하게 연결되는, 발현 카세트.

청구항 30

식물 또는 식물 세포에서 전사를 개시할 수 있는 뉴클레오티드 서열을 포함하는 재조합 폴리뉴클레오티드를 포함하는 발현 카세트로서, 뉴클레오티드 서열은 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나로 이루어진 군으로부터 선택되고, 전사를 개시할 수 있는 뉴클레오티드 서열은 형태형성 유전자에 작동 가능하게 연결되는, 발현 카세트.

청구항 31

유전자이식 식물의 제조 방법으로서,

재조합 발현 카세트로 식물 세포를 형질전환시키는 단계이되,

(a) 조직-선호적 프로모터 카세트(상기 조직-선호적 프로모터 카세트는

(i) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열;

(ii) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열;

(iii) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열;

(iv) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 단편 또는 변이체이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 또는

(v) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 100 bp 이상의 단편이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열

로 이루어진 군으로부터 선택되는 뉴클레오티드 서열을 포함하고, (i) 내지 (v)의 뉴클레오티드 서열은 형태형성 유전자에 작동 가능하게 연결됨) 및

(b) 형질 유전자 카세트(상기 형질 유전자 카세트는 영양분 강화, 수확량 증가, 무생물 스트레스 내성, 가뭄 내성, 내한성, 제초제 내성, 해충 저항성, 병원체 저항성, 곤충 저항성, 질소 이용 효율(NUE), 병해 저항성, 또는 대사 경로 변경 능력을 부여하는 유전자 산물을 암호화하는 이종의 관심 폴리뉴클레오티드를 포함함)를 포함하는 재조합 발현 카세트로 식물 세포를 형질전환시키는 단계;

상기 재조합 발현 카세트를 갖는 유전자이식 식물 세포를 선별하는 단계; 및

유전자이식 식물 세포로부터 유전자이식 식물을 재생시키는 단계를 포함하는, 방법.

청구항 32

제31항에 있어서, 식물 세포는 외떡잎식물, 쌍떡잎식물, 또는 겉씨식물인, 방법.

청구항 33

제32항에 있어서, 외떡잎식물, 쌍떡잎식물, 또는 겉씨식물은 옥수수, 알팔파, 수수, 벼, 밀렛, 대두, 밀, 목화, 해바라기, 보리, 귀리, 호밀, 아마, 사탕수수, 바나나, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 소나무, 미송, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 대나무, 라이밀, 멜론, 또는 배추속으로 이루어진 군으로부터 선택되는, 방법.

청구항 34

제31항에 있어서, 형태형성 유전자는 WUS/WOX 호메오박스 폴리펩티드를 암호화하고,

WUS/WOX 호메오박스 폴리펩티드는 서열 번호 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 또는 148 중 어느 하나의 아미노산 서열을 포함하거나, 서열 번호 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 또는 147 중 어느 하나의 뉴클레오티드 서열에 의해 암호화되는, 방법.

청구항 35

제31항에 있어서, 형태형성 유전자는 식물 대사, 기관 발육, 줄기 세포 발육, 세포 성장 자극, 기관형성, 재생, 체세포 배발생, 체세포배 성숙 촉진, 정단 분열조직의 발생 및/또는 발육, 싹 분열조직의 발생 및/또는 발육, 싹의 발생 및/또는 발육, 또는 이들의 조합에 관여하는 유전자 산물을 암호화하는, 방법.

청구항 36

제31항에 있어서, 상기 발현 카세트는 FLP, FLPe, KD, Cre, SSV1, 람다 Int, 파이 C31 Int, HK022, R, B2, B3, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, 또는 U153으로 이루어진 군으로부터 선택되는 부위-특이적 재조합효소를 암호화하는 뉴클레오티드 서열을 포함하는 부위-특이적 재조합효소 카세트를 추가로 포함하고, 부위-특이적 재조합효소는 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터에 작동 가능하게 연결되는, 방법.

청구항 37

제36항에 있어서, 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터는 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, 또는 LEA-D34로 이루어진 군으로부터 선택되는, 방법.

청구항 38

제37항에 있어서, 상기 재조합 발현 카세트로부터 상기 조직-선호적 프로모터 카세트 및 상기 부위-특이적 재조합효소 카세트를 절제하는 단계를 추가로 포함하는, 방법.

청구항 39

제38항의 방법에 의해 제조된 유전자이식 식물.

청구항 40

제39항의 유전자이식 식물의 종자로서, 상기 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 41

제31항에 있어서, 상기 조직-선호적 프로모터 카세트는 제1 T-DNA를 포함하고 상기 형질 유전자 카세트는 제2 T-DNA를 포함하는, 방법.

청구항 42

제41항에 있어서, 제1 T-DNA 및 제2 T-DNA는 식물 세포를 형질전환시키기 위한 동일한 박테리아 균주에 존재하는, 방법.

청구항 43

제42항에 있어서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법.

청구항 44

제43항의 방법에 의해 제조된 유전자이식 식물.

청구항 45

제44항의 유전자이식 식물의 종자로서, 상기 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 46

제41항에 있어서, 제1 T-DNA는 제1 박테리아 균주에 존재하고 제2 T-DNA는 제2 박테리아 균주에 존재하며, 제1 박테리아 균주와 제2 박테리아 균주는 식물 세포를 형질전환시키기 위한 비율로 혼합되는, 방법.

청구항 47

제46항에 있어서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법.

청구항 48

제47항의 방법에 의해 제조된 유전자이식 식물.

청구항 49

제48항의 유전자이식 식물의 종자로서, 상기 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 50

체세포배 성숙 효율을 향상시키는 방법으로서,

재조합 발현 카세트로 식물 세포를 형질전환시키는 단계이되,

(a) 조직-선호적 프로모터 카세트(상기 조직-선호적 프로모터 카세트는

(i) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열;

(ii) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열;

(iii) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열;

(iv) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 단편 또는 변이체이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열;

(v) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 100 bp 이상의 단편이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열

로 이루어진 군으로부터 선택되는 뉴클레오티드 서열을 포함하고, (a) 내지 (e)의 뉴클레오티드 서열은 형태형성 유전자에 작동 가능하게 연결됨) 및

(b) 형질 유전자 카세트(상기 형질 유전자 카세트는 영양분 강화, 수확량 증가, 무생물 스트레스 내성, 가뭄 내성, 내한성, 제초제 내성, 해충 저항성, 병원체 저항성, 곤충 저항성, 질소 이용 효율(NUE), 병해 저항성, 또는 대사 경로 변경 능력을 부여하는 유전자 산물을 암호화하는 이종의 관심 폴리뉴클레오티드를 포함함)를 포함하는 재조합 발현 카세트로 식물 세포를 형질전환시키는 단계;

상기 재조합 발현 카세트를 갖는 유전자이식 식물 세포를 선별하는 단계; 및

유전자이식 식물 세포로부터 유전자이식 식물을 재생시키는 단계를 포함하고, 상기 재조합 발현 카세트로 인해 상기 재조합 발현 카세트를 포함하지 않는 유전자이식 식물 세포에 비해 체세포배 성숙 효율이 향상되는, 방법.

청구항 51

제50항에 있어서, 식물 세포는 외떡잎식물, 쌍떡잎식물, 또는 겉씨식물인, 방법.

청구항 52

제51항에 있어서, 외떡잎식물, 쌍떡잎식물, 또는 겉씨식물은 옥수수, 알팔파, 수수, 벼, 밀렛, 대두, 밀, 목화, 해바라기, 보리, 귀리, 호밀, 아마, 사탕수수, 바나나, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 소나무, 미송, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 대나무, 라이밀, 멜론, 또는 배추속으로 이루어진 군으로부터 선택되는, 방법.

청구항 53

제50항에 있어서, 형태형성 유전자는 WUS/WOX 호메오박스 폴리펩티드를 암호화하고,

WUS/WOX 호메오박스 폴리펩티드는 서열 번호 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 또는 148 중 어느 하나의 아미노산 서열을 포함하거나, 서열 번호 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 또는 147 중 어느 하나의 뉴클레오티드 서열에 의해 암호화되는, 방법.

청구항 54

제50항에 있어서, 형태형성 유전자는 식물 대사, 기관 발육, 줄기 세포 발육, 세포 성장 자극, 기관형성, 재생, 체세포 배발생, 체세포배 성숙 촉진, 정단 분열조직의 발생 및/또는 발육, 싹 분열조직의 발생 및/또는 발육, 싹의 발생 및/또는 발육, 또는 이들의 조합에 관여하는 유전자 산물을 암호화하는, 방법.

청구항 55

제50항에 있어서, 상기 발현 카세트는 FLP, FLPe, KD, Cre, SSV1, 람다 Int, 파이 C31 Int, HK022, R, B2, B3, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, 또는 U153으로 이루어진 군으로부터 선택되는 부위-특이적 재조합효소를 암호화하는 뉴클레오티드 서열을 포함하는 부위-특이적 재조합효소 카세트를 추가로 포함하고, 부위-특이적 재조합효소는 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터에 작동 가능하게 연결되는, 방법.

청구항 56

제55항에 있어서, 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터는 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, 또는 LEA-D34로 이루어진 군으로부터 선택되는, 방법.

청구항 57

제56항에 있어서, 상기 재조합 발현 카세트로부터 상기 조직-선호적 프로모터 카세트 및 상기 부위-특이적 재조합효소 카세트를 절제하는 단계를 추가로 포함하는, 방법.

청구항 58

제57항의 방법에 의해 제조된 유전자이식 식물.

청구항 59

제58항의 유전자이식 식물의 종자로서, 상기 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 60

제50항에 있어서, 상기 조직-선호적 프로모터 카세트는 제1 T-DNA를 포함하고 상기 형질 유전자 카세트는 제2 T-DNA를 포함하는, 방법.

청구항 61

제60항에 있어서, 제1 T-DNA 및 제2 T-DNA는 식물 세포를 형질전환시키기 위한 동일한 박테리아 균주에 존재하는, 방법.

청구항 62

제61항에 있어서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법.

청구항 63

제62항의 방법에 의해 제조된 유전자이식 식물.

청구항 64

제63항의 유전자이식 식물의 종자로서, 상기 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 65

제60항에 있어서, 제1 T-DNA는 제1 박테리아 균주에 존재하고 제2 T-DNA는 제2 박테리아 균주에 존재하며, 제1 박테리아 균주와 제2 박테리아 균주는 식물 세포를 형질전환시키기 위한 비율로 혼합되는, 방법.

청구항 66

제65항에 있어서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법.

청구항 67

제66항의 방법에 의해 제조된 유전자이식 식물.

청구항 68

제67항의 유전자이식 식물의 종자로서, 상기 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 69

유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서,

재조합 발현 카세트로 쌍떡잎식물 세포 또는 겉씨식물 세포를 형질전환시키는 단계이되,

(a) 조직-선호적 프로모터 카세트(상기 조직-선호적 프로모터 카세트는

(i) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열;

(ii) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열;

(iii) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열;

(iv) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나이고 식물 세포에서 전사를 개시하는 단편 또는 변이체;

(v) 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 100 bp 이상의 단편이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열의 100 bp 이상의 단편

으로 이루어진 군으로부터 선택되는 뉴클레오티드 서열을 포함하고, 식물 세포에서 전사를 개시하는 (i) 내지 (v)의 뉴클레오티드 서열은 WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열에 작동 가능하게 연결됨) 및

(b) 형질 유전자 카세트(상기 형질 유전자 카세트는 영양분 강화, 수확량 증가, 무생물 스트레스 내성, 가뭄 내성, 내한성, 제초제 내성, 해충 저항성, 병원체 저항성, 곤충 저항성, 질소 이용 효율(NUE), 병해 저항성, 또는 대사 경로 변경 능력을 부여하는 유전자 산물을 암호화하는 이종의 관심 폴리뉴클레오티드를 포함함)를 포함하는 재조합 발현 카세트로 쌍떡잎식물 세포 또는 겉씨식물 세포를 형질전환시키는 단계;

각각의 형질전환된 식물 세포에서 상기 재조합 발현 카세트를 발현시켜 체세포배 또는 싹을 형성하는 단계; 및

체세포배를 발아시키거나 싹을 배양하여, 이종의 관심 폴리뉴클레오티드를 포함하는 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물을 형성하는 단계를 포함하는, 방법.

청구항 70

제69항에 있어서, 체세포배 또는 싹은 쌍떡잎식물 세포 또는 겉씨식물 세포의 형질전환 개시 후 약 21일 내지 약 28일 내에 형성되는, 방법.

청구항 71

제69항에 있어서, 겉씨식물 세포는 소나무 및 미송으로 이루어진 군으로부터 선택되는, 방법.

청구항 72

제69항에 있어서, 쌍떡잎식물 세포는 알팔파, 대두, 목화, 해바라기, 아마, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 멜론, 또는 배추속으로 이루어진 군으로부터 선택되는, 방법.

청구항 73

제69항에 있어서, WUS/WOX 호메오박스 폴리펩티드는 서열 번호 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 또는 148 중 어느 하나의 아미노산 서열을 포함하거나, 서열 번호 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 또는 147 중 어느 하나의 뉴클레오티드 서열에 의해 암호화되는, 방법.

청구항 74

제69항에 있어서, WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열은 식물 대사, 기관 발육, 줄기 세포 발육, 세포 성장 자극, 기관형성, 재생, 체세포 배발생, 체세포배 성숙 촉진, 정단 분열조직의 발생 및/또는 발육, 싹 분열조직의 발생 및/또는 발육, 싹의 발생 및/또는 발육, 또는 이들의 조합에 관여하는 유전자 산물을 암호화하는, 방법.

청구항 75

제69항에 있어서, 상기 발현 카세트는 FLP, FLPe, KD, Cre, SSV1, 람다 Int, 파이 C31 Int, HK022, R, B2, B3, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, 또는 U153으로 이루어진 군으로부터 선택되는 부위-특이적 재조합효소를 암호화하는 뉴클레오티드 서열을 포함하는 부위-특이적 재조합효소 카세트를 추가로 포함하고, 부위-특이적 재조합효소는 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터에

작동 가능하게 연결되는, 방법.

청구항 76

제75항에 있어서, 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터는 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, 또는 LEA-D34로 이루어진 군으로부터 선택되는, 방법.

청구항 77

제76항에 있어서, 상기 재조합 발현 카세트로부터 상기 조직-선호적 프로모터 카세트 및 상기 부위-특이적 재조합효소 카세트를 절제하는 단계를 추가로 포함하는, 방법.

청구항 78

제77항의 방법에 의해 제조된 유전자이식 식물.

청구항 79

제78항의 유전자이식 식물의 종자로서, 상기 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 80

제69항에 있어서, 상기 조직-선호적 프로모터 카세트는 제1 T-DNA를 포함하고 상기 형질 유전자 카세트는 제2 T-DNA를 포함하는, 방법.

청구항 81

제80항에 있어서, 제1 T-DNA 및 제2 T-DNA는 식물 세포를 형질전환시키기 위한 동일한 박테리아 균주에 존재하는, 방법.

청구항 82

제81항에 있어서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법.

청구항 83

제82항의 방법에 의해 제조된 유전자이식 식물.

청구항 84

제83항의 유전자이식 식물의 종자로서, 상기 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 85

제80항에 있어서, 제1 T-DNA는 제1 박테리아 균주에 존재하고 제2 T-DNA는 제2 박테리아 균주에 존재하며, 제1 박테리아 균주와 제2 박테리아 균주는 식물 세포를 형질전환시키기 위한 비율로 혼합되는, 방법.

청구항 86

제85항에 있어서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법.

청구항 87

제86항의 방법에 의해 제조된 유전자이식 식물.

청구항 88

제86항의 유전자이식 식물의 종자로서, 상기 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 89

유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서,

(a) 이종의 관심 유전자를 포함하는 형질 유전자 카세트 및 WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열을 포함하는 형태형성 유전자 카세트를 포함하는 재조합 발현 카세트로 쌍떡잎식물 외식편 또는 겉씨식물 외식편의 세포를 형질전환시키는 단계;

(b) 각각의 형질전환된 세포에서 (a)의 재조합 발현 카세트를 발현시켜 체세포배 또는 싹을 형성하는 단계; 및

(c) 체세포배를 발아시키거나 싹을 배양하여 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물을 형성하는 단계를 포함하는 방법.

청구항 90

제89항에 있어서, 체세포배 또는 싹은 세포의 형질전환 개시 후 약 21일 내지 약 28일 내에 형성되는, 방법.

청구항 91

제89항에 있어서, 쌍떡잎식물 또는 겉씨식물은 알팔파, 대두, 목화, 해바라기, 아마, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 소나무, 미송, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 멜론, 또는 배추속으로 이루어진 군으로부터 선택되는, 방법.

청구항 92

제89항에 있어서, WUS/WOX 호메오박스 폴리펩티드는 서열 번호 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 또는 148 중 어느 하나의 아미노산 서열을 포함하거나, 서열 번호 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 또는 147 중 어느 하나의 뉴클레오티드 서열에 의해 암호화되는, 방법.

청구항 93

제89항에 있어서, WUS/WOX 호메오박스 폴리펩티드는 식물 대사, 기관 발육, 줄기 세포 발육, 세포 성장 자극, 기관형성, 재생, 체세포 배발생, 체세포배 성숙 촉진, 정단 분열조직의 발생 및/또는 발육, 싹 분열조직의 발생 및/또는 발육, 싹의 발생 및/또는 발육, 또는 이들의 조합에 관여하는, 방법.

청구항 94

제89항에 있어서, WUS/WOX 호메오박스 폴리펩티드는 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터로 이루어진 군으로부터 선택되는 프로모터에 작동 가능하게 연결되는, 방법.

청구항 95

제94항에 있어서, 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터는 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, LEA-D34, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일한 뉴클레오티드 서열, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일한 뉴클레오티드 서열, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 단편 또는 변이체, 또는 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 100 bp 이상의 단편으로 이루어진 군으로부터 선택되는, 방법.

청구항 96

제89항에 있어서, 이종의 관심 유전자는 영양분 강화, 수확량 증가, 무생물 스트레스 내성, 가뭄 내성, 내한성, 제초제 내성, 해충 저항성, 병원체 저항성, 곤충 저항성, 질소 이용 효율(NUE), 병해 저항성, 또는 대사 경로

변경 능력을 부여하는 유전자 산물을 암호화하는, 방법.

청구항 97

제89항에 있어서, 상기 재조합 발현 카세트는 FLP, FLPe, KD, Cre, SSV1, 람다 Int, 파이 C31 Int, HK022, R, B2, B3, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, 또는 U153으로 이루어진 군으로부터 선택되는 부위-특이적 재조합효소를 암호화하는 뉴클레오티드 서열을 포함하는 부위-특이적 재조합효소 카세트를 추가로 포함하고, 부위-특이적 재조합효소는 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터에 작동 가능하게 연결되는, 방법.

청구항 98

제97항에 있어서, 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터는 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, 또는 LEA-D34로 이루어진 군으로부터 선택되는, 방법.

청구항 99

제98항에 있어서, 상기 재조합 발현 카세트로부터 상기 형태형성 유전자 카세트 및 상기 부위-특이적 재조합효소 카세트를 절제하는 단계를 추가로 포함하는, 방법.

청구항 100

제99항의 방법에 의해 제조된 유전자이식 식물.

청구항 101

제100항의 유전자이식 식물의 종자로서, 상기 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 102

제89항에 있어서, 상기 형태형성 유전자 카세트는 제1 T-DNA를 포함하고 상기 형질 유전자 카세트는 제2 T-DNA를 포함하는, 방법.

청구항 103

제102항에 있어서, 제1 T-DNA 및 제2 T-DNA는 쌍떡잎식물 세포를 형질전환시키기 위한 동일한 박테리아 균주에 존재하는, 방법.

청구항 104

제103항에 있어서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법.

청구항 105

제104항의 방법에 의해 제조된 유전자이식 식물.

청구항 106

제105항의 유전자이식 식물의 종자로서, 상기 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 107

제102항에 있어서, 제1 T-DNA는 제1 박테리아 균주에 존재하고 제2 T-DNA는 제2 박테리아 균주에 존재하며, 제1 박테리아 균주와 제2 박테리아 균주는 식물 세포를 형질전환시키기 위한 비율로 혼합되는, 방법.

청구항 108

제107항에 있어서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법.

청구항 109

제108항의 방법에 의해 제조된 유전자이식 식물.

청구항 110

제109항의 유전자이식 식물의 종자로서, 상기 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자.

청구항 111

제89항에 있어서, 발아시키는 단계는 체세포배 또는 싹을 성숙 배지 또는 발아 배지로 옮겨 유전자이식 식물을 형성하는 단계를 포함하는, 방법.

발명의 설명

기 술 분 야

[0001] 본 발명은 식물 분자 생물학 분야, 보다 구체적으로는 식물의 유전자 발현 조절에 관한 것이다.

[0002] **관련 출원의 상호 참조**

[0003] 본 출원은 2017년 9월 25일 출원된 미국 가출원 62/562,663호의 우선권을 주장하며, 그 전문은 본원에 참조로 포함된다.

[0004] **전자적으로 제출된 서열 목록에 대한 참조**

[0005] 서열 목록의 공식 사본은 2018년 8월 29일 작성되어 본 명세서와 동시에 제출된 995,556 바이트 크기의 파일명 20180829_7453WOPCT_ST25의 ASCII 형식 서열 목록으로서 EFS-웹을 통해 전자적으로 제출되었다. 이 ASCII 형식의 문서에 포함된 서열 목록은 본 명세서의 일부이며, 그 전체가 본원에 참조로 포함된다.

배 경 기 술

[0006] 식물 숙주에서의 이종 DNA 서열의 발현은 식물 숙주 내에서 기능적인 프로모터를 포함해 작동 가능하게 연결되는 프로모터의 존재에 의존적이다. 프로모터 서열의 선택은 유기체 내에서 이종 DNA 서열이 발현되는 시기 및 위치를 결정할 것이다. 특정 조직 또는 기관에서의 발현이 요구되는 경우, 조직-선호적 프로모터가 사용될 수 있다. 자극에 대한 반응으로 유전자 발현이 요구되는 경우, 유도성 프로모터가 조절 요소로서 선택된다. 반면, 식물의 세포 전체에 걸쳐 연속적인 발현이 요구되는 경우, 항시성 프로모터가 사용된다. 유전자이식 식물에서 이종 뉴클레오티드 서열의 다양한 수준의 발현을 유도하기 위해 코어 프로모터 서열로부터의 상류 및/또는 하류의 추가적인 조절 서열이 형질전환 벡터의 발현 구축물에 포함될 수 있다.

[0007] 흔히 식물의 특정 조직 또는 기관에서 DNA 서열을 발현시키는 것이 바람직하다. 예를 들어, 세포 증식을 촉진하는 형태형성 유전자에 작동 가능하게 연결되는 조직-선호적 프로모터의 사용은 형질전환 과정 중 유전자이식 이벤트의 효율적 재생에 유용하다. 이러한 조직-선호적 프로모터는 또한, 원하는 식물 조직에서 형질 유전자 및/또는 병원체 저항성 단백질을 발현시켜 식물 수확량 및 병원체에 대한 저항성을 향상시키는 데 유용하다. 대안적으로, 원하는 표현형을 달성하기 위해 식물 조직 내에서 천연 DNA 서열의 발현을 억제하는 것이 바람직할 수 있다. 이 경우, 이러한 억제는 안티센스 서열의 발현이 천연 DNA 서열의 mRNA의 번역을 방해하는 RNA 전사체를 생성하도록 안티센스 뉴클레오티드 서열에 작동 가능하게 연결되는 조직-선호적 프로모터를 포함하도록 식물을 형질전환시켜 달성될 수 있다.

[0008] 또한, 예를 들어 세포 분열 또는 신장과 같은 특정 성장 또는 발육 단계에 있는 식물 조직에서 DNA 서열을 발현시키는 것이 바람직할 수 있다. 이러한 DNA 서열은 식물 성장 과정을 촉진하거나 억제하는 데 사용되어 식물의 성장 속도 또는 구조에 영향을 미칠 수 있다.

[0009] 따라서, 조직-선호적 프로모터, 특히 아그로박테리움-매개 형질전환 직후 시험관내 성장 및 형태형성을 자극하도록 유전자를 폭발적으로 발현시킨 후, 이소성 과발현이 비정상적인 성장 및 발육을 유발하는 식물 조직에서 감소되고 사실상 "제거"되는, 형태형성 유전자를 포함한 성장 자극 유전자의 제어된 발현을 위한 조절 요소로서 작용할 수 있는 프로모터의 단리 및 특성화가 필요하다.

발명의 내용

[0010] 식물에서 유전자 발현을 조절하기 위한 조성물 및 방법이 제공된다. 보다 구체적으로, 본 발명의 프로모터는 식물 조직의 표피 L1(외층)에서의 조직-선호적 발현을 부여한다. 본 발명의 특정 양태는, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 뉴클레오티드 서열의 단편 또는 변이체이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열의 단편 또는 변이체; 및 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 뉴클레오티드 서열의 100개 이상의 연속 뉴클레오티드이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열의 100개 이상의 연속 뉴클레오티드로 이루어진 군으로부터 선택되는 뉴클레오티드 서열을 갖는 조직-선호적 프로모터를 포함하는 형태형성 유전자 카세트를 포함하는 핵산 분자로서, 조직-선호적 프로모터가 형태형성 유전자에 작동 가능하게 연결되는, 핵산 분자를 포함한다. 형태형성 유전자 카세트를 포함하는 발현 카세트가 또한 포함된다. 형태형성 유전자 카세트를 포함하는 발현 카세트를 포함하는 벡터가 또한 포함된다.

[0011] 또한, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 뉴클레오티드 서열의 단편 또는 변이체이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열의 단편 또는 변이체; 및 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 뉴클레오티드 서열의 100개 이상의 연속 뉴클레오티드이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열의 100개 이상의 연속 뉴클레오티드로 이루어진 군으로부터 선택되는 뉴클레오티드 서열을 갖는 조직-선호적 프로모터를 포함하는 형태형성 유전자 카세트를 포함하는 발현 카세트를 포함하고, 조직-선호적 프로모터가 형태형성 유전자에 작동 가능하게 연결되는, 식물 세포 및 식물이 제공된다. 형태형성 유전자 카세트를 포함하는 발현 카세트를 포함하는 식물 세포 및 식물은 외떡잎식물, 쌍떡잎식물, 및 겉씨식물을 포함한다. 형태형성 유전자 카세트를 포함하는 발현 카세트를 포함하는 식물 세포 및 식물은 외떡잎식물, 쌍떡잎식물, 및 겉씨식물(옥수수, 알팔파, 수수, 벼, 밀렛, 대두, 밀, 목화, 해바라기, 보리, 귀리, 호밀, 아마, 사탕수수, 바나나, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 소나무, 미송, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 대나무, 라이밀, 멜론, 및 배추속을 포함하나, 이에 한정되지 않음)을 포함한다. 본 발명은 또한, 형태형성 유전자 카세트의 형태형성 유전자가 WUS/WOX 호메오박스 폴리펩티드를 암호화하는(WUS/WOX 호메오박스 폴리펩티드는 서열 번호 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 또는 148 중 어느 하나의 아미노산 서열을 포함하거나, 서열 번호 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 또는 147 중 어느 하나의 뉴클레오티드 서열에 의해 암호화됨), 형태형성 유전자 카세트를 포함하는 발현 카세트를 포함하는 식물 세포 또는 식물을 제공한다. 형태형성 유전자 카세트의 형태형성 유전자가 식물 대사, 기관 발육, 줄기 세포 발육, 세포 성장 자극, 기관형성, 재생, 체세포 배발생 개시, 체세포배 성숙 촉진, 정단 분열조직의 발생 및/또는 발육, 싹 분열조직의 발생 및/또는 발육, 싹의 발생 및/또는 발육, 또는 이들의 조합에 관여하는 유전자 산물을 암호화하는, 형태형성 유전자 카세트를 포함하는 발현 카세트를 포함하는 식물 세포 또는 식물이 또한 제공된다.

[0012] 본 발명은 또한, 발현 카세트가 영양분 강화, 수확량 증가, 무생물 스트레스 내성, 가뭄 내성, 내한성, 제초제 내성, 해충 저항성, 병원체 저항성, 곤충 저항성, 질소 이용 효율(NUE), 병해 저항성, 또는 대사 경로 변경 능력을 부여하는 유전자 산물을 암호화하는 이종 폴리뉴클레오티드를 포함하는 형질 유전자 카세트를 추가로 포함하는, 형태형성 유전자 카세트를 포함하는 발현 카세트를 포함하는 식물 세포 및 식물을 제공한다. 본 발명은 또한, 발현 카세트가 영양분 강화, 수확량 증가, 무생물 스트레스 내성, 가뭄 내성, 내한성, 제초제 내성, 해충 저항성, 병원체 저항성, 곤충 저항성, 질소 이용 효율(NUE), 병해 저항성, 또는 대사 경로 변경 능력을 부여하는 유전자 산물을 암호화하는 이종 폴리뉴클레오티드를 포함하는 형질 유전자 카세트, 및 FLP, FLPe, KD, Cre, SSV1, 람다 Int, 파이 C31 Int, HK022, R, B2, B3, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, 또는 U153으로 이루어진 군으로부터 선택되는 부위-특이적 재조합효소를 암호화하는 뉴클레오티드 서열을 포함하는

부위-특이적 재조합효소 카세트를 추가로 포함하고, 부위-특이적 재조합효소가 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터에 작동 가능하게 연결되는, 형태형성 유전자 카세트를 포함하는 발현 카세트를 포함하는 식물 세포 및 식물을 제공한다. UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, 또는 LEA-D34로 이루어진 군으로부터 선택되는 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 및 발육 조절 프로모터가 또한 제공된다. 발현 카세트의 형태형성 유전자 카세트 및 부위-특이적 재조합효소 카세트가 식물 세포 및 식물에서 일시적으로 발현되고, 발현 카세트의 형질 유전자 카세트가 식물 세포 및 식물의 게놈에 안정적으로 통합되는, 식물 세포 및 식물이 또한 제공된다. 발현 카세트의 형태형성 유전자 카세트 및 부위-특이적 재조합효소 카세트가 식물 세포 및 식물로부터 절제되고, 발현 카세트의 형질 유전자 카세트가 식물 세포 및 식물의 게놈에 안정적으로 통합되는, 식물 세포 및 식물이 또한 제공된다. 발현 카세트의 형질 유전자 카세트를 포함하는 식물 종자가 또한 제공된다.

[0013] 본 발명은 또한, 식물 또는 식물 세포에서 전사를 개시할 수 있는 뉴클레오티드 서열을 포함하는 재조합 폴리뉴클레오티드를 포함하는 발현 카세트로서, 뉴클레오티드 서열이 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나로 이루어진 군으로부터 선택되는 뉴클레오티드 서열의 100개 이상의 연속 뉴클레오티드를 가지며, 전사를 개시할 수 있는 뉴클레오티드 서열이 형태형성 유전자에 작동 가능하게 연결되는, 발현 카세트를 제공한다.

[0014] 식물 또는 식물 세포에서 전사를 개시할 수 있는 기능적 단편 또는 변이체를 포함하는 재조합 폴리뉴클레오티드를 포함하는 발현 카세트로서, 기능적 단편 또는 변이체가 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나로 이루어진 군으로부터 선택되는 뉴클레오티드 서열로부터 유도되고, 전사를 개시할 수 있는 기능적 단편 또는 변이체가 형태형성 유전자에 작동 가능하게 연결되는, 발현 카세트가 또한 제공된다.

[0015] 본 발명은 또한, 식물 또는 식물 세포에서 전사를 개시할 수 있는 뉴클레오티드 서열을 포함하는 재조합 폴리뉴클레오티드를 포함하는 발현 카세트로서, 뉴클레오티드 서열이 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나로 이루어진 군으로부터 선택되는 뉴클레오티드 서열과 적어도 70%의 동일성을 가지며, 전사를 개시할 수 있는 뉴클레오티드 서열이 형태형성 유전자에 작동 가능하게 연결되는, 발현 카세트를 제공한다.

[0016] 식물 또는 식물 세포에서 전사를 개시할 수 있는 뉴클레오티드 서열을 포함하는 재조합 폴리뉴클레오티드를 포함하는 발현 카세트로서, 뉴클레오티드 서열이 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나로 이루어진 군으로부터 선택되는 뉴클레오티드 서열과 적어도 95%의 동일성을 가지며, 전사를 개시할 수 있는 뉴클레오티드 서열이 형태형성 유전자에 작동 가능하게 연결되는, 발현 카세트가 또한 제공된다.

[0017] 식물 또는 식물 세포에서 전사를 개시할 수 있는 뉴클레오티드 서열을 포함하는 재조합 폴리뉴클레오티드를 포함하는 발현 카세트로서, 뉴클레오티드 서열이 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나로 이루어진 군으로부터 선택되고, 전사를 개시할 수 있는 뉴클레오티드 서열이 형태형성 유전자에 작동 가능하게 연결되는, 발현 카세트가 또한 제공된다.

[0018] 유전자이식 식물의 제조 방법으로서, (a) 조직-선호적 프로모터 카세트, 및 (b) 영양분 강화, 수확량 증가, 무생물 스트레스 내성, 가뭄 내성, 내한성, 제초제 내성, 해충 저항성, 병원체 저항성, 곤충 저항성, 질소 이용 효율(NUE), 병해 저항성, 또는 대사 경로 변경 능력을 부여하는 유전자 산물을 암호화하는 이종의 관심 폴리뉴클레오티드를 포함하는 형질 유전자 카세트를 포함하는 재조합 발현 카세트로 식물 세포를 형질전환시키는 단계(조직-선호적 프로모터 카세트는, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 단편 또는 변이체이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 또는 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 100 bp 이상의 단편이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열로 이루어진 군으로부터 선택되는 뉴클레오티드 서열을 포함하고, 뉴클레오티드 서열은 형태형성 유전자에 작동 가능하게 연결됨); 재조합 발현 카세트를 갖는 유전자이식 식물 세포를 선별하는 단계; 및 유전자이식 식물 세포로부터 유전자이식 식물을 재생시키는 단계를 포함하는 방법이 또한 제공된다.

[0019] 본 발명의 유전자이식 식물 제조 방법에 유용한 외떡잎식물 세포, 쌍떡잎식물 세포, 및 겉씨식물 세포가 또한 제공된다. 본 발명의 방법에 유용한 식물 세포는 외떡잎식물, 쌍떡잎식물, 및 겉씨식물(옥수수, 알팔파, 수수, 벼, 밀렛, 대두, 밀, 목화, 해바라기, 보리, 귀리, 호밀, 아마, 사탕수수, 바나나, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 소나무, 미송, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 대나무, 라이밀, 멜론, 및 배추속을 포함하나, 이에 한정되지 않음)을 포함한다.

[0020] 본 발명은 또한, 유전자이식 식물의 제조 방법으로서, 본원에 개시된 조직-선호적 프로모터 카세트를 포함하는 재조합 발현 카세트로 식물 세포를 형질전환시키는 단계를 포함하고, 형태형성 유전자가 WUS/WOX 호메오박스 폴리펩티드를 암호화하는(WUS/WOX 호메오박스 폴리펩티드는 서열 번호 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 또는 148 중 어느 하나의 아미노산 서열을 포함하거나, 서열 번호 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 또는 147 중 어느 하나의 뉴클레오티드 서열에 의해 암호화됨), 방법을 제공한다. 유전자이식 식물의 제조 방법으로서, 본원에 개시된 조직-선호적 프로모터 카세트를 포함하는 재조합 발현 카세트로 식물 세포를 형질전환시키는 단계를 포함하고, 형태형성 유전자가 식물 대사, 기관 발육, 줄기 세포 발육, 세포 성장 자극, 기관형성, 재생, 체세포 배발생 개시, 체세포배 성숙 촉진, 정단 분열조직의 발생 및/또는 발육, 싹 분열조직의 발생 및/또는 발육, 싹의 발생 및/또는 발육, 또는 이들의 조합에 관여하는 유전자 산물을 암호화하는, 방법이 또한 제공된다.

[0021] 유전자이식 식물의 제조 방법으로서, 본원에 개시된 조직-선호적 프로모터 카세트를 포함하는 재조합 발현 카세트로 식물 세포를 형질전환시키는 단계를 포함하고, 재조합 발현 카세트가 FLP, FLPe, KD, Cre, SSV1, 람다 Int, 파이 C31 Int, HK022, R, B2, B3, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, 또는 U153으로 이루어진 군으로부터 선택되는 부위-특이적 재조합효소를 암호화하는 뉴클레오티드 서열을 포함하는 부위-특이적 재조합효소 카세트를 추가로 포함하고, 부위-특이적 재조합효소가 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터에 작동 가능하게 연결되는, 방법이 또한 제공된다. 유전자이식 식물의 제조 방법으로서, 본원에 개시된 조직-선호적 프로모터 카세트 및 부위-특이적 재조합효소 카세트를 포함하는 재조합 발현 카세트로 식물 세포를 형질전환시키는 단계를 포함하고, 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터가 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, 또는 LEA-D34로 이루어진 군으로부터 선택되는, 방법이 또한 제공된다.

[0022] 유전자이식 식물의 제조 방법으로서, 재조합 발현 카세트로부터 조직-선호적 프로모터 카세트 및 부위-특이적 재조합효소 카세트를 절제하는 단계를 추가로 포함하는 방법이 또한 제공된다. 본원에 개시된 방법에 의해 제조되는 유전자이식 식물이 또한 제공된다. 본원에 개시된 방법에 의해 제조된 유전자이식 식물로부터 생성되고, 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자가 또한 제공된다.

[0023] 유전자이식 식물의 제조 방법으로서, 조직-선호적 프로모터 카세트가 제1 T-DNA를 포함하고 형질 유전자 카세트가 제2 T-DNA를 포함하는, 방법이 또한 제공된다. 유전자이식 식물의 제조 방법으로서, 제1 T-DNA 및 제2 T-DNA가 식물 세포를 형질전환시키기 위한 동일한 박테리아 균주에 존재하는, 방법이 또한 제공된다. 유전자이식 식물의 제조 방법으로서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법이 또한 제공된다. 이렇게 제조된 유전자이식 식물이 또한 제공된다. 이렇게 제조된 유전자이식 식물의 종자로서, 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자가 또한 제공된다.

[0024] 유전자이식 식물의 제조 방법으로서, 제1 T-DNA가 제1 박테리아 균주에 존재하고 제2 T-DNA가 제2 박테리아 균주에 존재하며, 제1 박테리아 균주와 제2 박테리아 균주가 식물 세포를 형질전환시키기 위한 비율로 혼합되는, 방법이 또한 제공된다. 유전자이식 식물의 제조 방법으로서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법이 또한 제공된다. 이렇게 제조된 유전자이식 식물이 또한 제공된다. 이렇게 제조된 유전자이식 식물의 종자로서, 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자가 또한 제공된다.

[0025] 체세포배 성숙 효율을 향상시키는 방법으로서, (a) 조직-선호적 프로모터 카세트, 및 (b) 영양분 강화, 수확량 증가, 무생물 스트레스 내성, 가뭄 내성, 내한성, 제초제 내성, 해충 저항성, 병원체 저항성, 곤충 저항성, 질소 이용 효율(NUE), 병해 저항성, 또는 대사 경로 변경 능력을 부여하는 유전자 산물을 암호화하는 이종의 관심

폴리뉴클레오티드를 포함하는 형질 유전자 카세트를 포함하는 재조합 발현 카세트로 식물 세포를 형질전환시키는 단계(조직-선호적 프로모터 카세트는, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 단편 또는 변이체이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 또는 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 100 bp 이상의 단편이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열로 이루어진 군으로부터 선택되는 뉴클레오티드 서열을 포함하고, 뉴클레오티드 서열은 형태형성 유전자에 작동 가능하게 연결됨); 재조합 발현 카세트를 갖는 유전자이식 식물 세포를 선별하는 단계; 및 유전자이식 식물 세포로부터 유전자이식 식물을 재생시키는 단계를 포함하고, 재조합 발현 카세트로 인해 재조합 발현 카세트를 포함하지 않는 유전자이식 식물 세포에 비해 체세포배 성숙 효율이 향상되는, 방법.

[0026] 본 발명의 체세포배 성숙 효율을 향상시키는 방법에 유용한 외떡잎식물 세포, 쌍떡잎식물 세포, 및 겉씨식물 세포가 또한 제공된다. 본 발명의 방법에 유용한 식물 세포는 외떡잎식물, 쌍떡잎식물, 및 겉씨식물(옥수수, 알팔파, 수수, 벼, 밀렛, 대두, 밀, 목화, 해바라기, 보리, 귀리, 호밀, 아마, 사탕수수, 바나나, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 소나무, 미송, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 대나무, 라이밀, 멜론, 및 배추속을 포함하나, 이에 한정되지 않음)을 포함한다.

[0027] 본 발명은 또한, 체세포배 성숙 효율을 향상시키는 방법으로서, 본원에 개시된 조직-선호적 프로모터 카세트를 포함하는 재조합 발현 카세트로 식물 세포를 형질전환시키는 단계를 포함하고, 형태형성 유전자가 WUS/WOX 호메오박스 폴리펩티드를 암호화하는(WUS/WOX 호메오박스 폴리펩티드는 서열 번호 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 또는 148 중 어느 하나의 아미노산 서열을 포함하거나, 서열 번호 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 또는 147 중 어느 하나의 뉴클레오티드 서열에 의해 암호화됨), 방법을 제공한다. 체세포배 성숙 효율을 향상시키는 방법으로서, 본원에 개시된 조직-선호적 프로모터 카세트를 포함하는 재조합 발현 카세트로 식물 세포를 형질전환시키는 단계를 포함하고, 형태형성 유전자가 식물 대사, 기관 발육, 줄기 세포 발육, 세포 성장 자극, 기관형성, 재생, 체세포 배발생 개시, 체세포배 성숙 촉진, 정단 분열조직의 발생 및/또는 발육, 싹 분열조직의 발생 및/또는 발육, 싹의 발생 및/또는 발육, 또는 이들의 조합에 관여하는 유전자 산물을 암호화하는, 방법이 또한 제공된다.

[0028] 체세포배 성숙 효율을 향상시키는 방법으로서, 본원에 개시된 조직-선호적 프로모터 카세트를 포함하는 재조합 발현 카세트로 식물 세포를 형질전환시키는 단계를 포함하고, 재조합 발현 카세트가 FLP, FLPe, KD, Cre, SSV1, 람다 Int, 파이 C31 Int, HK022, R, B2, B3, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, 또는 U153으로 이루어진 군으로부터 선택되는 부위-특이적 재조합효소를 암호화하는 뉴클레오티드 서열을 포함하는 부위-특이적 재조합효소 카세트를 추가로 포함하고, 부위-특이적 재조합효소가 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터에 작동 가능하게 연결되는, 방법이 또한 제공된다. 체세포배 성숙 효율을 향상시키는 방법으로서, 본원에 개시된 조직-선호적 프로모터 카세트 및 부위-특이적 재조합효소 카세트를 포함하는 재조합 발현 카세트로 식물 세포를 형질전환시키는 단계를 포함하고, 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터가 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, 또는 LEA-D34로 이루어진 군으로부터 선택되는, 방법이 또한 제공된다.

[0029] 체세포배 성숙 효율을 향상시키는 방법으로서, 재조합 발현 카세트로부터 조직-선호적 프로모터 카세트 및 부위-특이적 재조합효소 카세트를 절제하는 단계를 추가로 포함하는 방법이 또한 제공된다. 본원에 개시된 방법에 의해 제조되는 유전자이식 식물이 또한 제공된다. 본원에 개시된 방법에 의해 제조된 유전자이식 식물로부터 생성되고, 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자가 또한 제공된다.

[0030] 체세포배 성숙 효율을 향상시키는 방법으로서, 조직-선호적 프로모터 카세트가 제1 T-DNA를 포함하고 형질 유전자 카세트가 제2 T-DNA를 포함하는, 방법이 또한 제공된다. 체세포배 성숙 효율을 향상시키는 방법으로서, 제1

T-DNA 및 제2 T-DNA가 식물 세포를 형질전환시키기 위한 동일한 박테리아 균주에 존재하는, 방법이 또한 제공된다. 체세포배 성숙 효율을 향상시키는 방법으로서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법이 또한 제공된다. 이렇게 제조된 유전자이식 식물이 또한 제공된다. 이렇게 제조된 유전자이식 식물의 종자로서, 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자가 또한 제공된다.

[0031] 체세포배 성숙 효율을 향상시키는 방법으로서, 제1 T-DNA가 제1 박테리아 균주에 존재하고 제2 T-DNA가 제2 박테리아 균주에 존재하며, 제1 박테리아 균주와 제2 박테리아 균주가 식물 세포를 형질전환시키기 위한 비율로 혼합되는, 방법이 또한 제공된다. 체세포배 성숙 효율을 향상시키는 방법으로서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법이 또한 제공된다. 이렇게 제조된 유전자이식 식물이 또한 제공된다. 이렇게 제조된 유전자이식 식물의 종자로서, 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자가 또한 제공된다.

[0032] 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, (a) 조직-선호적 프로모터 카세트, 및 (b) 영양분 강화, 수확량 증가, 무생물 스트레스 내성, 가뭄 내성, 내한성, 제초제 내성, 해충 저항성, 병원체 저항성, 곤충 저항성, 질소 이용 효율(NUE), 병해 저항성, 또는 대사 경로 변경 능력을 부여하는 유전자 산물을 암호화하는 이종의 관심 폴리뉴클레오티드를 포함하는 형질 유전자 카세트를 포함하는 재조합 발현 카세트로 쌍떡잎식물 세포 또는 겉씨식물 세포를 형질전환시키는 단계(조직-선호적 프로모터 카세트는, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 단편 또는 변이체이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 또는 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 100 bp 이상의 단편이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열로 이루어진 군으로부터 선택되는 뉴클레오티드 서열을 포함하고, 식물세포에서 전사를 개시하는 뉴클레오티드 서열은 WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열에 작동 가능하게 연결됨); 각각의 형질전환된 식물 세포에서 재조합 발현 카세트를 발현시켜 체세포배 또는 싹을 형성하는 단계; 및 체세포배를 발아시키거나 싹을 배양하여, 이종의 관심 폴리뉴클레오티드를 포함하는 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물을 형성하는 단계를 포함하는 방법이 또한 제공된다. 개시된 방법은 또한, 쌍떡잎식물 세포 또는 겉씨식물 세포의 형질전환 개시 후 약 21일 내지 약 28일 내에 체세포배 또는 싹의 형성을 제공한다.

[0033] 본 발명의 유전자이식 식물 제조 방법에 유용한 겉씨식물 세포가 또한 제공된다. 본 발명의 방법에 유용한 겉씨식물 세포(소나무 및 미송을 포함하나, 이에 한정되지 않음)가 또한 제공된다. 본 발명의 유전자이식 식물 제조 방법에 유용한 쌍떡잎식물 세포가 또한 제공된다. 본 발명의 방법에 유용한 쌍떡잎식물 세포(알팔파, 대두, 목화, 해바라기, 아마, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 멜론, 또는 배추속을 포함하나, 이에 한정되지 않음)가 또한 제공된다.

[0034] 본 발명은 또한, 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 본원에 개시된 조직-선호적 프로모터 카세트를 포함하는 재조합 발현 카세트로 쌍떡잎식물 세포 또는 겉씨식물 세포를 형질전환시키는 단계를 포함하고, WUS/WOX 호메오박스 폴리펩티드가 서열 번호 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 또는 148 중 어느 하나의 아미노산 서열을 포함하거나, WUS/WOX 호메오박스 폴리펩티드가 서열 번호 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 또는 147 중 어느 하나의 뉴클레오티드 서열에 의해 암호화되는, 방법을 제공한다. 유전자이식 식물의 제조 방법으로서, 본원에 개시된 조직-선호적 프로모터 카세트를 포함하는 재조합 발현 카세트로 식물 세포를 형질전환시키는 단계를 포함하고, WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열이 식물 대사, 기관 발육, 줄기 세포 발육, 세포 성장 자극, 기관형성, 재생, 체세포 배발생 개시, 체세포배 성숙 촉진, 정단 분열조직의 발생 및/또는 발육, 싹 분열조직의 발생 및/또는 발육, 싹의 발생 및/또는 발육, 또는 이들의 조합에 관여하는 유전자 산물을 암호화하는, 방법이 또한 제공된다.

[0035] 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 본원에 개시된 조직-선호적 프로모터 카세트를 포함하는 재조합 발현 카세트로 쌍떡잎식물 세포 또는 겉씨식물 세포를 형질전환시키는 단계를 포함하고, 재조합 발현 카세트가 FLP, FLPe, KD, Cre, SSV1, 람다 Int, 파이 C31 Int, HK022, R, B2, B3, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, 또는 U153으로 이루어진 군으로부터 선택되는 부위-특이적 재조

합효소를 암호화하는 뉴클레오티드 서열을 포함하는 부위-특이적 재조합효소 카세트를 추가로 포함하고, 부위-특이적 재조합효소가 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터에 작동 가능하게 연결되는, 방법이 또한 제공된다. 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 본원에 개시된 조직-선호적 프로모터 카세트 및 부위-특이적 재조합효소 카세트를 포함하는 재조합 발현 카세트로 쌍떡잎식물 세포 또는 겉씨식물 세포를 형질전환시키는 단계를 포함하고, 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터가 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, 또는 LEA-D34로 이루어진 군으로부터 선택되는, 방법이 또한 제공된다.

[0036] 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 재조합 발현 카세트로부터 조직-선호적 프로모터 카세트 및 부위-특이적 재조합효소 카세트를 절제하는 단계를 추가로 포함하는 방법이 또한 제공된다. 본원에 개시된 방법에 의해 제조되는 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물이 또한 제공된다. 본원에 개시된 방법에 의해 제조된 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물로부터 생성되고, 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자가 또한 제공된다.

[0037] 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 조직-선호적 프로모터 카세트가 제1 T-DNA를 포함하고 형질 유전자 카세트가 제2 T-DNA를 포함하는, 방법이 또한 제공된다. 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 제1 T-DNA 및 제2 T-DNA가 식물 세포를 형질전환시키기 위한 동일한 박테리아 균주에 존재하는, 방법이 또한 제공된다. 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법이 또한 제공된다. 이렇게 제조된 유전자이식 식물이 또한 제공된다. 이렇게 제조된 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 종자로서, 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자가 또한 제공된다.

[0038] 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 제1 T-DNA가 제1 박테리아 균주에 존재하고 제2 T-DNA가 제2 박테리아 균주에 존재하며, 제1 박테리아 균주와 제2 박테리아 균주가 쌍떡잎식물 세포 또는 겉씨식물 세포를 형질전환시키기 위한 비율로 혼합되는, 방법이 또한 제공된다. 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법이 또한 제공된다. 이렇게 제조된 유전자이식 식물이 또한 제공된다. 이렇게 제조된 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 종자로서, 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자가 또한 제공된다.

[0039] 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, (a) 이종의 관심 유전자를 포함하는 형질 유전자 카세트 및 WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열을 포함하는 형태형성 유전자 카세트를 포함하는 재조합 발현 카세트로 쌍떡잎식물 외식편 또는 겉씨식물 외식편의 세포를 형질전환시키는 단계; (b) 각각의 형질전환된 세포에서 (a)의 재조합 발현 카세트를 발현시켜 체세포배 또는 싹을 형성하는 단계; 및 (c) 체세포배를 발아시키거나 싹을 배양하여 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물을 형성하는 단계를 포함하는 방법이 또한 제공된다. 개시된 방법은 또한, 쌍떡잎식물 세포 또는 겉씨식물 세포의 형질전환 개시 후 약 21일 내지 약 28일 내에 체세포배 또는 싹의 형성을 제공한다.

[0040] 본 발명의 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물 제조 방법에 유용한 쌍떡잎식물 세포 및 겉씨식물 세포가 또한 제공된다. 본 발명의 방법에 유용한 식물 세포는 쌍떡잎식물 및 겉씨식물(알팔파, 대두, 목화, 해바라기, 아마, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 소나무, 미송, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 멜론, 또는 배추속을 포함하나, 이에 한정되지 않음)을 포함한다.

[0041] 본 발명은 또한, 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, WUS/WOX 호메오박스 폴리펩티드가 서열 번호 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 또는 148 중 어느 하나의 아미노산 서열을 포함하거나, WUS/WOX 호메오박스 폴리펩티드가 서열 번호 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 또는 147 중 어느 하나의 뉴클레오티드 서열에 의해 암호화되는, 방법을 제공한다. 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, WUS/WOX 호메오박스 폴리펩티드가 식물 대사, 기관 발육,

줄기 세포 발육, 세포 성장 자극, 기관형성, 재생, 체세포 배발생 개시, 체세포배 성숙 촉진, 정단 분열조직의 발생 및/또는 발육, 싹 분열조직의 발생 및/또는 발육, 싹의 발생 및/또는 발육, 또는 이들의 조합에 관여하는 유전자 산물을 암호화하는 뉴클레오티드에 의해 암호화되는, 방법이 또한 제공된다.

[0042] 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, WUS/WOX 호메오박스 폴리펩티드가 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터로 이루어진 군으로부터 선택되는 프로모터에 작동 가능하게 연결되는, 방법이 또한 제공된다. 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터는 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, LEA-D34, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일한 뉴클레오티드 서열, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일한 뉴클레오티드 서열, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 단편 또는 변이체, 또는 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 100 bp 이상의 단편으로 이루어진 군으로부터 선택되는, 방법이 또한 제공된다.

[0043] 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 이종의 관심 폴리뉴클레오티드가 영양분 강화, 수확량 증가, 무생물 스트레스 내성, 가뭄 내성, 내한성, 제초제 내성, 해충 저항성, 병원체 저항성, 곤충 저항성, 질소 이용 효율(NUE), 병해 저항성, 또는 대사 경로 변경 능력을 부여하는 유전자 산물을 암호화하는, 방법이 또한 제공된다.

[0044] 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 재조합 발현 카세트는 FLP, FLPe, KD, Cre, SSV1, 람다 Int, 파이 C31 Int, HK022, R, B2, B3, Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, 또는 U153으로 이루어진 군으로부터 선택되는 부위-특이적 재조합효소를 암호화하는 뉴클레오티드 서열을 포함하는 부위-특이적 재조합효소 카세트를 추가로 포함하고, 부위-특이적 재조합효소가 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터에 작동 가능하게 연결되는, 방법이 또한 제공된다. 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터는 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, 또는 LEA-D34로 이루어진 군으로부터 선택되는, 방법이 또한 제공된다.

[0045] 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 재조합 발현 카세트로부터 형태형성 유전자 카세트 및 부위-특이적 재조합효소 카세트를 절제하는 단계를 추가로 포함하는 방법이 또한 제공된다. 본원에 개시된 방법에 의해 제조되는 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물이 또한 제공된다. 본원에 개시된 방법에 의해 제조된 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물로부터 생성되고, 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자가 또한 제공된다.

[0046] 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 형태형성 유전자 카세트가 제1 T-DNA를 포함하고 형질 유전자 카세트가 제2 T-DNA를 포함하는, 방법이 또한 제공된다. 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 제1 T-DNA 및 제2 T-DNA가 식물 세포를 형질전환시키기 위한 동일한 박테리아 균주에 존재하는, 방법이 또한 제공된다. 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법이 또한 제공된다. 이렇게 제조된 유전자이식 쌍떡잎식물 및 유전자이식 겉씨식물이 또한 제공된다. 이렇게 제조된 유전자이식 쌍떡잎식물 및 유전자이식 겉씨식물의 종자로서, 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자가 또한 제공된다.

[0047] 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 제1 T-DNA가 제1 박테리아 균주에 존재하고 제2 T-DNA가 제2 박테리아 균주에 존재하며, 제1 박테리아 균주와 제2 박테리아 균주가 식물 세포를 형질전환시키기 위한 비율로 혼합되는, 방법이 또한 제공된다. 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 제2 T-DNA로부터 제1 T-DNA를 분리시키는 단계를 추가로 포함하는 방법이 또한 제공된다. 이렇

게 제조된 유전자이식 식물이 또한 제공된다. 이렇게 제조된 유전자이식 쌍떡잎식물 및 유전자이식 겉씨식물의 종자로서, 재조합 발현 카세트의 형질 유전자 카세트를 포함하는 종자가 또한 제공된다.

[0048] 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물의 제조 방법으로서, 발아시키는 단계가 체세포배 또는 싹을 성숙 배지 또는 발아 배지로 옮겨 유전자이식 쌍떡잎식물 또는 유전자이식 겉씨식물을 형성하는 단계를 포함하는, 방법이 또한 제공된다.

도면의 간단한 설명

[0049] 도 1은 WUS 발현(HBS2 (PHP80734; 서열 번호 113), HBS3(PHP81343; 서열 번호 116), LTP3(PHP80730; 서열 번호 112), MATE1(PHP80736; 서열 번호 114) 및 NED1(PHP81060; 서열 번호 115))을 유도하는 프로모터 GM-HBSTART2(HBS2; 서열 번호 108), GM-HBSTART3(HBS3; 서열 번호 1), GM-LTP3(LTP3; 서열 번호 124), GM-MATE1(MATE1; 서열 번호 109), GM-NED1(NED1; 서열 번호 110), 및 TAG-RFP 대조군(WUS 유전자 없음)(PHP80728; 서열 번호 111)의 체세포배 성숙 효율 평균을 나타낸다.

도 2의 A, 도 2의 B, 및 도 2의 C는 백색광에서 찍은 사진이다. 도 2의 D, 도 2의 E, 및 도의 F는 유전자이식 이벤트를 나타내기 위해 형광에서 찍은 사진이다. 도 2의 A, 도 2의 B, 도 2의 C, 도 2의 D, 도 2의 E, 및 도 2의 F는 동시(아그로박테리움 감염 후 5~6주차)에 찍은 사진이다. TAG-RFP 대조군 처리(대조군 TAG-RFP; PHP80728; 서열 번호 111)인 도 2의 A 및 도 2의 D에 나타낸 체세포배는 작고 발육부전을 보인다. LTP3 PRO::WUS(PHP80730; 서열 번호 112) 처리인 도 2의 B 및 도 2의 E에 나타낸 체세포배는 TAG-RFP 대조군 처리에서보다 약 2배 더 크다. HBS3 PRO::WUS(PHP81343; 서열 번호 116) 처리인 도 2의 C 및 도 2의 F에 나타낸 체세포배는 TAG-RFP 대조군 처리에서보다 약 5배 더 크다.

도 3a 및 도 3b는 GM-HBS3 PRO::WUS 이벤트의 정상적인 발육 및 종자 세트를 보여준다.

발명을 실시하기 위한 구체적인 내용

[0050] 전부는 아니지만 일부 가능한 양태가 도시된 첨부 도면을 참조하여 이하 본 발명을 보다 상세하게 설명한다. 실제, 본 발명은 여러 다양한 형태로 구현될 수 있으며, 본 발명이 본원에 제시된 양태로 제한되는 것으로 해석되어서는 안 되고, 오히려 이들 양태는 본 발명이 해당되는 법적 요건을 충족하도록 제공된다.

[0051] 하기 설명 및 관련 도면에 제시된 교시의 이점을 가지며 개시된 조성물 및 방법과 관련된 당업자는 본원에 개시된 많은 변형예 및 다른 양태를 인식할 것이다. 따라서, 본 발명이 개시된 특정 양태로 제한되는 것은 아니며 변형예 및 다른 양태도 첨부된 청구범위의 범주 내에 포함되는 것으로 이해되어야 한다. 본원에서 특정 용어가 사용되지만, 이들은 일반적이고 설명적인 의미로만 사용되고 제한의 목적으로 사용되지 않는다.

[0052] 또한, 본원에 사용된 용어는 단지 특정 양태를 설명하기 위한 것이고 제한적 의미가 아닌 것으로 이해되어야 한다. 본 명세서 및 청구범위에 사용된 "포함하는"이란 용어는 "~로 이루어진"의 양태를 포함할 수 있다. 달리 정의되지 않는 한, 본원에 사용된 모든 기술 용어 및 과학 용어는 개시된 조성물 및 방법이 속하는 기술 분야의 당업자가 일반적으로 이해하는 것과 동일한 의미를 갖는다. 본 명세서 및 하기 청구범위에서, 본원에 정의될 다수의 용어가 참조될 것이다.

[0053] 재생 가능한 식물 구조는 완전히 기능적인 가임 식물을 형성할 수 있는 다세포 구조(예컨대, 이에 한정되는 것은 아니지만, 싹 분열조직, 싹, 체세포배, 배발생 캘러스, 체세포 분열조직, 및/또는 기관형성 캘러스)로서 정의된다.

[0054] 체세포배는 구형 및 전이기 배아의 형성, 배축과 배반의 형성, 및 지질과 전분의 축적을 포함한 접합배의 발육과 유사한 발육기를 통해 진행되는 다세포 구조로서 정의된다. 접합배에서 유래된 단일 체세포배는 발아하여, 원래 단일 세포에서 유래될 수 있는 단일 비키메라 식물을 생성한다.

[0055] 배발생 캘러스는 성숙한 가임 식물로 재생될 수 있는 증식하는 1차 및 2차 체세포배와 마주하는 미분화 또는 부분 미분화 세포의 이쇄성 또는 비이쇄성 혼합물로서 정의된다.

[0056] 체세포 분열조직은, 원시세포로 플랭킹되고 혈관 시원세포와 마주하는 미분화 정단 돔을 갖는 것을 특징으로 하는, 종자-유래 배아의 일부인 정단 분열조직과 유사한 다세포 구조로서 정의되며, 정점 돔은 지상 생장 식물을 발생시킨다. 이러한 체세포 분열조직은 단일의 또는 융합된 클러스터의 분열조직을 형성할 수 있다.

[0057] 기관형성 캘러스는 분화된 성장 식물 구조(정단 분열조직, 뿌리 분열조직, 잎, 및 뿌리를 포함하나, 이에 한정

되지 않음)의 치밀한 혼합물로서 정의된다.

[0058] 발아는 계속 성장하여 식물을 생성하는 묘목을 형성하기 위한 재생 가능한 구조의 성장이다.

[0059] 유전자이식 식물은 이식유전자를 함유하는 성숙한 가임 식물로서 정의된다.

[0060] 본 발명은 형태형성 유전자에 작동 가능하게 연결되는 조직-선호적 프로모터를 포함하는 핵산 분자가 얻어지는 조성물과 방법, 및 이들의 이용 방법에 관한 것이다. 본 발명의 핵산 분자 조성물은 형태형성 유전자에 작동 가능하게 연결되는 GM-HBSTART3(서열 번호 1), GM-HBSTART3(TRUNCATED)(서열 번호 2), AT-ML1(서열 번호 3), GM-ML1-유사(서열 번호 4), GM-ML1-유사(TRUNCATED)(서열 번호 5), ZM-HBSTART3(서열 번호 6), OS-HBSTART3(서열 번호 7), AT-PDF1 P2(서열 번호 8), GM-PDF1(서열 번호 9), GM-PDF1(TRUNCATED)(서열 번호 10), SB-PDF1(서열 번호 11), OS-PDF1(서열 번호 12), OS-PDF1(TRUNCATED)(서열 번호 13), PT-PDF1(서열 번호 14), PT-PDF1(TRUNCATED)(서열 번호 15), SI-PDF1(서열 번호 16), SI-PDF1(TRUNCATED)(서열 번호 17), AT-PDF2(서열 번호 18), GM-PDF2(서열 번호 19), GM-PDF2(TRUNCATED)(서열 번호 20), ZM-GL1(서열 번호 21), AT-PDF2a(서열 번호 22), AT-PDF2a(TRUNCATED)(서열 번호 23), GM-PDF2a(서열 번호 24), GM-PDF2a(TRUNCATED)(서열 번호 25), OS-PDF2(서열 번호 26), OS-PDF2(TRUNCATED)(서열 번호 27), PT-PDF2(서열 번호 28), PT-PDF2(TRUNCATED)(서열 번호 29), VV-PDF2(서열 번호 30), VV-PDF2(TRUNCATED)(서열 번호 31), ZM-PDF2(서열 번호 32), SI-PDF2(서열 번호 33), SI-PDF2(TRUNCATED)(서열 번호 34), VV-PDF2a(서열 번호 35), PT-PDF2a(서열 번호 36), PT-PDF2a(TRUNCATED)(서열 번호 37), MT-PDF2(서열 번호 38), MT-PDF2(TRUNCATED)(서열 번호 39), AT-HDG2(서열 번호 40), GM-HDG2(서열 번호 41), GM-HDG2(TRUNCATED)(서열 번호 42), SB-HDG2(서열 번호 43), SB-HDG2(TRUNCATED)(서열 번호 44), AT-CER6(서열 번호 45), AT-CER60(서열 번호 46), AT-CER60(TRUNCATED)(서열 번호 47), GM-CER6(서열 번호 48), GM-CER6(TRUNCATED)(서열 번호 49), PT-CER6(서열 번호 50), PT-CER6(TRUNCATED)(서열 번호 51), VV-CER6(서열 번호 52), VV-CER6(TRUNCATED)(서열 번호 53), SB-CER6(서열 번호 54), ZM-CER6(서열 번호 55), SI-CER6(서열 번호 56), SI-CER6(TRUNCATED)(서열 번호 57), OS-CER6(서열 번호 58), OS-CER6(TRUNCATED)(서열 번호 59), GM-HBSTART2(서열 번호 108), GM-MATE1(서열 번호 109), GM-NED1(서열 번호 110), GM-LTP3(서열 번호 124), AT-ML1(TRUNCATED)(서열 번호 125), AT-CER6(TRUNCATED1)(서열 번호 126), AT-PDF1(TRUNCATED)(서열 번호 149), AT-PDF2(TRUNCATED)(서열 번호 150), AT-HDG2(TRUNCATED)(서열 번호 151), AT-ANL2(서열 번호 152), 및 AT-CER6(TRUNCATED2)(서열 번호 189)으로 알려진 조직-선호적 프로모터에 대한 뉴클레오티드 서열을 포함한다. 본 발명은 형태형성 유전자에 작동 가능하게 연결되는 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189에 제시된 뉴클레오티드 서열 중 적어도 하나와 이의 단편 및 변이체를 포함하는 핵산 분자를 제공한다. 조성물은 형태형성 유전자에 작동 가능하게 연결되는 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189에 제시된 뉴클레오티드 서열 중 적어도 하나의 뉴클레오티드 서열을 포함하는 핵산 분자를 포함하는 발현 카세트, DNA 구축물, 및 벡터를 추가로 포함한다.

[0061] 본원에 사용된 용어 "본원에 개시된 조직-선호적 프로모터"는, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 뉴클레오티드 서열의 단편 또는 변이체이고 식물 세포에서 전사를 개시하는 단편 또는 변이체; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 뉴클레오티드 서열의 100개 이상의 연속 뉴클레오티드이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열의 100개 이상의 연속 뉴클레오티드; 및 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 뉴클레오티드 서열의 100 bp 이상의 단편이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열의 100 bp 이상의 단편으로 이루어진 군으로부터 선택되는 뉴클레오티드 서열을 의미한다.

[0062] 본원에 사용된 용어 "형태형성 유전자"는 이소성 발현시 식물을 생성할 수 있는 체세포-유래 구조의 형성을 자극하는 유전자를 의미한다. 보다 정확하게, 형태형성 유전자의 이소성 발현은 식물을 생성할 수 있는 체세포배 또는 싹 분열조직과 같은 기관형성 구조의 드노보 형성을 자극한다. 이러한 자극된 드노보 형성은 형태형성 유전자가 발현되는 세포, 또는 인접 세포에서 일어난다. 형태형성 유전자는 다른 유전자, 또는 식물 조직의 호르몬 수준에 영향을 미치는 유전자의 발현을 조절하는 전사 인자일 수 있으며, 이들 유전자는 모두 형태형성적 변화를 자극할 수 있다. 형태형성 유전자는 식물의 게놈에 안정적으로 통합되거나, 일시적으로 발현될 수 있다. 본 발명의 조직-선호적 프로모터는 식물 대사, 기관 발육, 줄기 세포 발육, 세포 성장 자극, 기관형성, 재생, 체세포 배발생 개시, 체세포배 성숙 촉진, 정단 분열조직의 발생 및/또는 발육, 싹 분열조직의 발생 및/또는 발

육, 싹의 발생 및/또는 발육, 또는 이들의 조합에 관여하는 형태형성 유전자, 예컨대 WUS/WOX 유전자(WUS1, WUS2, WUS3, WOX2A, WOX4, WOX5, 또는 WOX9)를 발현시키는 데 사용된다(미국 특허 7,348,468 및 7,256,322, 미국 특허 출원 공보 20170121722 및 20070271628; Laux et al. (1996) Development 122:87-96; Mayer et al. (1998) Cell 95:805-815; van der Graaff et al., 2009, Genome Biology 10:248; Dolzblasz et al., 2016, Mol. Plant 19:1028-39 참조). WUS/WOX의 조절은 식물 대사, 기관 발육, 줄기 세포 발육, 세포 성장 자극, 기관형성, 재생, 체세포 배발생 개시, 체세포배 성숙 촉진, 정단 분열조직의 발생 및/또는 발육, 싹 분열조직의 발생 및/또는 발육, 싹의 발생 및/또는 발육, 또는 이들의 조합 등, 식물 및/또는 식물 조직 표현형을 조절할 것으로 예상된다. 애기장대 WUS의 발현은 체세포배로 분화될 수 있는 생장 조직에서 줄기 세포를 유도할 수 있다(Zuo, et al. (2002) Plant J 30:349-359). 또한, 이와 관련하여 MYB118 유전자(미국 특허 7,148,402 참조), MYB115 유전자(Wang et al. (2008) Cell Research 224-235 참조), BABYBOOM 유전자(BBM; Boutilier et al. (2002) Plant Cell 14:1737-1749 참조), 또는 CLAVATA 유전자(예를 들어, 미국 특허 7,179,963 참조)가 관심의 대상이다.

[0063] 본 발명에 유용한 형태형성 유전자는 본원에 개시된 것뿐만 아니라 당업계에 알려진 WUS/WOX 유전자를 포함하나, 이에 한정되지 않는다. 형태형성 유전자는 AT-WUS(서열 번호 60), LJ-W(서열 번호 62), GM-W(서열 번호 64), CS-WUS(서열 번호 66), CR-WUS(서열 번호 68), AA-WUS(서열 번호 70), RS-WUS(서열 번호 72), BN-WUS(서열 번호 74), BO-WU(서열 번호 76), HA-WUS(서열 번호 78), PT-WUS(서열 번호 80), VV-WUS(서열 번호 82), AT-WUS(대두 최적화)(서열 번호 84), LJ-WUS(대두 최적화)(서열 번호 86), MT-WUS(대두 최적화)(서열 번호 88), PY-WUS(대두 최적화)(서열 번호 90), PV-WUS(대두 최적화)(서열 번호 92), ZM-WUS1(서열 번호 94), ZM-WUS2(서열 번호 96), ZM-WUS3(서열 번호 98), ZM-WOX2A(서열 번호 100), ZM-WOX4(서열 번호 102), ZM-WOX5A(서열 번호 104), ZM-WOX9(서열 번호 106), GG- WUS(서열 번호 127), MD-WUS(서열 번호 129), ME-WUS(서열 번호 131), KF-WUS(서열 번호 133), GH-WUS(서열 번호 135), ZOSMA-WUS(서열 번호 137), AMBTR-WUS(서열 번호 139), AC-WUS(서열 번호 141), AH-WUS(서열 번호 143), CUCSA-WUS(서열 번호 145), 및 PINTA-WUS(서열 번호 147) 등, 본원에 개시된 WUS/WOX 유전자를 포함하나, 이에 한정되지 않는다.

[0064] 본원에 개시된 WUS/WOX 유전자는 AT-WUS(서열 번호 61), LJ-W(서열 번호 63), GM-W(서열 번호 65), CS-WUS(서열 번호 67), CR-WUS(서열 번호 69), AA-WUS(서열 번호 71), RS-WUS(서열 번호 73), BN-WUS(서열 번호 75), BO-WU(서열 번호 77), HA-WUS(서열 번호 79), PT-WUS(서열 번호 81), VV-WUS(서열 번호 83), AT-WUS(서열 번호 85), LJ-WUS(서열 번호 87), MT-WUS(서열 번호 89), PY-WUS(서열 번호 91), PV-WUS(서열 번호 93), ZM-WUS1(서열 번호 95), ZM-WUS2(서열 번호 97), ZM-WUS3(서열 번호 99), ZM-WOX2A(서열 번호 101), ZM-WOX4(서열 번호 103), ZM-WOX5A(서열 번호 105), ZM-WOX9(서열 번호 107), GG- WUS(서열 번호 128), MD-WUS(서열 번호 130), ME-WUS(서열 번호 132), KF-WUS(서열 번호 134), GH-WUS(서열 번호 136), ZOSMA-WUS(서열 번호 138), AMBTR-WUS(서열 번호 130), AC-WUS(서열 번호 142), AH-WUS(서열 번호 144), CUCSA-WUS(서열 번호 146), 및 PINTA-WUS(서열 번호 148)를 포함하는 WUS/WOX 호메오박스 폴리펩티드를 암호화한다.

[0065] 본원에 개시된 WUS/WOX 유전자는 WUS/WOX 호메오박스 폴리펩티드를 암호화하는 것들을 포함하며, WUS/WOX 호메오박스 폴리펩티드는 서열 번호 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 또는 148 중 어느 하나의 아미노산 서열을 포함하거나, WUS/WOX 호메오박스 폴리펩티드는 서열 번호 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 또는 147 중 어느 하나의 뉴클레오티드 서열에 의해 암호화된다.

[0066] 본 발명에 유용한 다른 형태형성 유전자는 LEC1(Lotan et al., 1998, Cell 93:1195-1205), LEC2(Stone et al., 2008, PNAS 105:3151-3156; Belide et al., 2013, Plant Cell Tiss. Organ Cult 113:543-553), KN1/STM(Sinha et al., 1993. Genes Dev 7:787-795), 아그로박테리움의 IPT 유전자(Ebinuma and Komamine, 2001, In vitro Cell. Dev Biol - Plant 37:103-113), 모노프테로스-델타(Ckurshumova et al., 2014, New Phytol. 204:556-566), 아그로박테리움 AV-6b 유전자(Wabiko and Minemura 1996, Plant Physiol. 112:939-951), 아그로박테리움 IAA-h 및 IAA-m 유전자의 조합(Endo et al., 2002, Plant Cell Rep., 20:923-928), 애기장대 SERK 유전자(Hecht et al., 2001, Plant Physiol. 127:803-816), 애기장대 AGL15 유전자(Harding et al., 2003, Plant Physiol. 133:653-663)를 포함하나, 이에 한정되지 않는다.

[0067] 본원에 사용된 용어 "전사 인자"는 프로모터의 DNA 서열에 결합하여 발현을 상향 조절하거나 하향 조절함으로써 특정 유전자의 전사 속도를 제어하는 단백질을 의미한다. 형태형성 유전자이기도 한 전사 인자의 예는 AP2/EREBP 계열의 멤버(BBM(ODP2), plethora 및 aintegumenta 하위계열, CAAT-박스 결합 단백질, 예컨대 LEC1

및 HAP3 포함), 그리고 MYB, bHLH, NAC, MADS, bZIP 및 WRKY 계열의 멤버를 포함한다.

[0068] 일 양태에서, 재조합 발현 카세트 또는 구축물은 WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열을 포함한다. 다양한 양태에서, WUS/WOX 호메오박스를 암호화하는 뉴클레오티드 서열의 발현은 형질전환의 개시 후 약 21일 내지 약 28일 동안 일어난다.

[0069] 일 양태에서, WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열은 부위-특이적 재조합효소에 의한 절제의 표적이 될 수 있다. 따라서, WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열의 발현은 형질전환 후 원하는 시기에 절제에 의해 제어될 수 있다. 부위-특이적 재조합효소를 사용하여 WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열의 발현을 제어하는 경우, 발현 구축물은 절제될 폴리뉴클레오티드 서열을 플랭킹하는 적절한 부위-특이적 절제 부위(예를 들어, Cre 재조합효소가 사용될 경우 Cre lox 부위)를 포함하는 것으로 이해된다. WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열을 포함하는 발현 구축물의 동일 위치에 부위-특이적 재조합효소가 존재할 필요는 없다. 그러나, 일 양태에서, 발현 구축물은 부위-특이적 재조합효소를 암호화하는 뉴클레오티드 서열을 추가로 포함한다.

[0070] WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열의 발현을 제어하는 데 사용되는 부위-특이적 재조합효소는 다양한 적합한 부위-특이적 재조합효소로부터 선택될 수 있다. 예를 들어, 다양한 양태에서, 부위-특이적 재조합효소는 FLP, FLPe, KD, Cre, SSV1, 람다 Int, 파이 C31 Int, HK022, R, B2(Nern et al., (2011) PNAS Vol. 108, No. 34 pp 14198-14203), B3(Nern et al., (2011) PNAS Vol. 108, No. 34 pp 14198-14203), Gin, Tn1721, CinH, ParA, Tn5053, Bxb1, TP907-1, 또는 U153이다. 부위-특이적 재조합효소는 불안정화된 융합 폴리펩티드일 수 있다. 불안정화된 융합 폴리펩티드는 TETR(G17A)~CRE 또는 ESR(G17A)~CRE일 수 있다.

[0071] 일 양태에서, 부위-특이적 재조합효소를 암호화하는 뉴클레오티드 서열은 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 또는 발육 조절 프로모터에 작동 가능하게 연결된다. 적합한 항시성 프로모터, 유도성 프로모터, 조직-특이적 프로모터, 및 발육 조절 프로모터는 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO (ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1, DR5, XVE, GLB1, OLE, LTP2(Kalla et al., 1994. Plant J. 6:849-860 및 US5525716), HSP17.7, HSP26, HSP18A, AT-HSP811, AT-HSP811L, GM-HSP173B, 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, 또는 LEA-D34를 포함한다.

[0072] 일 양태에서, 부위-특이적 재조합효소에 작동 가능하게 연결되는 화학 유도성 프로모터는 XVE이다. 화학 유도성 프로모터는 테트라사이클린 리프레서(TETR), 에타메트설푸론 리프레서(ESR), 또는 클로르설푸론 리프레서(CR)에 의해 억제될 수 있고, 테트라사이클린-관련 또는 설포닐우레아 리간드의 첨가시 탈억제가 일어난다. 리프레서는 TETR일 수 있고, 테트라사이클린-관련 리간드는 독시사이클린 또는 무수테트라사이클린이다(Gatz, C., Frohberg, C. and Wendenburg, R. (1992) Stringent repression and homogeneous de-repression by tetracycline of a modified CaMV 35S promoter in intact transgenic tobacco plants, Plant J. 2, 397-404).

[0073] 대안적으로, 리프레서는 ESR일 수 있고, 설포닐우레아 리간드는 에타메트설푸론, 클로르설푸론, 메트설푸론-메틸, 설포메투론 메틸, 클로리무론 에틸, 니코설푸론, 프리미설푸론, 트리베누론, 설포설푸론, 트리플록시설푸론, 포람설푸론, 요오도설푸론, 프로설푸론, 티펜설푸론, 림설푸론, 메소설푸론, 또는 할로설푸론이다(본원에 전문이 참조로 포함되는 US20110287936).

[0074] 일 양태에서, 발현 구축물이 부위-특이적 재조합효소 절제 부위를 포함하는 경우, WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열은 옥신 유도성 프로모터, 발육 조절 프로모터, 조직-특이적 프로모터, 또는 항시성 프로모터에 작동 가능하게 연결될 수 있다. 이와 관련하여 유용한 예시적 옥신 유도성 프로모터, 발육 조절 프로모터, 조직-특이적 프로모터, 및 항시성 프로모터는 UBI, LLDAV, EVCV, DMMV, BSV(AY) PRO, CYMV PRO FL, UBIZM PRO, SI-UB3 PRO, SB-UBI PRO(ALT1), USB1ZM PRO, ZM-GOS2 PRO, ZM-H1B PRO(1.2 KB), IN2-2, NOS, 35S의 135 버전, ZM-ADF PRO(ALT2), AXIG1(본원에 전문이 참조로 포함되는 US 6,838,593), DR5, XVE, GLB1, OLE, LTP2, HSP17.7, HSP26, HSP18A, AT-HSP811(Takahashi, T, et al., (1992) Plant Physiol. 99 (2): 383-390), AT-HSP811L(Takahashi, T, et al., (1992) Plant Physiol. 99 (2): 383-390), GM-HSP173B(Schöffl, F., et al. (1984) EMBO J. 3(11): 2491-2497), 테트라사이클린, 에타메트설푸론, 또는 클로르설푸론에 의해 활성화된 프로모터, PLTP, PLTP1, PLTP2, PLTP3, SDR, LGL, LEA-14A, LEA-D34, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나, 서열 번호 1~59, 108~110, 124~126, 149~152, 및

189 중 적어도 하나와 적어도 95% 동일한 뉴클레오티드 서열, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일한 뉴클레오티드 서열, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 단편 또는 변이체, 또는 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 100 bp 이상의 단편을 포함한다.

[0075] WUS/WOX 호메오박스 폴리펩티드를 암호화하는 뉴클레오티드 서열의 발현에 적절한 지속 시간은 본원에 개시된 조직-선호적 프로모터(서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일한 뉴클레오티드 서열, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일한 뉴클레오티드 서열, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 단편 또는 변이체, 또는 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 100 bp 이상의 단편을 포함하나, 이에 한정되지 않음)를 사용함으로써 달성될 수 있다. 유전자이식 식물을 제조하기 위해 형태형성 유전자 카세트 및 형질 유전자 카세트를 사용하는 경우, 형질 유전자만을 함유하는 유전자이식 식물을 제공하기 위해 공동 형질전환 식물의 형질 유전자좌로부터 형태형성 유전자좌를 분리하는 능력을 갖는 것이 바람직하다. 이는 아그로박테리움 투메파시엔스 2 T-DNA 바이너리 시스템을 이용하여 달성될 수 있으며, 이 일반적인 주제에는 두 가지 변형예가 있다(Miller et al., 2002 참조). 예를 들어, 첫 번째 변형예는, 형태형성 유전자 및 제초제 선택(즉, HRA)을 위한 발현 카세트가 제1 T-DNA 내에 들어있고 형질 유전자 카세트가 제2 T-DNA 내에 들어있는 2 T-DNA 벡터이고, 두 T-DNA는 모두 하나의 바이너리 벡터에 존재한다. 2 T-DNA 플라스미드를 함유하는 아그로박테리움에 의해 식물 세포가 형질전환되는 경우, 형질전환된 세포의 많은 퍼센트가 서로 다른 게놈 위치(예를 들어, 서로 다른 염색체)에 통합된 두 T-DNA를 모두 포함한다. 두 번째 방법에서는, 예를 들어 각각 두 T-DNA 중 하나(형태형성 유전자 T-DNA 또는 형질 유전자 T-DNA)를 함유하는 2개의 아그로박테리움 균주가 일정 비율로 혼합되고, 그 혼합물이 형질전환에 사용된다. 이러한 혼합 아그로박테리움 방법을 이용한 형질전환 후, 재생된 유전자이식 이벤트가 보통 별개의 게놈 위치에서 두 T-DNA를 모두 함유하는 경우가 높은 빈도로 관찰된다. 두 공동 형질전환 방법 모두에 대해, 생성된 유전자이식 이벤트의 많은 비율에서, 2개의 T-DNA 좌위가 독립적으로 분리되고, T-DNA 좌위가 서로 분리된 자손 T1 식물이 쉽게 확인될 수 있으며, 결과적으로 형태형성 유전자/제초제 유전자가 없는 형질 유전자 함유 자손 종자가 재생되는 것으로 관찰된다. 문헌[Miller et al. Transgenic Res 11(4):381-96] 참조.

[0076] 본원에 제공된 방법은 관심 뉴클레오티드 서열이 통합된 재생 가능한 식물 세포를 생성하기 위해 박테리아-매개 및/또는 유전자총-매개 유전자 전달의 사용을 필요로 한다. 본 발명의 방법에 유용한 박테리아 균주는 병원성이 제거된(disarmed) *Agrobacteria*, *Ochrobactrum* 박테리아, 또는 *Rhizobiaceae* 박테리아를 포함하나, 이에 한정되지 않는다. 일 양태에서, 상이한 박테리아 균주는 (i) 병원성이 제거된 *Agrobacteria*와 *Ochrobactrum* 박테리아, (ii) 병원성이 제거된 *Agrobacteria*와 *Rhizobiaceae* 박테리아, 및 (iii) *Rhizobiaceae* 박테리아와 *Ochrobactrum* 박테리아로부터 선택된다.

[0077] 본 발명의 방법에 유용한, 병원성이 제거된 *Agrobacteria*는 AGL-1, EHA105, GV3101, LBA4404, 및 LBA4404 THY-를 포함하나, 이에 한정되지 않는다.

[0078] 본 발명의 방법에 유용한 *Ochrobactrum* 박테리아 균주는 *Ochrobactrum haywardense* H1 NRRL Deposit B-67078, *Ochrobactrum cytisi*, *Ochrobactrum daejeonense*, *Ochrobactrum oryzae*, *Ochrobactrum tritici* LBNL124-A-10, HTG3-C-07, *Ochrobactrum pecoris*, *Ochrobactrum ciceri*, *Ochrobactrum gallinifaecis*, *Ochrobactrum grignonense*, *Ochrobactrum guangzhouense*, *Ochrobactrum haematophilum*, *Ochrobactrum intermedium*, *Ochrobactrum lupini*, *Ochrobactrum pituitosum*, *Ochrobactrum pseudintermedium*, *Ochrobactrum pseudogrignonense*, *Ochrobactrum rhizosphaerae*, *Ochrobactrum thiophenivorans*, 및 *Ochrobactrum tritici*를 포함하나, 이에 한정되지 않는다.

[0079] 본 발명의 방법에 유용한 *Rhizobiaceae* 박테리아 균주는 *Rhizobium lusitanum*, *Rhizobium rhizogenes*, *Agrobacterium rubi*, *Rhizobium multihospitium*, *Rhizobium tropici*, *Rhizobium miluonense*, *Rhizobium leguminosarum*, *Rhizobium leguminosarum* bv. *trifolii*, *Rhizobium leguminosarum* bv. *phaseoli*, *Rhizobium leguminosarum*. bv. *viciae*, *Rhizobium leguminosarum* Madison, *Rhizobium leguminosarum* USDA2370, *Rhizobium leguminosarum* USDA2408, *Rhizobium leguminosarum* USDA2668, *Rhizobium leguminosarum* 2370G, *Rhizobium leguminosarum* 2370LBA, *Rhizobium leguminosarum* 2048G, *Rhizobium leguminosarum* 2048LBA, *Rhizobium leguminosarum* bv. *phaseoli* 2668G, *Rhizobium leguminosarum* bv. *phaseoli* 2668LBA, *Rhizobium leguminosarum* RL542C, *Rhizobium etli* USDA 9032, *Rhizobium etli* bv. *phaseoli*, *Rhizobium endophyticum*, *Rhizobium tibeticum*, *Rhizobium etli*, *Rhizobium pisi*, *Rhizobium phaseoli*, *Rhizobium fabae*, *Rhizobium*

hainanense, *Arthrobacter viscosus*, *Rhizobium alamii*, *Rhizobium mesosinicum*, *Rhizobium sullae*, *Rhizobium indigoferae*, *Rhizobium gallicum*, *Rhizobium yanglingense*, *Rhizobium mongolense*, *Rhizobium oryzae*, *Rhizobium loessense*, *Rhizobium tubonense*, *Rhizobium cellulosilyticum*, *Rhizobium soli*, *Neorhizobium galegae*, *Neorhizobium vignae*, *Neorhizobium huautlense*, *Neorhizobium alkalisoli*, *Aureimonas altamirensis*, *Aureimonas frigidaquae*, *Aureimonas ureilytica*, *Aurantimonas coralicida*, *Fulvimarina pelagi*, *Martelella mediterranea*, *Allorhizobium undicola*, *Allorhizobium vitis*, *Allorhizobium borbor*, *Beijerinckia fluminensis*, *Agrobacterium larrymoorei*, *Agrobacterium radiobacter*, *Rhizobium selenitireducens corrig*, *Rhizobium rosettiformans*, *Rhizobium daejeonense*, *Rhizobium aggregatum*, *Pararhizobium capsulatum*, *Pararhizobium giardinii*, *Ensifer mexicanus*, *Ensifer terangae*, *Ensifer saheli*, *Ensifer kostiensis*, *Ensifer kummerowiae*, *Ensifer fredii*, *Sinorhizobium americanum*, *Ensifer arboris*, *Ensifer garamanticus*, *Ensifer meliloti*, *Ensifer numidicus*, *Ensifer adhaerens*, *Sinorhizobium* sp., *Sinorhizobium meliloti* SD630, *Sinorhizobium meliloti* USDA1002, *Sinorhizobium fredii* USDA205, *Sinorhizobium fredii* SF542G, *Sinorhizobium fredii* SF4404, 및 *Sinorhizobium fredii SM542C*를 포함하나, 이에 한정되지 않는다. 본원에 전문이 참조로 포함되는 US 9,365,859 참조.

[0080] 일 양태에서, 제1 박테리아 균주와 제2 박테리아 균주는 50:50의 비율로 존재한다. 일 양태에서, 제1 박테리아 균주와 제2 박테리아 균주는 25:75의 비율로 존재한다. 일 양태에서, 제1 박테리아 균주와 제2 박테리아 균주는 10:90의 비율로 존재한다. 일 양태에서, 제1 박테리아 균주와 제2 박테리아 균주는 5:95의 비율로 존재한다. 일 양태에서, 제1 박테리아 균주와 제2 박테리아 균주는 1:99의 비율로 존재한다. 일 양태에서, 제1 박테리아 균주와 제2 박테리아 균주는 상이한 박테리아 균주이다.

[0081] 본 발명의 프로모터는 식물에서 전사의 개시를 가능하게 하는 뉴클레오티드 서열을 포함한다. 특정 양태에서, 프로모터는 조직-선호적 방식으로 전사의 개시를 가능하게 한다. 본 발명의 구축물은 형태형성 유전자에 작동 가능하게 연결되는 본원에 개시된 조직-선호적 프로모터를 포함한다.

[0082] 본원에 개시된 조직-선호적 프로모터는 또한, 관심 식물에서 이종의 폴리뉴클레오티드 또는 관심 폴리뉴클레오티드를 후속 발현시키기 위한 발현 카세트 또는 벡터의 구축물에 사용되거나, 다른 프로모터의 단리를 위한 프로브로서 사용될 수 있다. 본 발명은 형태형성 유전자에 작동 가능하게 연결되는 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나에 제시된 프로모터 뉴클레오티드 서열을 포함(선택적으로, 이종의 폴리뉴클레오티드 또는 관심 폴리뉴클레오티드를 추가로 포함)하는 단리된 DNA 구축물을 제공한다.

[0083] 본 발명의 양태는, 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 95% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나와 적어도 70% 동일하고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 뉴클레오티드 서열의 단편 또는 변이체이고 식물 세포에서 전사를 개시하는 단편 또는 변이체; 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 뉴클레오티드 서열의 100개 이상의 연속 뉴클레오티드이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열의 100개 이상의 연속 뉴클레오티드; 및 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 뉴클레오티드 서열의 100 bp 이상의 단편이고 식물 세포에서 전사를 개시하는 뉴클레오티드 서열의 100 bp 이상의 단편으로 이루어진 군으로부터 선택되는 뉴클레오티드 서열을 갖는 프로모터를 포함(선택적으로, 이종의 폴리뉴클레오티드 또는 관심 폴리뉴클레오티드를 추가로 포함)하는 핵산 분자로서, 프로모터가 형태형성 유전자에 작동 가능하게 연결되는, 핵산 분자를 포함한다. 또한, 핵산을 함유하는 프로모터를 포함하는 발현 카세트, 발현 카세트를 포함하는 벡터, 및 발현 카세트를 포함하는 식물 세포가 구현된다. 다른 양태는 발현 카세트가 일시적으로 발현되거나 식물 세포 또는 식물의 게놈에 안정적으로 통합되는 외떡잎 또는 쌍떡잎 식물 세포 또는 식물, 및 상기 발현 카세트를 포함하는 외떡잎 또는 쌍떡잎 식물을 포함하며, 외떡잎 또는 쌍떡잎 식물 세포 또는 식물은 옥수수, 알팔파, 수수, 벼, 밀렛, 대두, 밀, 목화, 해바라기, 보리, 귀리, 호밀, 아마, 사탕수수, 바나나, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 소나무, 미송, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 대나무, 라이밀, 멜론, 및 배추속을 포함한다.

[0084] 또한, 상기 발현 카세트가 식물의 게놈에 안정적으로 통합된 식물, 발현 카세트를 포함하는 식물 종자가 구현된다. 또한, 이종의 폴리뉴클레오티드 또는 관심 폴리뉴클레오티드의 유전자 또는 유전자 산물이 영양분 강화, 수확량 증가, 무생물 스트레스 내성, 가뭄 내성, 내한성, 제초제 내성, 해충 저항성, 병원체 저항성, 곤충 저항성, 질소 이용 효율(NUE), 병해 저항성, 또는 대사 경로 변경 능력을 부여하는 식물이 구현된다. 이종의 폴

리뉴클레오티드 또는 관심 폴리뉴클레오티드의 발현이 상기 식물의 표현형을 변경하는 식물이 또한 구현된다. 프로모터 활성을 갖는 기능적 단편을 포함하는 재조합 폴리뉴클레오티드를 포함하는 발현 카세트로서, 단편이 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189로 이루어진 군으로부터 선택되는 뉴클레오티드 서열로부터 유도되고, 프로모터 활성을 갖는 기능적 단편이 형태형성 유전자에 작동 가능하게 연결되는, 발현 카세트가 또한 구현된다.

[0085] 본 발명은 단리되거나 실질적으로 정제된 핵산 조성물을 포함한다. "단리" 또는 "정제"된 핵산 분자 또는 이의 생물학적 활성 부분에는, 재조합 기법에 의해 생성될 때 다른 세포 물질 또는 배양 배지가 실질적으로 없거나, 화학적으로 합성될 때 화학적 전구체 또는 다른 화학물질이 실질적으로 없다. "단리된" 핵산에는, 핵산이 유도되는 유기체의 게놈 DNA에서 자연적으로 핵산을 플랭킹하는(즉, 핵산의 5' 말단 및 3' 말단에 위치한) 서열(단백질 암호화 서열 포함)이 실질적으로 없다. 예를 들어, 다양한 양태에서, 단리된 핵산 분자는 핵산이 유도되는 세포의 게놈 DNA에서 자연적으로 핵산 분자를 플랭킹하는 뉴클레오티드 서열을 약 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, 또는 0.1 kb 미만으로 함유할 수 있다. 본 발명의 서열은 각각의 전사 개시 부위를 플랭킹하는 5' 미번역 영역으로부터 단리될 수 있다.

[0086] 개시된 프로모터 뉴클레오티드 서열의 단편 및 변이체도 본 발명에 포함된다. 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 프로모터 서열의 단편 및 변이체가 본 발명의 DNA 구축물에 사용될 수 있다. 본원에 사용된 용어 "단편"은 핵산 서열의 일부를 지칭한다. 프로모터 서열의 단편은 전사를 개시하는, 예컨대 항시적 방식으로 전사를 유도하는 생물학적 활성을 보유한다. 대안적으로, 혼성화 프로브로서 유용한 뉴클레오티드 서열의 단편이 생물학적 활성을 반드시 보유할 필요는 없다. 본원에 개시된 프로모터에 대한 뉴클레오티드 서열의 단편은 적어도 약 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825, 850, 875, 900, 925, 950, 975, 1000, 1025, 1050, 1075, 1100, 1125, 1150, 1175, 1200, 1225, 1250, 1275, 1300, 1325, 1350, 1375, 1400, 1425, 1450, 1475, 1500, 1525, 1550, 1575, 1600, 1625, 1650, 1675, 1700, 1725, 1750, 1775, 1800, 1825, 1850, 1875, 1900, 1925, 1950, 1975, 2000, 2025, 2050, 2075, 2100, 2125, 2150, 2175, 2200, 2225, 2250, 2275, 2300, 2325, 2350, 2375, 2400, 2425, 2450, 2475, 2500, 2525, 2550, 2575, 2600, 2625, 2650, 2675, 2700, 2725, 2750, 2775, 2800, 2825, 2850, 2875, 2900, 2925, 2950, 2975, 3000, 3025, 3050, 3075, 3100, 3125, 3150, 3175, 3200, 3225, 3250, 3275, 3300, 3325, 3350, 3375, 3400, 3425, 3450, 3475, 3500, 3525, 3550, 3575, 3600, 3625, 3650, 3675, 3700, 3725, 3750, 3775, 3800, 3825, 3850, 3875, 3900, 3925, 3950, 3975, 4000, 4025, 4050, 4075, 4100, 4125, 4150, 4175, 4200, 4225, 4250, 4275, 4300, 4325, 4350, 4375, 4400, 4425, 4450, 4475, 4500, 4525, 4550, 4575, 4600, 4625, 4650, 4675, 4700, 4725, 4750, 4775, 4800, 4825, 4850, 4875, 4900, 4925, 4950, 4975, 5000, 5025, 5050, 5075, 5100, 5125, 5150, 5175, 5200, 5225, 5250, 5275, 5300, 5325, 5350, 5375, 5400, 5425, 5450, 5475, 5500, 5525, 5550, 5575, 5600, 5625, 5650, 5675, 5700, 5725, 5750, 5775, 5800, 5825, 5850, 5875, 5900, 5925, 5950, 5975, 6000, 6025, 6050, 6075, 6100, 6125, 6150, 6175, 6200, 또는 6225개의 뉴클레오티드 내지 서열 번호 1~59, 108~110, 124~126, 149~152, 및 189 중 적어도 하나의 전장의 범위일 수 있다. 프로모터의 생물학적 활성 부분은 본 발명의 프로모터 서열의 일부를 단리하고, 그 부분의 전사 활성을 평가함으로써 제조될 수 있다.

[0087] 본원에 사용된 용어 "변이체"는 본원에 개시된 프로모터 서열과 실질적으로 유사한 서열을 의미한다. 변이체는 천연 폴리뉴클레오티드 내의 하나 이상의 내부 부위에서의 하나 이상의 뉴클레오티드의 결실 및/또는 첨가, 및/또는 천연 폴리뉴클레오티드의 하나 이상의 부위에서의 하나 이상의 뉴클레오티드의 치환을 포함한다. 본원에 사용된 바와 같이, "천연" 뉴클레오티드 서열은 자연발생적 뉴클레오티드 서열을 포함한다. 뉴클레오티드 서열의 경우, 자연발생적 변이체는 주지의 분자 생물학 기법을 이용하여, 예를 들어 본원에 요약된 바와 같은 중합효소 연쇄 반응(PCR) 및 혼성화 기법으로 동정될 수 있다.

[0088] 변이체 뉴클레오티드 서열은 또한, 예를 들어 부위-특이적 돌연변이 유도를 이용해 생성된 것과 같은 합성적으로 유도된 뉴클레오티드 서열을 포함한다. 일반적으로, 본원에 개시된 뉴클레오티드 서열의 변이체는 기본 파라미터를 사용해 본원에 기술된 서열 정렬 프로그램으로 결정시, 해당 뉴클레오티드 서열과 적어도 40%, 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, to 95%, 96%, 97%, 98%, 99% 이상의 서열 동일성을 가질 것이다. 본원에 개시된 뉴클레오티드 서열의 생물학적 활성 변이체도 포함된다. 생물학적 활성 변이체는, 예를 들어 하나 이상의 뉴클레오티드 치환, 결실, 또는 삽입을 갖는 본원에 개시된 뉴클레오티드 서열의 천연 프로모터 서열을 포함한다. 프로모터 활성은 노던 블롯 분석, 전사 융합에서 얻은 리포터 활성 측정 등과 같은

기법을 이용해 측정될 수 있다. 예를 들어, 본원에 전문이 참조로 포함되는 문헌[Sambrook, *et al.*, (1989) Molecular Cloning: A Laboratory Manual (2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.)] 참조(이하 "Sambrook"이라 함). 대안적으로, 프로모터 단편 또는 변이체의 제어하에 생성된 녹색 형광 단백질(GFP) 또는 황색 형광 단백질(YFP) 등과 같은 리포터 유전자의 수준이 측정될 수 있다. 예를 들어, 본원에 전문이 참조로 포함되는 문헌[Matz et al. (1999) Nature Biotechnology 17:969-973]; 미국 특허 번호 6,072,050; 문헌[Nagai, *et al.*, (2002) *Nature Biotechnology* 20(1):87-90] 참조. 변이체 뉴클레오티드 서열은 또한, DNA 셔플링과 같은 변이원성 재조합성 절차로부터 유도된 서열을 포함한다. 이러한 절차에서, 프로모터에 대한 하나 이상의 상이한 뉴클레오티드 서열을 조작하여 새로운 프로모터를 생성할 수 있다. 이러한 방식으로, 실질적인 서열 동일성을 가지며 시험관내 또는 생체내에서 상동 재조합될 수 있는 서열 영역을 포함하는 관련 서열 폴리뉴클레오티드의 집단으로부터 재조합 폴리뉴클레오티드의 라이브러리가 생성된다. 이러한 DNA 셔플링 전략은 당업계에 알려져 있다. 예를 들어, 본원에 전문이 참조로 포함되는 문헌[Stemmer, (1994) *Proc. Natl. Acad. Sci. USA* 91:10747-10751; Stemmer, (1994) *Nature* 370:389 391; Crameri, *et al.*, (1997) *Nature Biotech.* 15:436-438; Moore, *et al.*, (1997) *J. Mol. Biol.* 272:336-347; Zhang, *et al.*, (1997) *Proc. Natl. Acad. Sci. USA* 94:4504-4509; Crameri, *et al.*, (1998) *Nature* 391:288-291]과 미국 특허 번호 5,605,793 및 5,837,458 참조.

[0089] 돌연변이 유도 및 뉴클레오티드 서열 변경을 위한 방법은 당업계에 잘 알려져 있다. 예를 들어, 본원에 전문이 참조로 포함되는 문헌[Kunkel, (1985) *Proc. Natl. Acad. Sci. USA* 82:488-492; Kunkel, *et al.*, (1987) *Methods in Enzymol.* 154:367-382]; 미국 특허 번호 4,873,192; 문헌[Walker and Gaastra, eds. (1983) Techniques in Molecular Biology (MacMillan Publishing Company, New York)], 및 거기에 인용된 참고문헌 참조.

[0090] 본 발명의 뉴클레오티드 서열은 다른 유기체, 구체적으로는 다른 식물, 보다 구체적으로는 다른 외떡잎식물 또는 쌍떡잎식물로부터 해당 서열을 단리하는 데 사용될 수 있다. 이러한 방식으로, PCR, 혼성화 등과 같은 방법을 사용해, 본원에 제시된 서열과의 서열 상동성에 기초하여 이러한 서열을 동정할 수 있다. 본원에 제시된 전체 서열 또는 이들의 단편과의 서열 동일성에 기초하여 단리된 서열은 본 발명에 포함된다.

[0091] PCR 접근법에서, 임의의 관심 식물로부터 추출된 cDNA 또는 게놈 DNA로부터 해당 DNA 서열을 증폭시키기 위해 PCR 반응에 사용하기 위한 올리고뉴클레오티드 프라이머가 설계될 수 있다. PCR 프라이머 설계 및 PCR 클로닝 방법은 일반적으로 당업계에 알려져 있으며, 상기 Sambrook에 개시되어 있다. 또한, 본원에 전문이 참조로 포함되는 문헌[Innis, *et al.*, eds. (1990) PCR Protocols: A Guide to Methods and Applications (Academic Press, New York); Innis and Gelfand, eds. (1995) PCR Strategies (Academic Press, New York); 및 Innis and Gelfand, eds. (1999) PCR Methods Manual (Academic Press, New York)] 참조. 공지된 PCR 방법은 쌍을 이룬 프라이머, 내부 프라이머, 단일 특이적 프라이머, 변성 프라이머, 유전자-특이적 프라이머, 벡터-특이적 프라이머, 부분적 불일치 프라이머 등을 이용하는 방법을 포함하나, 이에 한정되지 않는다.

[0092] 혼성화 기법에서는, 알려진 뉴클레오티드 서열의 전부 또는 일부를, 선택된 유기체로부터 클로닝된 게놈 DNA 단편 또는 cDNA 단편의 집단(즉, 게놈 또는 cDNA 라이브러리)에 존재하는 다른 해당 뉴클레오티드 서열에 선택적으로 혼성화하는 프로브로서 사용한다. 혼성화 프로브는 게놈 DNA 단편, cDNA 단편, RNA 단편, 또는 기타 올리고뉴클레오티드일 수 있으며, 검출 가능한 그룹, 예컨대 ^{32}P 또는 임의의 다른 검출 가능한 마커로 표지될 수 있다. 따라서, 예를 들어 혼성화 프로브는 본 발명의 프로모터에 기초한 합성 올리고뉴클레오티드의 표지에 의해 제조될 수 있다. 혼성화 프로브의 제조 및 게놈 라이브러리의 구축 방법은 일반적으로 당업계에 알려져 있으며, 상기 Sambrook에 개시되어 있다.

[0093] 예를 들어, 본원에 개시된 전체 프로모터 서열, 또는 이의 하나 이상의 부분이 해당 프로모터 서열 및 메신저 RNA에 특이적으로 혼성화할 수 있는 프로브로서 사용될 수 있다. 다양한 조건에서 특이적 혼성화를 달성하기 위해, 이러한 프로브는, 프로모터 서열 중에서 고유하고 일반적으로 약 10개 이상의 뉴클레오티드 길이 또는 약 20개 이상의 뉴클레오티드 길이인 서열을 포함한다. 이러한 프로브는 선택된 식물로부터 해당 프로모터 서열을 PCR로 증폭시키는 데 사용될 수 있다. 이 기법은 원하는 유기체로부터 추가의 코딩 서열을 단리하는 데 사용되거나, 유기체에 코딩 서열이 존재하는지 확인하기 위한 진단 분석법으로서 사용될 수 있다. 혼성화 기법은 도말된 DNA 라이브러리의 혼성화 스크리닝을 포함한다(플라크 또는 콜로니, 예를 들어 상기 Sambrook 참조).

[0094] 이러한 서열의 혼성화는 엄격한 조건에서 수행될 수 있다. 용어 "엄격한 조건" 또는 "엄격한 혼성화 조건"은 프로브가 다른 서열보다 표적 서열에 더 검출 가능한 정도(예를 들어, 백그라운드에 비해 적어도 2배)로 혼성화하

는 조건을 의미하는 것이다. 엄격한 조건은 서열 의존적이며 상황에 따라 다를 것이다. 혼성화 및/또는 세척 조건의 엄격성을 제어함으로써, 프로브에 100% 상보적인 표적 서열을 동정할 수 있다(상동성 프로빙). 대안적으로, 엄격 조건은 서열에서 일부 불일치를 허용하여 더 낮은 정도의 유사성이 검출되도록 조정될 수 있다(이종 프로빙). 일반적으로, 프로브는 약 1000개 미만의 뉴클레오티드 길이, 최적으로는 500개 미만의 뉴클레오티드 길이이다.

[0095] 일반적으로, 엄격한 조건은, 염 농도가 pH 7.0 내지 8.3에서 약 1.5 M 미만의 Na 이온, 일반적으로는 약 0.01 내지 1.0 M의 Na 이온 농도(또는 다른 염)이고, 온도가 짧은 프로브(예를 들어, 10 내지 50개의 뉴클레오티드)의 경우 약 30℃ 이상이고 긴 프로브(예를 들어, 50개 초과의 뉴클레오티드)의 경우 약 60℃ 이상인 조건일 것이다. 엄격한 조건은 포름아미드와 같은 불안정화제의 첨가로 달성될 수도 있다. 예시적인 낮은 엄격 조건은 37℃에서 30 내지 35% 포름아미드, 1 M NaCl, 1% SDS(소듐 도데실 설페이트)의 완충액을 이용한 혼성화, 및 50 내지 55℃에서 1배 내지 2배 SSC(20배 SSC=3.0 M NaCl/0.3 M 트리소듐 시트레이트)에서의 세척을 포함한다. 예시적인 적당한 엄격 조건은 37℃에서 40 내지 45% 포름아미드, 1.0 M NaCl, 1% SDS에서의 혼성화, 및 55 내지 60℃에서 0.5배 내지 1배 SSC에서의 세척을 포함한다. 예시적인 높은 엄격 조건은 37℃에서 50% 포름아미드, 1 M NaCl, 1% SDS에서의 혼성화, 및 60 내지 65℃에서 적어도 30분 동안 0.1배 SSC에서의 최종 세척을 포함한다. 혼성화를 위한 시간은 일반적으로 약 24시간 미만, 보통 약 4 내지 약 12시간이다. 세척을 위한 시간은 적어도 평형에 도달하기에 충분한 시간일 것이다.

[0096] 특이성은 일반적으로 혼성화 후 세척의 함수이며, 중요한 인자는 최종 세척액의 이온 강도 및 온도이다. DNA-DNA 하이브리드의 경우, 열 융점(T_m)은 다음의 방정식(Meinkoth and Wahl, (1984) *Anal. Biochem* 138:267 284)으로부터 근사될 수 있다: $T_m = 81.5℃ + 16.6 (\log M) + 0.41 (\% GC) - 0.61 (\% form) - 500/L$; 식에서 M은 1가 양이온의 몰 농도이고, % GC는 DNA 중의 구아노신과 시토신 뉴클레오티드의 백분율이고, % form은 혼성화 용액 중의 포름아미드의 백분율이고, L은 염기쌍에서의 하이브리드의 길이이다. T_m은 상보적인 표적 서열의 50%가 완벽하게 일치하는 프로브에 혼성화되는 (정의된 이온 강도 및 pH에서의) 온도이다. T_m은 1%의 불일치마다 약 1℃ 감소하므로, T_m, 혼성화, 및/또는 세척 조건은 원하는 동일성의 서열에 혼성화되도록 조정될 수 있다. 예를 들어, 90% 동일성을 갖는 서열이 요구되는 경우, T_m은 10℃ 감소될 수 있다. 일반적으로, 엄격한 조건은 정의된 이온 강도 및 pH에서 특정 서열 및 그 보체에 대한 T_m보다 약 5℃ 낮도록 선택된다. 그러나, 매우 엄격한 조건은 T_m보다 1, 2, 3, 또는 4℃ 낮은 온도에서의 혼성화 및/또는 세척을 사용할 수 있고, 적당히 엄격한 조건은 T_m보다 6, 7, 8, 9, 또는 10℃ 낮은 온도에서의 혼성화 및/또는 세척을 사용할 수 있고, 낮은 엄격 조건은 T_m보다 11, 12, 13, 14, 15, 또는 20℃ 낮은 온도에서의 혼성화 및/또는 세척을 사용할 수 있다. 방정식, 혼성화와 세척 조성, 및 원하는 T_m을 사용할 때, 당업자는 혼성화 및/또는 세척액의 엄격성의 변화가 본질적으로 기술되어 있음을 이해할 것이다. 원하는 불일치도에 따라 T_m이 45℃(수용액) 또는 32℃(포름아미드 용액) 미만이 되는 경우, 더 높은 온도가 사용될 수 있도록 SSC 농도를 증가시키는 것이 바람직하다. 핵산의 혼성화에 대한 광범위한 가이드는, 본원에 전문이 참조로 포함되는 문헌[Tijssen, (1993) Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes, Part I, Chapter 2 (Elsevier, New York); 및 Ausubel, *et al.*, eds. (1995) Current Protocols in Molecular Biology, Chapter 2 (Greene Publishing and Wiley-Interscience, New York)]에서 확인된다. 또한, 상기 Sambrook 참조. 따라서, 엄격한 조건에서 본원에 개시된 프로모터 서열 또는 이들의 단편에 혼성화되고 프로모터 활성을 갖는 단리된 서열은 본 발명에 포함된다.

[0097] 일반적으로, 본원에 개시된 프로모터 서열에 혼성화되고 프로모터 활성을 갖는 서열은, 개시된 서열과 적어도 40% 내지 50%의 상동성, 약 60%, 70%, 80%, 85%, 90%, 95% 내지 98% 이상의 상동성을 가질 것이다. 즉, 서열의 서열 유사성은 적어도 약 40% 내지 50%, 약 60% 내지 70%, 및 약 80%, 85%, 90%, 95% 내지 98%의 서열 유사성을 공유하는 범위일 수 있다.

[0098] 다음의 용어들은 둘 이상의 핵산과 폴리뉴클레오티드 간의 서열 관계를 설명하는 데 사용된다: (a) "기준 서열", (b) "비교창", (c) "서열 동일성", (d) "서열 동일성 백분율", 및 (e) "실질적 동일성".

[0099] 본원에 사용된 바와 같이, "기준 서열"은 서열 비교의 기준으로서 사용되는 정의된 서열이다. 기준 서열은, 예를 들어 전장 cDNA 또는 유전자 서열 또는 완전한 cDNA 또는 유전자 서열의 세그먼트로서, 특정 서열의 서브세

트 또는 전체일 수 있다.

[0100] 본원에 사용된 바와 같이, "비교창"은 폴리뉴클레오티드 서열의 연속적인 특정 세그먼트를 지칭하며, 비교창 내의 폴리뉴클레오티드 서열은 (삽입 또는 결실을 포함하지 않는) 기준 서열과 비교하여 두 서열의 최적 정렬을 위한 삽입 또는 결실(즉, 갭)을 포함할 수 있다. 일반적으로, 비교창은 20개 이상의 연속 뉴클레오티드 길이이며, 선택적으로 30, 40, 50, 100개 이상의 길이일 수 있다. 당업자는 폴리뉴클레오티드 서열에 갭이 포함됨으로 인한 기준 서열과의 높은 유사성을 피하기 위해 일반적으로 갭 페널티가 도입되어 일치의 수에서 제외된다는 것을 이해한다.

[0101] 비교를 위한 서열 정렬 방법은 당업계에 잘 알려져 있다. 따라서, 임의의 두 서열 간의 서열 동일성 백분율은 수학적 알고리즘을 사용하여 결정될 수 있다. 이러한 수학적 알고리즘의 비제한적인 예는, 본원에 전문이 참조로 포함되는 문헌[Myers and Miller, (1988) *CABIOS* 4:11-17]의 알고리즘; 문헌[Smith, *et al.*, (1981) *Adv. Appl. Math.* 2:482]의 알고리즘; 문헌[Needleman and Wunsch, (1970) *J. Mol. Biol.* 48:443-453]의 알고리즘; 문헌[Pearson and Lipman, (1988) *Proc. Natl. Acad. Sci.* 85:2444-2448]의 알고리즘; 문헌[Karlin and Altschul, (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877]에서 수정된 문헌[Karlin and Altschul, (1990) *Proc. Natl. Acad. Sci. USA* 872:264]의 알고리즘이다.

[0102] 이러한 수학적 알고리즘의 컴퓨터 구현은 서열 동일성을 결정하기 위해 서열의 비교에 이용될 수 있다. 이러한 구현은 PC/Gene 프로그램의 CLUSTAL(Intelligenetics에서 입수 가능, Mountain View, Calif.); ALIGN 프로그램(버전 2.0) 및 GCG Wisconsin Genetics Software Package®, 버전 10의 GAP, BESTFIT, BLAST, FASTA 및 TFASTA(Accelrys Inc.에서 입수 가능, 9685 Scranton Road, San Diego, Calif., USA)를 포함하나, 이에 한정되지 않는다. 이러한 프로그램을 이용한 정렬은 기본 파라미터를 사용하여 수행될 수 있다. CLUSTAL 프로그램은, 본원에 전문이 참조로 포함되는 문헌[Higgins, *et al.*, (1988) *Gene* 73:237-244; Higgins, *et al.*, (1989) CABIOS 5:151-153; Corpet, *et al.*, (1988) *Nucleic Acids Res.* 16:10881-90; Huang, *et al.*, (1992) *CABIOS* 8:155-65; 및 Pearson, *et al.*, (1994) *Meth. Mol. Biol.* 24:307-331]에 잘 설명되어 있다. ALIGN 프로그램은 상기 문헌[Myers and Miller, (1988)]의 알고리즘을 기반으로 한다. ALIGN 프로그램으로 아미노산 서열을 비교할 때 PAM120 가중치 잔기 표(weight residue table), 12의 갭 길이 페널티, 및 4의 갭 페널티를 사용할 수 있다. 본원에 전문이 참조로 포함되는 문헌[Altschul, *et al.*, (1990) *J. Mol. Biol.* 215:403]의 BLAST 프로그램은 상기 문헌[Karlin and Altschul, (1990)]의 알고리즘을 기반으로 한다. 본 발명의 단백질을 암호화하는 뉴클레오티드 서열에 상동적인 뉴클레오티드 서열을 얻기 위해 BLASTN 프로그램(점수=100, 단어 길이=12)으로 BLAST 뉴클레오티드 검색을 수행할 수 있다. 본 발명의 단백질 또는 폴리펩티드에 상동적인 아미노산 서열을 얻기 위해 BLASTX 프로그램(점수=50, 단어 길이=3)으로 BLAST 단백질 검색을 수행할 수 있다. 비교를 위한 갭 포함 정렬을 얻기 위해, 본원에 전문이 참조로 포함된 문헌[Altschul, *et al.*, (1997) *Nucleic Acids Res.* 25:3389]에 기재된 바와 같이 Gapped BLAST(BLAST 2.0)를 이용할 수 있다. 대안적으로, 분자 사이의 먼 관계를 감지하는 반복 검색을 수행하기 위해 PSI-BLAST(BLAST 2.0)를 사용할 수 있다. 상기 문헌[Altschul, *et al.*, (1997)] 참조. BLAST, Gapped BLAST, PSI-BLAST를 이용할 때, 각각의 프로그램(예를 들어, 뉴클레오티드 서열의 경우 BLASTN, 단백질의 경우 BLASTX)의 기본 파라미터를 사용할 수 있다. 미국 국립생물공학정보센터의 웹 사이트 ncbi.nlm.nih.gov 참조. 정렬은 검사에 의해 수동으로 수행될 수도 있다.

[0103] 달리 명시되지 않는 한, 본원에 제공된 서열 동일성/유사성 값은 하기 파라미터를 사용하여, GAP 버전 10을 이용해 얻은 값을 지칭한다. 50의 갭 가중치와 3의 길이 가중치를 사용한 뉴클레오티드 서열에 대한 동일성%와 유사성%, 및 nwsgapdna.cmp 점수 매트릭스; 8의 가중치 및 2의 길이 가중치를 사용한 아미노산 서열에 대한 동일성%와 유사성%, 및 BLOSUM62 점수 매트릭스; 또는 이들의 임의의 동등한 프로그램. 본원에 사용된 바와 같이, "동등한 프로그램"은 문제가 되는 임의의 두 서열에 대해, GAP 버전 10에 의해 생성된 해당 정렬과 비교할 때 동일한 뉴클레오티드 또는 아미노산 잔기 일치 및 동일한 서열 동일성 백분율을 갖는 정렬을 생성하는 임의의 서열 비교 프로그램이다.

[0104] GAP 프로그램은 상기 문헌[Needleman and Wunsch]의 알고리즘을 사용하여, 일치의 수를 최대화하고 갭의 수를 최소화하는 2개의 완전한 서열의 정렬을 찾는다. GAP는 가능성 있는 모든 정렬 및 갭 위치를 고려하여, 일치 염기의 수가 가장 많고 갭이 가장 적은 정렬을 생성한다. 이는 일치 염기의 단위에서 갭 생성 페널티 및 갭 확장 페널티를 제공할 수 있다. GAP는 삽입되는 각각의 갭에 대해 일치의 갭 생성 페널티 수의 이득을 만들어야 한다. 0보다 큰 갭 확장 페널티가 선택되는 경우, GAP는 삽입되는 각각의 갭에 대해 추가로 갭의 길이와 갭 확장 페널티의 곱의 이득을 만들어야 한다. 단백질 서열에 대한 GCG Wisconsin Genetics Software Package®의 버전 10에서의 기본 갭 생성 페널티 값과 갭 확장 페널티 값은 각각 8과 2이다. 뉴클레오티드 서열의 경우, 기

본 갭 확장 페널티는 3인데 반해 기본 갭 생성 페널티는 50이다. 갭 생성 및 갭 확장 페널티는 0 내지 200으로 이루어진 정수 군으로부터 선택되는 정수로 표현될 수 있다. 따라서, 예를 들어 갭 생성 및 갭 확장 페널티는 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 이상일 수 있다.

[0105] GAP는 최적 정렬 계열 중 하나의 멤버를 나타낸다. 이 계열의 멤버가 많을 수 있지만 더 좋은 품질을 갖는 다른 멤버는 없다. GAP는 정렬을 위한 4가지 성능 지수인 품질, 비율, 동일성, 및 유사성을 표시한다. 품질은 서열을 정렬하기 위해 최대화되는 측정 기준이다. 비율은 품질을 더 짧은 세그먼트에서의 염기의 수로 나눈 값이다. 동일성 백분율은 실제로 일치하는 기호의 백분율이다. 유사성 백분율은 실제로 유사한 기호의 백분율이다. 갭의 맞은편에 있는 기호는 무시된다. 한 쌍의 기호에 대한 점수 매트릭스 값이 유사성 임계값인 0.50 이상이면 유사성 점수가 매겨진다. GCG Wisconsin Genetics Software Package®의 버전 10에 사용되는 점수 매트릭스는 BLOSUM62이다(본원에 전문이 참조로 포함되는 문헌[Henikoff and Henikoff, (1989) *Proc. Natl. Acad. Sci. USA* 89:10915] 참조).

[0106] 본원에 사용된 바와 같이, 2개의 핵산 또는 폴리펩티드 서열과 관련하여 "서열 동일성" 또는 "동일성"은 특정 비교창에서 최대 상응도로 정렬되는 경우 동일한 두 서열 내의 잔기를 의미한다. 단백질과 관련하여 서열 동일성 백분율이 사용되는 경우, 동일하지 않은 잔기 위치는, 아미노산 잔기가 화학적 특성(예를 들어, 전하량 또는 소수성)이 유사한 다른 아미노산 잔기로 치환되어 분자의 기능적 특성을 변화시키지 않는 보존적 아미노산 치환에 의해 종종 상이한 것으로 인식된다. 서열 간에 보존적 치환이 다른 경우, 치환의 보존적 성질을 교정하도록 서열 동일성 백분율이 상향 조정될 수 있다. 이러한 보존적 치환이 다른 서열을 "서열 유사성" 또는 "유사성"을 갖는다고 한다. 이러한 조정을 위한 수단은 당업자에게 잘 알려져 있다. 일반적으로, 이는 보존적 치환을 완전 불일치가 아니라 부분적 불일치로 점수를 매김으로써 서열 동일성 백분율을 증가시키는 것을 수반한다. 따라서, 예를 들어 동일한 아미노산의 점수가 1이고 비보존적 치환의 점수가 0인 경우, 보존적 치환의 점수는 0과 1 사이이다. 보존적 치환의 점수는, 예를 들어 PC/GENE 프로그램(Intelligenetics, Mountain View, Calif.)에서 구현된 바와 같이 계산된다.

[0107] 본원에 사용된 바와 같이, "서열 동일성 백분율"은 비교창에서 최적으로 정렬된 2개의 서열을 비교하여 결정된 값을 의미하며, 비교창 내의 폴리뉴클레오티드 서열의 부분은 (삽입 또는 결실을 포함하지 않는) 기준 서열과 비교하여 두 서열의 최적 정렬을 위한 삽입 또는 결실(즉, 갭)을 포함할 수 있다. 백분율은, 동일한 핵산 염기 또는 아미노산 잔기가 두 서열 모두에서 발생하는 위치의 수를 결정하여 일치하는 위치의 수를 산출하고, 일치하는 위치의 수를 비교창 내의 위치의 총 수로 나누고, 그 결과에 100을 곱하여 서열 동일성 백분율을 산출함으로써 계산된다.

[0108] 폴리뉴클레오티드 서열의 "실질적 동일성"이란 용어는, 폴리뉴클레오티드가 표준 파라미터를 사용하여 정렬 프로그램을 이용해 기준 서열과 비교할 때 적어도 70%, 최적으로는 적어도 80%, 더 최적으로는 적어도 90%, 가장 최적으로는 적어도 95%의 서열 동일성을 갖는 서열을 포함함을 의미한다. 당업자는 코돈 변성, 아미노산 유사성, 리딩 프레임 포지셔닝 등을 고려하여, 2개의 뉴클레오티드 서열에 의해 암호화되는 단백질의 상응하는 동일성을 결정하기 위해 이들 값이 적절히 조정될 수 있음을 인식할 것이다. 이러한 목적을 위한 아미노산 서열의 실질적 동일성은 일반적으로, 적어도 60%, 70%, 80%, 90%, 및 적어도 95%의 서열 동일성을 의미한다.

[0109] 뉴클레오티드 서열이 실질적으로 동일하다는 또 다른 표시는 두 분자가 엄격한 조건에서 서로 혼성화되는 경우이다. 일반적으로, 엄격한 조건은 정의된 이온 강도 및 pH에서 특정 서열에 대한 T_m보다 약 5℃ 낮도록 선택된다. 그러나, 엄격한 조건은 본원에 달리 규정된 바와 같이, 원하는 엄격성의 정도에 따라 T_m보다 약 1℃ 내지 약 20℃ 낮은 범위의 온도를 포함한다. 엄격한 조건에서 서로 혼성화되지 않는 핵산은, 이들이 암호화하는 폴리펩티드가 실질적으로 동일한 경우, 여전히 실질적으로 동일하다. 이는, 예를 들어 유전자 코드에 의해 허용되는 최대 코돈 변성을 사용하여 핵산의 카피가 생성되는 경우에 발생할 수 있다. 2개의 핵산 서열이 실질적으로 동일하다는 하나의 표시는 제1 핵산에 의해 암호화된 폴리펩티드가 제2 핵산에 의해 암호화된 폴리펩티드와 면역학적으로 교차 반응성인 경우이다.

[0110] 본원에 개시된 조직-선호적 프로모터, 뿐만 아니라 이들의 변이체 및 단편은 식물의 유전자 조작에 유용하다(예를 들어 관심 표현형을 발현시키기 위해 형질전환 또는 유전자이식 식물을 제조하는 데 유용하다). 본원에 사용된 용어 "형질전환 식물" 및 "유전자이식 식물"은 게놈 내에 이종 폴리뉴클레오티드를 포함하는 식물을 지칭한다. 일반적으로, 이종 폴리뉴클레오티드는 폴리뉴클레오티드가 대대로 전달되도록 유전자이식 또는 형질전환 식물의 게놈 내에 안정적으로 통합된다. 이종 폴리뉴클레오티드는 단독으로 또는 재조합 DNA 구축물의 일부로서 게놈 내에 통합될 수 있다. 본원에 사용된 용어 "유전자이식"은 초기에 그렇게 변경된 유전자이식체뿐만 아니라

초기의 유전자이식체로부터 유성 교배 또는 무성 번식에 의해 생성된 것들을 포함하여, 이종 핵산의 존재에 의해 유전형이 변경된 임의의 세포, 세포주, 캘러스, 조직, 식물 부분 또는 식물을 포함하는 것으로 이해되어야 한다.

[0111] 유전자이식 "이벤트"는 관심 유전자를 포함하는 핵산 발현 카세트를 포함하는 이종 DNA 구축물을 이용한 식물 세포의 형질전환, 전달된 유전자를 식물의 게놈에 삽입함으로 인한 식물 집단의 재생, 및 특정 게놈 위치에 삽입되는 것을 특징으로 하는 식물의 선별에 의해 생성된다. 이벤트는 삽입된 유전자의 발현을 표현형적인 특징으로 한다. 유전자 수준에서, 이벤트는 식물의 유전자 구성의 일부이다. 용어 "이벤트"는 또한, 형질전환체와 다른 식물 간의 유성 교배에 의해 생성된 자손을 지칭하며, 여기서 자손은 이종 DNA를 포함한다.

[0112] 용어 "식물"은 전체 식물, 식물 기관, 식물 조직, 종자, 식물 세포, 종자 및 그 자손을 지칭한다. 식물 세포는 종자 세포, 현탁 배양물, 배아, 분열부, 캘러스 조직, 잎, 뿌리, 싹, 배우체, 포자체, 꽃가루, 및 미포자를 제한 없이 포함한다.

[0113] 식물 부분은 분화 및 미분화 조직(뿌리, 줄기, 싹, 잎, 꽃가루, 종자, 종양 조직 및 다양한 형태의 세포 및 배양물(예컨대, 단일 세포, 원형질체, 배아, 및 캘러스 조직)을 포함하나, 이에 한정되지 않음)을 포함한다. 식물 조직은 식물에, 또는 식물 기관, 조직, 또는 세포 배양물에 있을 수 있다.

[0114] 본 발명은 또한, 본원에 개시된 임의의 방법 또는 조성물에 의해 얻은 식물을 포함한다. 본 발명은 또한, 본원에 개시된 임의의 방법 또는 조성물에 의해 얻은 식물의 종자를 포함한다. 용어 "식물"은 전체 식물, 식물 기관(예컨대, 잎, 줄기, 뿌리 등), 식물 조직, 식물 세포, 식물 부분, 종자, 육아, 배아, 및 그 자손을 지칭한다. 식물 세포는 분화 또는 미분화 세포(예를 들어, 캘러스, 미분화 캘러스, 미숙배, 성숙배, 미숙 접합배, 미숙 자엽, 배축, 현탁 배양 세포, 원형질체, 잎, 잎 세포, 뿌리 세포, 체관부 세포, 및 꽃가루)일 수 있다. 식물 세포는 종자 세포, 현탁 배양물, 외식편, 미숙배, 배아, 접합배, 체세포배, 배발생 캘러스, 분열조직, 체세포 분열조직, 기관형성 캘러스, 원형질체, 성숙 이삭-유도 종자로부터 유도된 배아, 엽저, 성숙 식물의 잎, 잎끝, 미숙 꽃, 수술, 미숙 이삭, 수염, 자엽, 미숙 자엽, 배축, 분열부, 캘러스 조직, 잎 세포, 줄기 세포, 뿌리 세포, 싹 세포, 배우체, 포자체, 꽃가루, 및 미포자를 제한 없이 포함한다. 식물 부분은 분화 및 미분화 조직(뿌리, 줄기, 싹, 잎, 꽃가루, 종자, 종양 조직, 및 배양물의 다양한 형태의 세포(예컨대, 단일 세포, 원형질체, 배아, 및 캘러스 조직)을 포함하나, 이에 한정되지 않음)을 포함한다. 식물 조직은 식물에, 또는 식물 기관, 조직, 또는 세포 배양물에 있을 수 있다. 곡물은 종의 성장 또는 생식 이외의 목적을 위해 상업적 재배업자에 의해 생산되는 성숙 종자를 의미하는 것이다. 재생 식물의 자손, 변이체, 및 돌연변이체가 도입된 폴리뉴클레오티드를 포함하는 한, 이들 자손, 변이체, 및 돌연변이체도 본 발명의 범위 내에 포함된다.

[0115] 본 발명은 임의의 식물 종(외떡잎식물 및 쌍떡잎식물(옥수수, 알팔파, 수수, 벼, 밀렛, 대두, 밀, 목화, 해바라기, 보리, 귀리, 호밀, 아마, 사탕수수, 바나나, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 소나무, 미송, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 대나무, 라이밀, 멜론, 및 배추속을 포함하나, 이에 한정되지 않음)을 포함하나, 이에 한정되지 않음)의 형질전환에 사용될 수 있다. 외떡잎식물은 보리, 옥수수(콘), 밀렛(예컨대, 펄 밀렛(Pennisetum glaucum), 프로소 밀렛(Panicum miliaceum), 조(Setaria italica), 손가락조(Eleusine coracana)), 귀리, 벼, 호밀, Setaria 종, 수수, 라이밀, 또는 밀, 또는 잎과 줄기 작물(대나무, 마람 그라스, 왕포아풀, 갈대, 독보리, 사탕수수를 포함하나, 이에 한정되지 않음), 잔디, 관상용 그라스, 및 기타 그라스, 예컨대 스위치그라스 및 터프 그라스를 포함하나, 이에 한정되지 않는다. 대안적으로, 본 발명에 사용되는 쌍떡잎식물은 케일, 콜리플라워, 브로콜리, 겨자, 양배추, 완두, 클로버, 알팔파, 잠두, 토마토, 땅콩, 카사바, 대두, 카놀라, 해바라기, 잇꽃, 담배, 애기장대, 또는 목화를 포함하나, 이에 한정되지 않는다.

[0116] 고등 식물, 예를 들어 속씨식물 및 겉씨식물 강이 본 발명에 사용될 수 있다. 본 발명에 유용한 적합한 종의 식물은 쥐꼬리망초과, 부추과, 알스트로에메리아과, 수선화과, 협죽도과, 야자나무과, 국화과, 매자나무과, 홍목과, 십자화과, 파인애플과, 삼과, 석죽과, 개비자나무과, 명아주과, 콜키쿰과, 박과, 마과, 마황과, 코카나무과, 대극과, 콩과, 꿀풀과, 아마과, 석송과, 아욱과, 멜란티움과, 파초과, 도금양과, 니사나무과, 양비귀과, 소나무과, 질경이과, 벼과, 장미과, 꼭두서니과, 버드나무과, 무환자나무과, 가지과, 주목과, 차나무과, 및 포도과 식물일 수 있다. 닥풀(Abelmoschus) 속, 침엽수(Abies) 속, 관상수(Acer) 속, 겨이삭(Agrostis) 속, 부추(Allium) 속, 알스트로메리아(Alstroemeria) 속, 아나나스(Ananas,) 속, 천심련(Andrographis) 속, 쇠풀(Andropogon) 속, 쑥(Artemisia) 속, 물대(Arundo) 속, 아트로파(Atropa) 속, 매자나무(Berberis) 속, 베타(Beta) 속, 빅사(Bixa) 속, 브라시카(Brassica) 속, 칼렌둘라(Calendula) 속, 카멜리아(Camellia) 속, 캠프노

테카(Camptotheca) 속, 칸나비스(Cannabis) 속, 캡시쿰(Capsicum) 속, 카르타무스(Carthamus) 속, 카타란투스(Catharanthus) 속, 세팔로탁수스(Cephalotaxus) 속, 크리산테눔(Chrysanthemum) 속, 신코나(Cinchona) 속, 시트룰러스(Citrullus) 속, 코페아(Coffea) 속, 콜키쿰(Colchicum) 속, 콜레우스(Coleus) 속, 쿠쿠미스(Cucumis) 속, 쿠쿠비타(Cucurbita) 속, 시노돈(Cynodon) 속, 다투라(Datura) 속, 디안투스(Dianthus) 속, 디지탈리스(Digitalis) 속, 디오스코레아(Dioscorea) 속, 엘라에이스(Elaeis) 속, 에페드라(Ephedra) 속, 에리안투스(Erianthus) 속, 에리톡실룸(Erythroxylum) 속, 에페드라(Eucalyptus) 속, 페스투카(Festuca) 속, 프라가리아(Fragaria) 속, 갈란투스(Galanthus) 속, 글리신(Glycine) 속, 고십피움(Gossypium) 속, 헬리안투스(Helianthus) 속, 헤비아(Hevea) 속, 호르데움(Hordeum) 속, 히오스시아무스(Hyoscyamus) 속, 자트로파(Jatropha) 속, 락투카(Lactuca) 속, 리눔(Linum) 속, 롤리움(Lolium) 속, 루피누스(Lupinus) 속, 리코페드시콘(Lycopersicon) 속, 리코포디움(Lycopodium) 속, 만니핫(Manihot) 속, 메디카고(Medicago) 속, 멘타(Mentha) 속, 미스칸투스(Miscanthus) 속, 무사(Musa) 속, 니코티아나(Nicotiana) 속, 오리자(Oryza,) 속, 파니쿰(Panicum) 속, 파파비르(Papaver) 속, 파르테니움(Parthenium) 속, 펜니세툼(Pennisetum) 속, 페투니아(Petunia) 속, 팔라리스(Phalaris) 속, 플레움(Phleum) 속, 피누스(Pinus) 속, 포아(Poa) 속, 포인세티아(Poinsettia) 속, 포퓰러스(Populus) 속, 라우울피아(Rauwolfia) 속, 리시누스(Ricinus) 속, 로사(Rosa) 속, 사카룸(Saccharum) 속, 살릭스(Salix) 속, 산구이나리아(Sanguinaria) 속, 스코폴리아(Scopolia) 속, 호밀(Secale) 속, 가지(Solanum) 속, 수수(Sorghum) 속, 스파르티나(Spartina) 속, 스피나세아(Spinacea) 속, 타나세툼(Tanacetum) 속, 주목(Taxus) 속, 테오브로마(Theobroma) 속, 라이밀(Triticosecale) 속, 트리티쿰(Triticum) 속, 유니올라(Uniola) 속, 여로(Veratrum) 속, 빈카(Vinca) 속, 포도나무(Vitis) 속 및 옥수수(Zea) 속의 구성원 식물이 본 발명의 방법에 사용될 수 있다.

[0117] 농업, 원예, 바이오매스 생산(액체 연료 분자 및 기타 화학물질 생산용), 및/또는 임업에 중요하거나 도움이 되는 식물이 본 발명의 방법에 사용될 수 있다. 비제한적인 예는, 예를 들어 Panicum virgatum(스위치그라스), Miscanthus giganteus(억새), Saccharum 종(사탕수수, 에너지케인), Populus balsamifera(포플러), 목화(Gossypium barbadense, Gossypium hirsutum), Helianthus annuus(해바라기), Medicago sativa(알팔파), Beta vulgaris(사탕무), 수수(Sorghum bicolor, Sorghum vulgare), Erianthus 종, Andropogon gerardii(큰 푸른줄기), Pennisetum purpureum(코끼리풀), Phalaris arundinacea(리이드케너리그라스), Cynodon dactylon(우산잔디), Festuca arundinacea(톨페스큐), Spartina pectinata(프레리 코드-그라스), Arundo donax(물대), Secale cereale(호밀), Salix 종(버드나무), Eucalyptus 종(유칼립투스, E. grandis(및 이의 잡종, "urograndis"로 알려짐), E. globulus, E. camaldulensis, E. tereticornis, E.viminalis, E. nitens, E. saligna 및 E. urophylla 포함), Triticosecale 종(밀속 - 밀 X 호밀), 대나무, Carthamus tinctorius(잇꽃), Jatropha curcas(자트로파), Ricinus communis(아주까리), Elaeis guineensis(야자), Linum usitatissimum(아마), Manihot esculenta(카사바), Lycopersicon esculentum(토마토), Lactuca sativa(상추), Phaseolus vulgaris(껍질콩), Phaseolus limensis(리마콩), Lathyrus 종(완두콩), Musa paradisiaca(바나나), Solanum tuberosum(감자), Brassica 종(B. napus(카놀라), B. rapa, B. juncea), Brassica oleracea(브로콜리, 콜리플라워, 미니양배추), Camellia sinensis(차), Fragaria ananassa(딸기), Theobroma cacao(코코아), Coffea arabica(커피), Vitis vinifera(포도), Ananas comosus(파인애플), Capsicum annum(고추 및 피망), Arachis hypogaea(땅콩), Ipomoea batatus(고구마), Cocos nucifera(코코넛), Citrus 종(감귤 나무), Persea americana(아보카도), 무화과(Ficus casica), 구아바(Psidium guajava), 망고(Mangifera indica), 올리브(Olea europaea), Carica papaya(파파야), Anacardium occidentale(캐슈), Macadamia integrifolia(마카디아 나무), Prunus amygdalus(아몬드), Allium cepa(양파), Cucumis melo(머스크멜론), Cucumis sativus(오이), Cucumis cantalupensis(칸탈루프), Cucurbita maxima(호박), Cucurbita moschata(호박), Spinacea oleracea(시금치), Citrullus lanatus(수박), Abelmoschus esculentus(오크라), Solanum melongena(가지), Cyamopsis tetragonoloba(구아콩), Ceratonia siliqua(구주콩), Trigonella foenum-graecum(호로파), Vigna radiata(녹두), Vigna unguiculata(동부), Vicia faba(잠두), Cicer arietinum(병아리콩), Lens culinaris(렌즈콩), Papaver somniferum(양귀비), Papaver orientale, Taxus baccata, Taxus brevifolia, Artemisia annua, Cannabis sativa, Camptotheca acuminate, Catharanthus roseus, Vinca rosea, Cinchona officinalis, Colchicum autumnale, Veratrum californica., Digitalis lanata, Digitalis purpurea, Dioscorea 종, Andrographis paniculata, Atropa belladonna, Datura stomonium, Berberis 종, Cephalotaxus 종, Ephedra sinica, Ephedra 종, Erythroxylum coca, Galanthus wornorii, Scopolia 종, Lycopodium serratum(뱀톱), Lycopodium 종, Rauwolfia serpentina, Rauwolfia 종, Sanguinaria canadensis, Hyoscyamus 종, Calendula officinalis, Chrysanthemum parthenium, Coleus forskohlii, Tanacetum parthenium, Parthenium argentatum

(구아율), Hevea 종(고무), Mentha spicata(민트), Mentha piperita(민트), Bixa orellana(아치오테), Alstroemeria 종, Rosa 종(장미), Rhododendron 종(아젤리아), Macrophylla hydrangea(수국), Hibiscus rosasanensis(하이비스커스), Tulipa 종(튤립), Narcissus 종(수선화), Petunia hybrida (피튜니아), Dianthus caryophyllus(카네이션), Euphorbia pulcherrima(포인세티아), chrysanthemum, Nicotiana tabacum(담배), Lupinus albus(루핀), Uniola paniculata(귀리), 벤트 그라스(Agrostis 종), Populus tremuloides(사시나무), Pinus 종(소나무), Abies 종(미송), Acer 종(단풍나무), Hordeum vulgare(보리), Poa pratensis(왕포아풀), Lolium 종(독보리), Phleum pratense(티모시), 및 침엽수를 포함한다.

[0118] 침엽수가 본 발명에 사용될 수 있으며, 예를 들어, 소나무, 예컨대 테다 소나무(Pinus taeda), 슬래시 소나무(Pinus elliotii), 폰데로사 소나무(Pinus ponderosa), 로지폴 소나무(Pinus contorta), 및 몬테레이 소나무(Pinus radiata); 미송(Pseudotsuga menziesii); 캐나다 솔송나무(Tsuga canadensis); 미국 솔송나무(Tsuga heterophylla); 마운틴 헴록(Tsuga mertensiana); 미국 낙엽송 또는 낙엽송(Larix occidentalis); 시트카 가문비나무(Picea glauca); 거대 가문비나무(Sequoia sempervirens); 전나무, 예컨대 유럽 전나무(Abies amabilis) 및 발삼 전나무(Abies balsamea); 및 삼나무, 예컨대 미국 삼나무(Thuja plicata) 및 알래스카 측백나무(Chamaecyparis nootkatensis)를 포함한다.

[0119] 터프 그라스가 본 발명에 사용될 수 있으며, 새포아풀(Poa annua); 쥐보리(Lolium multiflorum); 좀포아풀(Poa compressa); 콜로니얼 벤트그라스(Agrostis tenuis); 크리핑 벤트그라스(Agrostis palustris); 마초풀(Agropyron desertorum); 페어웨이 휘트그라스(Agropyron cristatum); 하드훼스큐(Festuca longifolia); 왕포아풀(Poa pratensis); 오리새(Dactylis glomerata); 다년생 독보리(Lolium perenne); 레드훼스큐(Festuca rubra); 흰겨이삭(Agrostis alba); 큰새포아풀(Poa trivialis); 김의털(Festuca ovina); 스무스부롬그라스(Bromus inermis); 티모시(Phleum pratense); 벨벳 벤트그라스(Agrostis canina); 위핑 알카리그라스(Puccinellia distans); 웨스턴 휘트그라스(Agropyron smithii); 성어거스틴 그라스(Stenotaphrum secundatum); 조이시아 그라스(Zoysia spp.); 바히아 그라스(Paspalum notatum); 카펫 그라스(Axonopus affinis); 센티페드 그라스(Eremochloa ophiuroides); 키쿠유 그라스(Pennisetum clandesinum); 씨소어 파스팔름(Paspalum vaginatum); 블루 그라마(Bouteloua gracilis); 버팔로 그라스(Buchloe dactyloids); 시데오에이트 그라마(Bouteloua curtipendula)를 포함하나, 이에 한정되지 않는다.

[0120] 특정 양태에서, 본 발명의 식물은 경작 식물(예를 들어, 콘, 알팔파, 해바라기, 배추속, 대두, 목화, 잇꽃, 땅콩, 벼, 수수, 밀, 밀렛, 담배 등)이다. 본 발명에 유용한 다른 식물은 보리, 귀리, 호밀, 아마, 사탕수수, 바나나, 카사바, 강낭콩, 동부, 토마토, 감자, 비트, 포도, 유칼립투스, 포플러, 소나무, 미송, 감귤류, 파파야, 카카오, 오이, 사과, 고추류, 대나무, 라이밀, 및 멜론을 포함한다.

[0121] 본 발명의 프로모터 서열에 의해 발현되는 추가의 이종 코딩 서열, 이종 폴리뉴클레오티드, 및 관심 폴리뉴클레오티드가 식물의 표현형을 변화시키기 위해 사용될 수 있다. 식물의 유전자 발현을 수정하는 것, 식물의 병원체 또는 곤충 방어 메커니즘을 변경하는 것, 제초제에 대한 식물 내성을 증가시키는 것, 환경 스트레스에 대응하도록 식물 발육을 변경하는 것, 염, 온도(더위와 추위), 가뭄 등에 대한 식물의 반응을 조절하는 것을 포함하여, 표현형의 다양한 변화가 관심의 대상이다. 이러한 결과는 적절한 유전자 산물을 포함하는 이종의 관심 뉴클레오티드 서열을 발현시킴으로써 달성될 수 있다. 특정 양태에서, 이종의 관심 뉴클레오티드 서열은, 식물 또는 식물 부분에서 발현 수준이 증가되는 내인성 식물 서열이다. 하나 이상의 내인성 유전자 산물, 특히 호르몬, 수용체, 신호전달 분자, 효소, 운반체, 또는 보조 인자의 발현을 변경하거나, 식물의 영양분 섭취에 영향을 주어 결과를 달성할 수 있다. 본원에 개시된 프로모터에 의해 제공되는 조직-선호적 프로모터는 유전자 산물 발현을 변경할 수 있다. 이러한 변화는 형질전환 식물의 표현형을 변화시킨다. 특정 양태에서, 발현 패턴이 조직-선호적이므로, 발현 패턴은 여러 유형의 스크리닝에 유용하다.

[0122] 본 발명에 대한 관심 뉴클레오티드 서열의 일반적인 범주는, 예를 들어 징크 핑거와 같은 정보 관련 유전자, 키나제와 같은 신호전달 관련 유전자, 및 열충격 단백질과 같은 하우스키핑 관련 유전자를 포함한다. 이식유전자의 보다 구체적인 범주는, 예를 들어 작물학적 특성, 곤충 저항성, 병해 저항성, 제초제 저항성, 환경 스트레스 저항성(추위, 염, 가뭄 등에 대한 내성 변경), 및 곡물 특성에 대한 중요한 형질을 암호화하는 유전자를 포함한다. 이식유전자의 또 다른 범주는 식물 및 기타 진핵 생물뿐만 아니라 원핵 생물의 효소, 보조인자, 및 호르몬과 같은 외인성 산물의 발현을 유도하는 유전자를 포함한다. 임의의 관심 유전자 또는 폴리뉴클레오티드는 본 발명의 프로모터에 작동 가능하게 연결될 수 있고 식물에서 발현될 수 있는 것으로 인식된다.

[0123] 다수의 관심 유전자가 본 발명의 프로모터에 작동 가능하게 연결되어 식물에서 발현될 수 있다. 예를 들어,

WUS/WOX 유전자에 곤충 저항성 형질이 쌓일 수 있고, 또한 하나 이상의 추가적 투입 형질(예를 들어, 제초제 저항성, 진균 저항성, 바이러스 저항성, 스트레스 내성, 병해 저항성, 웅성 불임성, 줄기 강도 등) 또는 산출 형질(예를 들어, 증가된 수확량, 변성된 전분, 개선된 오일 프로파일, 균형을 이룬 아미노산, 높은 라이신 또는 메티오닌, 증가된 소화력, 개선된 섬유질, 가뭄 저항성, 영양분 강화 등)이 쌓일 수 있다.

[0124] 본 발명의 프로모터는 곡물의 품질, 예컨대 오일의 수준(올레산의 함량 증가)과 유형, 라이신과 황의 수준, 셀룰로스의 수준, 및 전분과 단백질 함량을 증가시키는 필수 아미노산의 포화 및 불포화된 품질과 양에 영향을 미치는 작물학적으로 중요한 형질에 작동 가능하게 연결될 수 있다. 본 발명의 프로모터는 콘에서 호르도티오닌 단백질 변형을 제공하는 유전자에 작동 가능하게 연결될 수 있으며, 이러한 유전자는, 본원에 전문이 참조로 포함되는 미국 특허 번호 5,990,389; 5,885,801; 5,885,802 및 5,703,049에 기재되어 있다. 본 발명의 프로모터가 작동 가능하게 연결될 수 있는 유전자의 다른 예는, 1996년 3월20일 출원된 미국 특허 번호 5,850,016에 기재된 대두 2S 알부민에 의해 암호화되는 라이신 및/또는 황이 풍부한 종자 단백질, 및 보리의 키모트립신 억제제(Williamson, *et al.*, (1987) *Eur. J. Biochem* 165:99-106)이며, 이들의 개시 내용 전체는 본원에 참조로 포함된다.

[0125] 본 발명의 프로모터는, 근충, 거세미, 유럽옥수수좀 등과 같이 수확량에 큰 방해가 되는 해충에 대한 저항성을 암호화하는 곤충 저항성 유전자에 작동 가능하게 연결될 수 있다. 이러한 유전자는, 예를 들어, 바실러스 투링기엔시스 독성 단백질 유전자(미국 특허 번호 5,366,892; 5,747,450; 5,736,514; 5,723,756; 5,593,881, 및 Geiser, *et al.*, (1986) *Gene* 48:109)를 포함하며, 이들의 개시 내용 전체는 본원에 참조로 포함된다. 본 발명의 프로모터에 작동 가능하게 연결될 수 있는 병해 저항성 형질을 암호화하는 유전자는, 예를 들어 후모니신을 해독하는 것과 같은 독소제거 유전자(미국 특허 번호 5,792,931); 약독성(avr) 및 병해 저항성(R) 유전자(Jones, *et al.*, (1994) *Science* 266:789; Martin, *et al.*, (1993) *Science* 262:1432; 및 Mindrinos, *et al.*, (1994) *Cell* 78:1089)를 포함하며, 이들 문헌의 전문은 본원에 참조로 포함된다.

[0126] 본 발명의 프로모터에 작동 가능하게 연결될 수 있는 제초제 저항성 형질은, 아세토락테이트 신타제(ALS)의 작용을 억제하는 작용을 하는 제초제, 특히 설포닐우레아형 제초제에 대한 저항성을 코딩하는 유전자(예를 들어, 이러한 저항성으로 이어지는 돌연변이, 특히 S4 및/또는 Hra 돌연변이를 함유하는 아세토락테이트 신타제(ALS) 유전자), 글루타민 신타제의 작용을 억제하는 작용을 하는 제초제에 대한 저항성을 코딩하는 유전자, 예컨대, 포스피노트리신 또는 바스타(예를 들어, bar 유전자), 글리포세이트에 대한 저항성을 코딩하는 유전자(예를 들어, EPSPS 유전자 및 GAT 유전자; 예를 들어 본원에 전문이 참조로 포함되는 미국 특허 출원 공개 번호 2004/0082770 및 WO 03/092360 참조), 또는 당업계에 알려진 기타 이러한 유전자를 포함한다. bar 유전자는 제초제 바스타에 대한 저항성을 암호화하고, nptII 유전자는 항생제 카나마이신 및 제네티신에 대한 저항성을 암호화하고, ALS 유전자 돌연변이체는 제초제 클로르설푸론에 대한 저항성을 암호화하며, 이들 모두는 본 발명의 프로모터에 작동 가능하게 연결될 수 있다.

[0127] 글리포세이트 저항성은 본 발명의 프로모터에 작동 가능하게 연결될 수 있는 돌연변이 5-에놀피루브릴-3-포스피키메이트 신타제(EPSP) 및 aro A 유전자에 의해 부여된다. 예를 들어, 글리포세이트 저항성을 부여할 수 있는 EPSPS 형태의 뉴클레오티드 서열이 개시되어 있는 미국 특허 번호 4,940,835(Shah, *et al.*) 참조. 미국 특허 번호 5,627,061(Barry, *et al.*)에도 본 발명의 프로모터에 작동 가능하게 연결될 수 있는 EPSPS 효소를 암호화하는 유전자가 기재되어 있다. 또한, 본원에 전문이 참조로 포함되는 미국 특허 번호 6,248,876 B1; 6,040,497; 5,804,425; 5,633,435; 5,145,783; 4,971,908; 5,312,910; 5,188,642; 4,940,835; 5,866,775; 6,225,114 B1; 6,130,366; 5,310,667; 4,535,060; 4,769,061; 5,633,448; 5,510,471; Re. 36,449; RE 37,287 E 및 5,491,288과 국제 공보 WO 97/04103; WO 97/04114; WO 00/66746; WO 01/66704; WO 00/66747 및 WO 00/66748 참조. 글리포세이트 저항성은 또한, 본원에 전문이 참조로 포함되는 미국 특허 번호 5,776,760 및 5,463,175에 보다 상세히 기재된 바와 같이 글리포세이트 산화환원 효소를 암호화하는, 본 발명의 프로모터에 작동 가능하게 연결될 수 있는 유전자를 발현하는 식물에게 부여된다. 글리포세이트 저항성은 또한, 글리포세이트 N-아세틸트랜스퍼라제를 암호화하는, 본 발명의 프로모터에 작동 가능하게 연결될 수 있는 유전자의 과발현에 의해 식물에게 부여될 수 있다. 또한, 예를 들어 본원에 전문이 참조로 포함되는 미국 특허 출원 일련번호 11/405,845 및 10/427,692 참조.

[0128] 본 발명의 프로모터에 작동 가능하게 연결되는 불임성 유전자도 DNA 구축물에서 암호화될 수 있고, 물리적 제웅(detasseling)에 대한 대안을 제공한다. 이러한 방식으로 사용되는 유전자의 예는 웅성 조직-선호적 유전자, 및 본원에 전문이 참조로 포함되는 미국 특허 번호 5,583,210에 기재된 QM과 같은 웅성 불임성 표현형을 갖는 유전자를 포함한다. 본 발명의 프로모터에 작동 가능하게 연결될 수 있는 다른 유전자는 키나제 및 웅성 또는 자성

배우체 발육에 유독한 화합물을 암호화하는 것을 포함한다.

[0129] 예를 들어, 에탄올 생성을 위한 전분을 증가시키거나 단백질의 발현을 제공할 수 있는, 본 발명의 프로모터에 작동 가능하게 연결되는 유전자 또는 유전자에 상업적 형질도 암호화될 수 있다. 형질전환 식물의 다른 중요한 상업적 용도는, 본원에 전문이 참조로 포함되는 미국 특허 번호 5,602,321에 기재된 것과 같은 폴리머 및 바이오플라스틱의 제조이다. 본 발명의 프로모터에 작동 가능하게 연결될 수 있는 β-케토티올라제, PHBase(폴리하이드록시부티레이트 신타제), 및 아세토아세틸-CoA 환원효소와 같은 유전자는(본원에 전문이 참조로 포함되는 문헌[Schubert, *et al.*, (1988) *J. Bacteriol.* 170:5837-5847] 참조) 폴리하이드록시알카노에이트(PHA)의 발현을 촉진시킨다.

[0130] 본 발명의 프로모터에 작동 가능하게 연결될 수 있는 기타 적용 가능한 유전자 및 이들의 관련 표현형의 예는 바이러스 외피 단백질 및/또는 RNA를 암호화하는 유전자, 또는 바이러스 저항성을 부여하는 기타 바이러스 또는 식물 유전자; 진균 저항성을 부여하는 유전자; 수확량 향상을 촉진하는 유전자; 및 추위, 가뭄으로 인한 탈수, 열과 염분, 독성 금속 또는 미량 원소 등과 같은 스트레스에 대한 저항성을 제공하는 유전자를 포함한다.

[0131] 비제한적인 예시로서, 다음은 본 발명의 프로모터 서열에 작동 가능하게 연결될 수 있는 유전자 유형의 다른 예의 목록이다.

[0132] 1. 곤충이나 병해에 대한 저항성을 부여하고 다음을 암호화하는 이식유전자

[0133] (A) 식물 병해 저항성 유전자. 식물 방어는 보통 식물의 병해 저항성 유전자(R)의 산물과 병원체의 상응하는 약독성(Avr) 유전자의 산물 간의 특정 상호작용에 의해 활성화된다. 식물 품종은 클로닝된 저항성 유전자를 사용해 형질전환되어 특정 병원체 균주에 저항성이 있는 식물로 유전자 조작될 수 있다. 예를 들어, 본원에 전문이 참조로 포함되는 문헌[Jones, *et al.*, (1994) *Science* 266:789 (cloning of the tomato Cf-9 gene for resistance to *Cladosporium fulvum*); Martin, *et al.*, (1993) *Science* 262:1432 (tomato *Pto* gene for resistance to *Pseudomonas syringae* pv. tomato encodes a protein kinase); Mindrinos, *et al.*, (1994) *Cell* 78:1089 (*Arabidopsis RSP2* gene for resistance to *Pseudomonas syringae*); McDowell and Woffenden, (2003) *Trends Biotechnol.* 21(4):178-83 및 Toyoda, *et al.*, (2002) *Transgenic Res.* 11(6):567-82] 참조. 병해 저항성 식물은 야생형 식물에 비해 병원체에 대해 더 저항성이 있는 식물이다.

[0134] (B) *Bacillus thuringiensis* 단백질, 이의 유도체 또는 이를 모델링한 합성 폴리펩티드. 예를 들어, *Bt* 델타-내독소 유전의 클로닝 및 뉴클레오티드 서열이 개시되어 있는 문헌[Geiser, *et al.*, (1986) *Gene* 48:109] 참조. 또한 델타-내독소 유전자를 암호화하는 DNA 분자는, 예를 들어 ATCC 수탁번호 40098, 67136, 31995, 및 31998으로 American Type Culture Collection(Rockville, MD)에서 구입할 수 있다. 유전자 조작되는 *Bacillus thuringiensis* 이식유전자의 다른 예는 다음의 특허 및 특허 출원에 제시되어 있으며, 이 목적을 위해 본원에 참조로 포함된다. 본원에 전문이 참조로 포함되는 미국 특허 번호 5,188,960; 5,689,052; 5,880,275; WO 91/14778; WO 99/31248; WO 01/12731; WO 99/24581; WO 97/40162 및 미국 출원 일련번호 10/032,717; 10/414,637 및 10/606,320.

[0135] (C) 에크디스테로이드 및 유충 호르몬과 같은 곤충-특이적 호르몬 또는 페로몬, 이들의 변이체, 이들에 기초한 모방체, 또는 이들의 길항제 또는 작용제. 예를 들어, 유충 호르몬의 불활성화제인 클로닝된 유충 호르몬 에스테라제의 바큘로바이러스 발현에 대한 문헌[Hammock, *et al.*, (1990) *Nature* 344:458]의 개시 내용 참조(그 전문은 본원에 참조로 포함됨).

[0136] (D) 발현시 해당 해충의 생리를 방해하는 곤충-특이적 펩티드. 예를 들어, 본원에 전문이 참조로 포함되는 문헌[Regan, (1994) *J. Biol. Chem.* 269:9(발현 클로닝으로 곤충 이뇨 호르몬 수용체를 코딩하는 DNA를 생성); Pratt, *et al.*, (1989) *Biochem. Biophys. Res. Comm.*163:1243(*Diploptera puntata*에서 알로스타틴이 확인됨); Chattopadhyay, *et al.*, (2004) *Critical Reviews in Microbiology* 30(1):33-54; Zjawiony, (2004) *J Nat Prod* 67(2):300-310; Carlini and Grossi-de-Sa, (2002) *Toxicon* 40(11):1515-1539; Ussuf, *et al.*, (2001) *Curr Sci.* 80(7):847-853 및 Vasconcelos and Oliveira, (2004) *Toxicon* 44(4):385-403]의 개시 내용 참조. 또한, 곤충-특이적 독소를 암호화하는 유전자가 개시되어 있는 미국 특허 번호 5,266,317(Tomalski, *et al.*) 참조(그 전문은 본원에 참조로 포함됨).

[0137] (E) 모노테르펜, 세스퀴테르펜, 스테로이드, 하이드록삼산, 페닐프로파노이드 유도체, 또는 살충 활성을 갖는 다른 비단백질 분자의 과잉 축적을 초래하는 효소.

[0138] (F) 생물학적 활성 분자, 예를 들어 천연 당분해 효소, 단백질분해 효소, 지방분해 효소, 뉴클레아제,

시클라제, 트랜스아미나제, 에스테라제, 가수분해 효소, 포스파타제, 키나제, 포스포릴라제, 중합효소, 엘라스타제, 키티나제, 및 글루카나제의 변형(번역후 변형 포함)에 관여하는 효소(천연이든 합성이든 무관). 칼라제 유전자의 뉴클레오티드 서열이 개시되어 있는 PCT 출원 번호 WO 93/02197(Scott, *et al.*) 참조(그 전문은 본원에 참조로 포함됨). 키티나제-암호화 서열을 함유하는 DNA 분자는, 예를 들어 ATCC 수탁번호 39637 및 67152로부터 얻을 수 있다. 또한, 담배 구충 키티나제를 암호화하는 cDNA의 뉴클레오티드 서열이 교시되어 있는 문헌[Kramer, *et al.*, (1993) *Insect Biochem. Molec. Biol.* 23:691], 파슬리 *ubi*4-2 폴리유비퀴틴 유전자의 뉴클레오티드 서열을 제공하는 문헌[Kawalleck, *et al.*, (1993) *Plant Molec. Biol.* 21:673], 미국 특허 출원 일련번호 10/389,432, 10/692,367, 및 미국 특허 번호 6,563,020 참조(이들의 전문은 본원에 참조로 포함됨).

[0139] (G) 신호 변환을 자극하는 분자. 예를 들어, 녹두 칼모듈린 cDNA 클론의 뉴클레오티드 서열에 대한 문헌[Botella, *et al.*, (1994) *Plant Molec. Biol.* 24:757]의 개시 내용, 및 옥수수 칼모듈린 cDNA 클론에 대한 뉴클레오티드 서열을 제공하는 문헌[Griess, *et al.*, (1994) *Plant Physiol.*104:1467] 참조(이들의 전문은 본원에 참조로 포함됨).

[0140] (H) 소수성 모멘트 펩티드. 본원에 전문이 참조로 포함되는 PCT 출원 번호 WO 95/16776 및 미국 특허 번호 5,580,852(진균성 식물 병원체를 억제하는 타키플레신의 펩티드 유도체에 대한 개시 내용) 및 PCT 출원 번호 WO 95/18855 및 미국 특허 번호 5,607,914(병해 저항성을 부여하는 합성 항균 펩티드를 교시).

[0141] (I) 멤브레인 투과효소, 채널 형성제, 또는 채널 차단제. 예를 들어, *Pseudomonas solanacearum*에 저항성이 있는 유전자이식 담배 식물을 만드는 세크로핀-베타 용해성 펩티드 유사체의 이종 발현에 대한 문헌[Jaynes, *et al.*, (1993) *Plant Sci.* 89:43]의 개시 내용 참조(그 전문은 본원에 참조로 포함됨).

[0142] (J) 바이러스-침습성 단백질 또는 이로부터 유도된 복합 독소. 예를 들어, 형질전환 식물 세포 내 바이러스 외피 단백질의 축적은 바이러스 감염에 대한 저항성, 및/또는 외피 단백질 유전자가 유도되는 바이러스 및 관련 바이러스에 의한 병해 발생에 대한 저항성을 부여한다. 본원에 전문이 참조로 포함되는 문헌[Beachy, *et al.*, (1990) *Ann. Rev. Phytopathol.* 28:451] 참조. 외피 단백질-매개 저항성은 알팔파 모자이크 바이러스, 오이 모자이크 바이러스, 담배 줄무늬 바이러스, 감자 바이러스 X, 감자 바이러스 Y, 담배 에칭 바이러스(tobacco etch virus), 담배 래틀 바이러스(tobacco rattle virus), 및 담배 모자이크 바이러스에 대해 형질전환 식물에 부여된 바 있다(상기 문헌).

[0143] (K) 곤충-특이적 항체 또는 이로부터 유도된 면역독소. 따라서, 곤충 소화관에서의 중요한 대사 기능에 대해 표적화된 항체는 영향을 받은 효소를 불활성화시켜 곤충을 죽인다. 본원에 전문이 참조로 포함되는 문헌[Taylor, *et al.*, 요약 #497, SEVENTH INT'L SYMPOSIUM ON MOLECULAR PLANT-MICROBE INTERACTIONS (Edinburgh, Scotland, 1994)] (단일쇄 항체 단편의 생성을 통한 유전자이식 담배에서의 효소 불활성화) 참조.

[0144] (L) 바이러스-특이적 항체. 예를 들어, 재조합 항체 유전자를 발현하는 유전자이식 식물이 바이러스 공격으로부터 보호됨을 나타내는 문헌[Tavladoraki, *et al.*, (1993) *Nature* 366:469] 참조(그 전문은 본원에 참조로 포함됨).

[0145] (M) 병원체 또는 기생충에 의해 자연적으로 생성된 발육 억제 단백질. 따라서, 진균성 엔도 알파-1,4-D-폴리갈락투로나제는 식물 세포벽 호모-알파-1,4-D-갈락투로나제를 가용화함으로써 진균 군집화 및 식물 영양분 방출을 용이하게 한다. 본원에 전문이 참조로 포함되는 문헌[Lamb, *et al.*, (1992) *Bio/Technology* 10:1436] 참조. 콩 엔도폴리갈락투로나제-억제 단백질을 암호화하는 유전자의 클로닝 및 특성화는, 본원에 전문이 참조로 포함되는 문헌[Toubart, *et al.*, (1992) *Plant J.* 2:367]에 기재되어 있다.

[0146] (N) 식물에 의해 자연적으로 생성된 발육 억제 단백질. 예를 들어, 본원에 전문이 참조로 포함되는 문헌[Logemann, *et al.*, (1992) *Bio/Technology* 10:305]은 보리 리보좀-불활성화 유전자를 발현하는 유전자이식 식물이 진균성 병해에 대해 증가된 저항성을 가짐을 보여주었다.

[0147] (O) 전신 획득 저항성(SAR) 반응 및/또는 병인 관련 유전자에 관여하는 유전자. 본원에 전문이 참조로 포함되는 문헌[Briggs, (1995) *Current Biology* 5(2):128-131, Pieterse and Van Loon, (2004) *Curr. Opin. Plant Bio.* 7(4):456-64 및 Somssich, (2003) *Cell* 113(7):815-6].

[0148] (P) 항진균성 유전자(Cornelissen and Melchers, (1993) *Pl. Physiol.* 101:709-712 및 Parijs, *et al.*, (1991) *Planta* 183:258-264 및 Bushnell, *et al.*, (1998) *Can. J. of Plant Path.* 20(2):137-149). 또한, 본원에 전문이 참조로 포함되는 미국 특허 출원 번호 09/950,933 참조.

[0149] (Q) 후모니신, 뷰베리신, 모닐리포르민, 및 제랄레논 등에 대한 독소제거 유전자, 및 이들의 구조적으로 관련된 유도체. 예를 들어, 본원에 전문이 참조로 포함되는 미국 특허 번호 5,792,931 참조.

[0150] (R) 시스타틴 및 시스타틴 프로테이나제 억제제. 본원에 전문이 참조로 포함되는 미국 출원 일련번호 10/947,979 참조.

[0151] (S) 디펜신 유전자. 본원에 전문이 참조로 포함되는 WO03/000863 및 미국 출원 일련번호 10/178,213 참조.

[0152] (T) 선충류에 대한 저항성을 부여하는 유전자. 본원에 전문이 참조로 포함되는 WO 03/033651 및 문헌[Urwin, *et. al.*, (1998) *Planta* 204:472-479, Williamson (1999) *Curr Opin Plant Bio.* 2(4):327-31] 참조.

[0153] (U) 진균 *Colletotrichum graminiola*에 의해 발생하는 탄저병 줄기 썩음에 대한 저항성을 부여하는 rcg1과 같은 유전자. 본원에 전문이 참조로 포함되는 문헌[Jung, *et al.*, Generation-means analysis and quantitative trait locus mapping of Anthracnose Stalk Rot genes in Maize, *Theor. Appl. Genet.* (1994) 89:413-418], 뿐만 아니라 미국 가출원 번호 60/675,664 참조.

[0154] 2. 제초제에 대한 저항성을 부여하는 이식유전자, 예를 들어,

[0155] (A) 성장점 또는 분열조직을 억제하는 제초제, 예컨대 이미다졸리논 또는 설포닐우레아. 이 범주 내의 예시적 유전자는, 예를 들어 문헌[Lee, *et al.*, (1988) *EMBO J.* 7:1241] 및 문헌[Miki, *et al.*, (1990) *Theor. Appl. Genet.* 80:449]에 각각 기재된 바와 같이 돌연변이 ALS 및 AHAS 효소를 코딩한다. 또한, 본원에 전문이 참조로 포함되는 미국 특허 번호 5,605,011; 5,013,659; 5,141,870; 5,767,361; 5,731,180; 5,304,732; 4,761,373; 5,331,107; 5,928,937 및 5,378,824와 국제 공보 WO 96/33270 참조.

[0156] (B) 글리포세이트(돌연변이 5-에놀피루블-3-포스피키메이트 신타제(EPSP) 및 *aroA* 유전자에 의해 각각 부여되는 저항성), 기타 포스포노 화합물, 예컨대 글루포시네이트(포스피노트리신 아세틸 트랜스퍼라제(PAT) 및 *Streptomyces hygroscopicus* 포스피노트리신 아세틸 트랜스퍼라제 (*bar*) 유전자), 및 피리딘옥시 또는 페녹시 프로프리온산 및 시클로헥손(ACCase 억제제-암호화 유전자). 예를 들어, 글리포세이트 저항성을 부여할 수 있는 EPSPS 형태의 뉴클레오티드 서열이 개시되어 있는 미국 특허 번호 4,940,835(Shah, *et al.*) 참조. 미국 특허 번호 5,627,061(Barry, *et al.*)에도 EPSPS 효소를 암호화하는 유전자가 기재되어 있다. 또한, 본원에 전문이 참조로 포함되는 미국 특허 번호 6,566,587; 6,338,961; 6,248,876 B1; 6,040,497; 5,804,425; 5,633,435; 5,145,783; 4,971,908; 5,312,910; 5,188,642; 4,940,835; 5,866,775; 6,225,114 B1; 6,130,366; 5,310,667; 4,535,060; 4,769,061; 5,633,448; 5,510,471; Re. 36,449; RE 37,287 E 및 5,491,288과 국제 공보 EP1173580; WO 01/66704; EP1173581 및 EP1173582 참조. 글리포세이트 저항성은 또한, 본원에 전문이 참조로 포함되는 미국 특허 번호 5,776,760 및 5,463,175에 보다 상세히 기재된 바와 같이 글리포세이트 산화환원 효소를 암호화하는 유전자를 발현하는 식물에게 부여된다. 또한, 글리포세이트 저항성은 글리포세이트 N-아세틸트랜스퍼라제를 암호화하는 유전자의 과발현에 의해 식물에게 부여될 수 있다. 예를 들어, 본원에 전문이 참조로 포함되는 미국 특허 출원 일련번호 11/405,845 및 10/427,692와 PCT 출원 번호 US01/46227 참조. 돌연변이 *aroA* 유전자를 암호화하는 DNA 분자는 ATCC 수탁번호 39256으로 얻을 수 있으며, 이 돌연변이 유전자의 뉴클레오티드 서열은 본원에 전문이 참조로 포함되는 미국 특허 번호 4,769,061(Comai)에 개시되어 있다. 본원에 전문이 참조로 포함되는 EP 특허 출원 번호 0 333 033(Kumada, *et al.*), 및 미국 특허 번호 4,975,374(Goodman, *et al.*)에는 L-포스피노트리신과 같은 제초제에 대한 저항성을 부여하는 글루타민 신테타제 유전자의 뉴클레오티드 서열이 개시되어 있다. 포스피노트리신-아세틸-트랜스퍼라제 유전자의 뉴클레오티드 서열은, 포스피노트리신 아세틸 트랜스퍼라제 활성을 코딩하는 키메라 *bar* 유전자를 발현하는 유전자이식 식물의 제조를 설명하는 EP 특허 번호 0 242 246 및 0 242 236(Leemans, *et al.*), 문헌[De Greef, *et al.*, (1989) *Bio/Technology* 7:61]에 제공되어 있으며, 이들 문헌의 전문은 본원에 참조로 포함된다. 또한, 본원에 전문이 참조로 포함되는 미국 특허 번호 5,969,213; 5,489,520; 5,550,318; 5,874,265; 5,919,675; 5,561,236; 5,648,477; 5,646,024; 6,177,616 B1 및 5,879,903 참조. 세톡시딤 및 할록시포프와 같은 페녹시 프로프리온산 및 시클로헥손에 대한 저항성을 부여하는 예시적 유전자는 본원에 전문이 참조로 포함되는 문헌[Marshall, *et al.*, (1992) *Theor. Appl. Genet.* 83:435]에 기재된 *Acc1-S1, Acc1-S2* 및 *Acc1-S3* 유전자이다.

[0157] (C) 광합성을 억제하는 제초제, 예컨대 트리아진(*psbA* 및 *gs+* 유전자) 및 벤조니트릴(니트릴라제 유전자). 본원에 전문이 참조로 포함되는 문헌[Przibilla, *et al.*, (1991) *Plant Cell* 3:169]에는 돌연변이 *psbA* 유전자를 암호화하는 플라스미드를 이용한 *Chlamydomonas*의 형질전환이 기술되어 있다. 니트릴라제 유전자에 대한 뉴클레오티드 서열은 본원에 전문이 참조로 포함되는 미국 특허 번호 4,810,648(Stalker)에 기재되어 있으며, 이들 유

전자를 함유하는 DNA 분자는 ATCC 수탁번호 53435, 67441, 및 67442로 입수 가능하다. 글루타티온 S-트랜스퍼라제를 코딩하는 DNA의 클로닝 및 발현은 본원에 전문이 참조로 포함되는 문헌[Hayes, *et al.*, (1992) *Biochem. J.* 285:173]에 기술되어 있다.

[0158] (D) 이 효소를 발현하는 식물을 다양한 유형의 제초제에 저항성이 있도록 하는 것으로 밝혀진 아세토하이드록시산 신타제는 다양한 식물에 도입된 바 있다(예를 들어, 본원에 전문이 참조로 포함되는 문헌[Hattori, *et al.*, (1995) *Mol Gen Genet* 246:419] 참조). 제초제에 대한 저항성을 부여하는 기타 유전자는 랫트 시토크롬 P4507A1과 효모 NADPH-시토크롬 P450 산화환원 효소의 키메라 단백질을 암호화하는 유전자(Shiota, *et al.*, (1994) *Plant Physiol.* 106(1):17-23), 글루타티온 환원효소와 수퍼옥사이드 디스뮤타제에 대한 유전자(Aono, *et al.*, (1995) *Plant Cell Physiol* 36:1687), 및 다양한 포스포트랜스퍼라제에 대한 유전자(Datta, *et al.*, (1992) *Plant Mol Biol* 20:619)를 포함하며, 이들 문헌의 전문은 본원에 참조로 포함된다.

[0159] (E) 프로토포르피리노겐 산화효소(프로톡스)는 모든 식물 생존에 필요한 엽록소를 생성하는 데 필요하다. 프로톡스 효소는 다양한 제초제 화합물에 대한 표적 역할을 한다. 이러한 제초제는 또한, 존재하는 모든 상이한 종의 식물의 성장을 억제하여 이들을 완전히 파괴시킨다. 이러한 제초제에 저항성이 있는 변경된 프로톡스 활성을 갖는 식물의 개발은 본원에 전문이 참조로 포함되는 미국 특허 번호 6,288,306 B1; 6,282,837 B1 및 5,767,373; 국제 공개 번호 WO 01/12825에 기술되어 있다.

[0160] 3. 하기와 같은 변경된 곡물 특성을 부여하거나 이에 기여하는 이식유전자,

[0161] (A) 예를 들어, 하기에 의해 변경된 지방산

[0162] (1) 식물의 스테아르산 함량을 증가시키기 위한 스테아로일-ACP 불포화효소의 하향 조절. 본원에 전문이 참조로 포함되는 문헌[Knultzon, *et al.*, (1992) *Proc. Natl. Acad. Sci. USA* 89:2624] 및 WO99/64579(Genes for Desaturases to Alter Lipid Profiles in Corn) 참조,

[0163] (2) FAD-2 유전자 변형을 통한 올레산의 증가 및/또는 FAD-3 유전자 변형을 통한 리놀렌산의 감소(본원에 전문이 참조로 포함되는 미국 특허 번호 6,063,947; 6,323,392; 6,372,965, 및 WO 93/11245 참조),

[0164] (3) 본원에 전문이 참조로 포함되는 WO 01/12800에서와 같은, 공액 리놀렌산 또는 리놀레산 함량의 변경,

[0165] (4) LEC1, AGP, Dek1, Superal1, milps, 다양한 lpa 유전자(예컨대 lpa1, lpa3, hpt, 또는 hggt)의 변경. 예를 들어, 본원에 전문이 참조로 포함되는 WO 02/42424, WO 98/22604, WO 03/011015, 미국 특허 번호 6,423,886, 미국 특허 번호 6,197,561, 미국 특허 번호 6,825,397, 미국 특허 출원 공개 번호 2003/0079247, 2003/0204870, WO02/057439, WO03/011015 및 문헌[Rivera-Madrid, *et. al.*, (1995) *Proc. Natl. Acad. Sci.* 92:5620-5624] 참조.

[0166] (B) 예를 들어, 하기에 의해 변경된 인 함량

[0167] (1) 피타제-암호화 유전자의 도입은 형질전환 식물에 더 많은 유리 인산염을 첨가하여 피틴산염의 분해를 향상시킬 것이다. 예를 들어, *Aspergillus niger* 피타제 유전자의 뉴클레오티드 서열의 개시 내용에 대해 문헌[Van Hartingsveldt, *et al.*, (1993) *Gene* 127:87] 참조(그 전문은 본원에 참조로 포함됨).

[0168] (2) 피틴산염 함량을 감소시키는 유전자의 상향 조절. 옥수수에서, 이는 예를 들어 문헌[Raboy, et al., (1990) Maydica 35:383]에서와 같이, 낮은 수준의 피틴산을 특징으로 하는 옥수수 돌연변이체에서 확인되는 LPA 대립유전자와 같은 하나 이상의 대립유전자와 관련된 DNA를 클로닝한 후 재도입함으로써, 및/또는 본원에 전문이 참조로 포함되는 WO 02/059324, 미국 특허 출원 공개 번호 2003/0009011, WO 03/027243, 미국 특허 출원 공개 번호 2003/0079247, WO 99/05298, 미국 특허 번호 6,197,561, 미국 특허 번호 6,291,224, 미국 특허 번호 6,391,348, WO2002/059324, 미국 특허 출원 공개 번호 2003/0079247, WO98/45448, WO99/55882, WO01/04147에서와 같이 이노시톨 키나제 활성을 변경함으로써 달성될 수 있다.

[0169] (C) 예를 들어, 전분의 분지화 패턴에 영향을 미치는 효소에 대한 유전자 또는 NTR 및/또는 TRX와 같은 티오레독신을 변경하는 유전자(전문이 참조로 포함되는 미국 특허 번호 6,531,648 참조), 및/또는 cs27 또는 TUSC27 또는 en27과 같은 감마 제인 녹아웃 또는 돌연변이체(전문이 참조로 포함되는 미국 특허 번호 6,858,778 및 미국 특허 출원 공개 번호 2005/0160488 및 2005/0204418 참조)를 변경함으로써 변경된 탄수화물. 본원에 전문이 참조로 포함되는 문헌[Shiroza, *et al.*, (1988) *J. Bacteriol.* 170:810(스트렙토코구스 뮤턴트 프룩토실트랜스퍼라제 유전자의 뉴클레오티드 서열), Steinmetz, *et al.*, (1985) *Mol. Gen. Genet.* 200:220(바실러스 서브틸리스 레반수크라제 유전자의 뉴클레오티드 서열), Pen, *et al.*, (1992) *Bio/Technology* 10:292(바실러스 리케

니포미스 알파-아밀라제를 발현하는 유전자이식 식물의 제조), Elliot, *et al.*, (1993) *Plant Molec. Biol.* 21:515(토마토 인버타제 유전자의 뉴클레오티드 서열), Søgaard, *et al.*, (1993) *J. Biol. Chem.* 268:22480 (보리 알파-아밀라제 유전자의 부위-특이적 돌연변이 유도) 및 Fisher, *et al.*, (1993) *Plant Physiol.* 102:1045(옥수수 배젖 전분 분지화 효소 II)], WO 99/10498(UDP-D-자일로스 4-에피머라제, Fragile 1 및 2, Ref1, HCHL, C4H의 변형을 통한 개선된 소화성 및/또는 전분 추출), 미국 특허 번호 6,232,529(method of producing high oil seed by modification of starch levels (AGP)) 참조. 상기 언급된 지방산 변형 유전자는 또한, 전분과 오일의 경로의 상호관계를 통해 전분 함량 및/또는 조성에 영향을 주기 위해 사용될 수 있다.

[0170] (D) 토코페롤 또는 토코트리에놀의 변경과 같은 변경된 항산화제 함량 또는 조성. 예를 들어, 피틀 프레닐 트랜스퍼라제(ppt)의 변경을 통한 항산화제 수준의 조작을 수반하는 미국 특허 번호 6,787,683, 미국 특허 출원 공개 번호 2004/0034886, 및 호모겐티스산 게라닐 게라닐 트랜스퍼라제(hggt)의 변경을 통한 WO 00/68393 참조(이들의 전문은 본원에 참조로 포함됨).

[0171] (E) 변경된 필수 종자 아미노산. 예를 들어, 본원에 전문이 참조로 포함되는 미국 특허 번호 6,127,600(method of increasing accumulation of essential amino acids in seeds), 미국 특허 번호 6,080,913(binary methods of increasing accumulation of essential amino acids in seeds), 미국 특허 번호 5,990,389(높은 라이신), WO99/40209(alteration of amino acid compositions in seeds), WO99/29882(methods for altering amino acid content of proteins), 미국 특허 번호 5,850,016(alteration of amino acid compositions in seeds), WO98/20133(proteins with enhanced levels of essential amino acids), 미국 특허 번호 5,885,802(높은 메티오닌), 미국 특허 번호 5,885,801(높은 트레오닌), 미국 특허 번호 6,664,445(plant amino acid biosynthetic enzymes), 미국 특허 번호 6,459,019(증가된 라이신 및 트레오닌), 미국 특허 번호 6,441,274(plant tryptophan synthase beta subunit), 미국 특허 번호 6,346,403(methionine metabolic enzymes), 미국 특허 번호 5,939,599(높은 황), 미국 특허 번호 5,912,414(증가된 메티오닌), WO98/56935(plant amino acid biosynthetic enzymes), WO98/45458(engineered seed protein having higher percentage of essential amino acids), WO98/42831(증가된 라이신), 미국 특허 번호 5,633,436(황 아미노산 함량 증가), 미국 특허 번호 5,559,223(synthetic storage proteins with defined structure containing programmable levels of essential amino acids for improvement of the nutritional value of plants), WO96/01905(증가된 트레오닌), WO95/15392(증가된 라이신), 미국 특허 출원 공개 번호 2003/0163838, 미국 특허 출원 공개 번호 2003/0150014, 미국 특허 출원 공개 번호 2004/0068767, 미국 특허 번호 6,803,498, WO01/79516, 및 WO00/09706(Ces A: 셀룰로스 신타제), 미국 특허 번호 6,194,638(헤미셀룰로스), 미국 특허 번호 6,399,859 및 미국 특허 출원 공개 번호 2004/0025203(UDPGdH), 미국 특허 번호 6,194,638(RGP) 참조.

[0172] 4. 부위-특이적 DNA 통합을 위한 부위를 생성하는 유전자

[0173] 이는 FLP/FRT 시스템에서 사용될 수 있는 FRT 부위 및/또는 Cre/Loxp 시스템에서 사용될 수 있는 Lox 부위의 도입을 포함한다. 예를 들어, 본원에 전문이 참조로 포함되는 문헌[Lyznik, *et al.*, (2003) *Plant Cell Rep* 21:925-932] 및 WO 99/25821 참조. 사용될 수 있는 다른 시스템은 파지 Mu의 Gin 재조합효소(Maeser, *et al.*, 1991; Vicki Chandler, *The Maize Handbook* ch. 118 (Springer-Verlag 1994), 대장균의 Pin 재조합효소 (Enomoto, *et al.*, 1983), 및 pSR1 플라스미드의 R/RS 시스템(Araki, *et al.*, 1992)을 포함하며, 이들 문헌의 전문은 본원에 참조로 포함된다.

[0174] 5. 무생물 스트레스 저항성(개화, 이삭 및 종자 발육, 질소 이용 효율, 변경된 질소 반응성, 가뭄 저항성 또는 내성, 추위 저항성 또는 내성, 및 염 저항성 또는 내성을 포함하나, 이에 한정되지 않음) 및 스트레스하에서의 증가된 수확량에 영향을 미치는 유전자. 예를 들어, 말레이트의 변경을 통해 물 이용 효율이 변경됨을 보여주는 WO 00/73475; 식물에 대한 결빙, 높은 염분, 및 가뭄의 부정적인 영향을 완화시킬 뿐만 아니라 식물 표현형에 대한 기타 긍정적인 효과를 부여하는 데 효과적인 CBF 유전자 및 전사 인자를 포함한 유전자가 기재되어 있는 미국 특허 번호 5,892,009, 미국 특허 번호 5,965,705, 미국 특허 번호 5,929,305, 미국 특허 번호 5,891,859, 미국 특허 번호 6,417,428, 미국 특허 번호 6,664,446, 미국 특허 번호 6,706,866, 미국 특허 번호 6,717,034, WO2000060089, WO2001026459, WO2001035725, WO2001034726, WO2001035727, WO2001036444, WO2001036597, WO2001036598, WO2002015675, WO2002017430, WO2002077185, WO2002079403, WO2003013227, WO2003013228, WO2003014327, WO2004031349, WO2004076638, WO9809521, 및 WO9938977; 식물에서 아브시스산이 변경되어 수확량 증가 및/또는 무생물 스트레스에 대한 내성 증가와 같은 식물 표현형 개선으로 이어짐을 보여주는 미국 특허 출원 공개 번호 2004/0148654 및 WO01/36596; 사이토키닌 발현이 변형되어 가뭄 내성과 같은 스트레스 내성이 증가된 식물, 및/또는 수확량 증가로 이어짐을 보여주는 WO2000/006341, WO04/090143, 미국 특허 출원 일련번호

10/817483 및 미국 특허 번호 6,992,237 참조(이들의 전문은 본원에 참조로 포함됨). 또한, 본원에 전문이 참조로 포함되는 WO0202776, WO2003052063, JP2002281975, 미국 특허 번호 6,084,153, WO0164898, 미국 특허 번호 6,177,275 및 미국 특허 번호 6,107,547(질소 이용률 향상 및 변경된 질소 반응성) 참조. 에틸렌 변경에 대해서는, 본원에 전문이 참조로 포함되는 미국 특허 출원 공개 번호 2004/0128719, 미국 특허 출원 공개 번호 2003/0166197, 및 WO200032761 참조. 무생물 스트레스의 식물 전사 인자 또는 전사 조절 인자에 대해서는, 본원에 전문이 참조로 포함되는 미국 특허 출원 공개 번호 2004/0098764 또는 미국 특허 출원 공개 번호 2004/0078852 참조.

[0175] 6. 식물 성장에 영향을 미치고 수확량, 개화, 식물 성장, 및/또는 식물 구조와 같은 작물학적 형질에 영향을 미치는 기타 유전자 및 전사 인자가 식물에 도입 또는 혼입될 수 있다(예를 들어, 본원에 전문이 참조로 포함되는 WO97/49811(LHY), WO98/56918(ESD4), WO97/10339, 및 미국 특허 번호 6,573,430(TFL), 미국 특허 번호 6,713,663(FT), WO96/14414(CON), WO96/38560, WO01/21822(VRN1), WO00/44918(VRN2), WO99/49064(GI), WO00/46358(FRI), WO97/29123, 미국 특허 번호 6,794,560, 미국 특허 번호 6,307,126(GAI), WO99/09174 (D8 및 Rht), WO2004076638 및 WO2004031349(전사 인자) 참조).

[0176] 본원에 개시된 프로모터 서열에 작동 가능하게 연결되는 이종 뉴클레오티드 서열 및 이와 관련된 생물학적 활성 단편 또는 변이체는 표적 유전자에 대한 안티센스 서열일 수 있다. 용어 "안티센스 DNA 뉴클레오티드 서열"은 뉴클레오티드 서열의 5'에서 3'으로의 정상적인 방향에 대해 반대 방향인 서열을 의미하는 것이다. 식물 세포 내로 전달되는 경우, 안티센스 DNA 서열의 발현은 표적 유전자에 대한 DNA 뉴클레오티드 서열의 정상적인 발현을 막는다. 안티센스 뉴클레오티드 서열은 표적 유전자에 대한 DNA 뉴클레오티드 서열의 전사에 의해 생성되는 내인성 메신저 RNA(mRNA)에 상보적이고 혼성화될 수 있는 RNA 전사체를 암호화한다. 이 경우, 표적 유전자에 의해 암호화되는 천연 단백질의 생성은 원하는 표현형 반응을 달성하도록 억제된다. 서열이 해당 mRNA에 혼성화되고 그 발현을 방해하는 한, 안티센스 서열의 변형은 이루어질 수 있다. 이러한 방식으로, 해당 안티센스 서열과 70%, 80%, 85%의 서열 동일성을 갖는 안티센스 구축물이 사용될 수 있다. 또한, 안티센스 뉴클레오티드의 일부는 표적 유전자의 발현을 방해하는 데 사용될 수 있다. 일반적으로, 적어도 50개의 뉴클레오티드, 100개의 뉴클레오티드, 200개 이상의 뉴클레오티드의 서열이 사용될 수 있다. 따라서, 본원에 개시된 프로모터 서열은 안티센스 DNA 서열에 작동 가능하게 연결되어 식물에서 천연 단백질의 발현을 감소시키거나 억제할 수 있다.

[0177] "RNAi"는 유전자의 발현을 감소시키기 위한 일련의 관련 기술을 의미한다(예를 들어, 본원에 전문이 참조로 포함되는 미국 특허 번호 6,506,559 참조). 다른 명칭으로 언급되는 더 오래된 기술은 현재 동일한 메커니즘을 이용할 것으로 생각되지만, 문헌에서는 다른 명칭으로 사용된다. 이들은 표적 단백질의 발현을 억제할 수 있는 안티센스 RNA 전사체의 생성인 "안티센스 억제", 및 동일하거나 실질적으로 유사한 외래성 또는 내인성 유전자의 발현을 억제할 수 있는 센스 RNA 전사체의 생성을 의미하는 "보조 억제" 또는 "센스 억제"를 포함한다(본원에 전문이 참조로 포함되는 미국 특허 번호 5,231,020). 이러한 기술은 침묵될 표적 유전자에 상보적인 하나의 가닥을 갖는 이중가닥 RNA의 축적을 생기게 하는 구축물의 사용을 필요로 한다. 본 발명의 프로모터 서열은, 마이크로RNA 및 siRNA를 포함하는 RNA 간섭을 생기게 할 구축물의 발현을 유도하기 위해 사용될 수 있다.

[0178] 본원에 사용된 용어 "프로모터" 또는 "전사 개시 영역"은 특정 코딩 서열에 대해 적절한 전사 개시 부위에서 RNA 합성을 개시하도록 RNA 중합효소 II에 명령할 수 있는 TATA 박스 또는 DNA 서열을 대개 포함하는 DNA의 조절 영역을 의미한다. 프로모터는 RNA 합성을 개시하도록 RNA 중합효소 II에 명령할 수 있는 TATA 박스 또는 DNA 서열의 상류 또는 5'에 일반적으로 위치하는, 상류 프로모터 요소로 지칭되는 다른 인식 서열을 추가로 포함할 수 있고, 이들은 전사 개시 속도에 영향을 미친다. 본원에 개시된 프로모터 영역에 대한 뉴클레오티드 서열을 확인한 경우, 본원에서 확인된 특정 프로모터 영역으로부터 상류의 5' 미번역 영역에서 추가의 프로모터를 단리하고 동정하는 것은 최신 기술에 속하는 것으로 인식된다. 또한, 키메라 프로모터가 제공될 수 있다. 이러한 키메라는 이종 전사 조절 영역의 단편 및/또는 변이체에 융합된 프로모터 서열의 일부를 포함한다. 따라서, 본원에 개시된 프로모터 영역은 코딩 서열, 인핸서 등의 조직 및 일시적 발현을 담당하는 것들과 같은 상류 프로모터를 포함할 수 있다.

[0179] 본원에 사용된 용어 "조절 요소"는 또한, 항상 그런 것은 아니지만 일반적으로 구조 유전자의 코딩 서열의 상류(5')에 있는 DNA의 서열을 의미하며, 이는 RNA 중합효소 및/또는 특정 부위에서 전사를 개시하는 데 필요한 다른 인자에 대한 인식을 제공함으로써 코딩 영역의 발현을 제어하는 서열을 포함한다. 특정 부위에서의 개시를 보장하도록 RNA 중합효소 또는 다른 전사 인자에 대한 인식을 제공하는 조절 요소의 예는 프로모터 요소이다. 프로모터 요소는 전사의 개시를 담당하는 코어 프로모터 요소뿐만 아니라, 유전자 발현을 변형시키는 다른 조절 요소를 포함한다. 코딩 영역 서열의 인트론 또는 3' 내에 위치한 뉴클레오티드 서열은 또한 관심 코딩 영역의

발현 조절에 기여할 수 있음을 이해해야 한다. 적합한 인트론의 예는 옥수수 IVS6 인트론, 또는 옥수수 액틴 인트론을 포함하나, 이에 한정되지 않는다. 조절 요소는 또한, 전사 개시 부위의 하류(3') 및/또는 전사된 영역 내에 위치한 요소를 포함할 수 있다. 본 발명과 관련하여, 전사후 조절 요소는 전사 개시 후에 활성인 요소, 예를 들어 번역 및 전사 인핸서, 번역 및 전사 리프레서, 및 mRNA 안정성 결정 인자를 포함할 수 있다.

[0180] 본 발명의 프로모터 또는 이들의 변이체 또는 단편은 이종 조절 요소 또는 프로모터와 작동적으로 결합되어 이종 조절 요소의 활성을 조절할 수 있다. 이러한 조절은 이종 조절 요소의 전사 활성의 강화 또는 억제, 및/또는 전사후 이벤트의 조절을 포함한다. 예를 들어, 본 발명의 하나 이상의 프로모터 또는 이들의 단편은 항시성, 유도성, 또는 조직-특이적 프로모터 또는 이들의 단편과 작동적으로 결합되어 식물 세포의 원하는 조직 내에서 이러한 프로모터의 활성을 조절할 수 있다.

[0181] 본 발명의 프로모터 서열 또는 이들의 변이체 또는 단편은, 형태형성 유전자 및/또는 이종의 관심 뉴클레오티드 서열에 작동 가능하게 연결될 경우, 식물의 L1 조직에서 형태형성 유전자 및/또는 이종 뉴클레오티드 서열의 안정적 또는 일시적 발현을 유도할 수 있다.

[0182] 본 명세서 전체에 걸쳐 사용된 "이종 뉴클레오티드 서열", "이종의 관심 폴리뉴클레오티드", 또는 "이종 폴리뉴클레오티드"는 본 발명의 프로모터 서열에 작동 가능하게 연결되거나 이와 자연적으로 발생하지 않는 서열이다. 이러한 뉴클레오티드 서열은 프로모터 서열에 이종성이지만, 식물 숙주에 상동성이거나 고유하거나 이종성이거나 외래성일 수 있다. 마찬가지로, 프로모터 서열은 식물 숙주 및/또는 관심 폴리뉴클레오티드에 상동성이거나 고유하거나 이종성이거나 외래성일 수 있다.

[0183] 본 발명의 단리된 프로모터 서열은 이종 뉴클레오티드 서열의 다양한 발현 수준을 제공하도록 변형될 수 있다. 따라서, 전체 프로모터 영역보다 적게 이용될 수 있고, 관심 뉴클레오티드 서열의 발현을 유도하는 능력이 유지된다. mRNA의 발현 수준은 프로모터 서열의 일부의 결실에 따라 상이한 방식으로 변경될 수 있는 것으로 인식된다. mRNA 발현 수준은 감소될 수 있거나, 대안적으로, 예를 들어 절단 프로세스 중에 제거되는 (리프레서에 대해) 부정적인 조절 요소가 존재하는 경우, 프로모터 결손으로 인해 발현이 증가될 수 있다. 일반적으로, 단리된 프로모터 서열의 적어도 약 20개의 뉴클레오티드가 뉴클레오티드 서열의 발현을 유도하는 데 사용될 것이다.

[0184] 전사 수준을 높이기 위해 본 발명의 프로모터 영역과 함께 인핸서가 이용될 수 있는 것으로 인식된다. 인핸서는 프로모터 영역의 발현을 증가시키는 작용을 하는 뉴클레오티드 서열이다. 인핸서는 당업계에 알려져 있으며, SV40 인핸서 영역, 35S 인핸서 요소 등을 포함한다. 일부 인핸서는, 예를 들어 인핸서 없이 동일한 프로모터가 하나의 특정 조직 또는 소수의 특정 조직에서만 발현되는 경우 프로모터를 항시적으로 발현시킴으로써, 정상적인 프로모터 발현 패턴을 변경하는 것으로 알려져 있다.

[0185] 본 발명의 단리된 프로모터 서열의 변형은 이종 뉴클레오티드 서열의 다양한 발현을 제공할 수 있다. 따라서, 이들은 약한 프로모터 또는 강한 프로모터로 변형될 수 있다. 일반적으로, "약한 프로모터"는 낮은 수준의 코딩 서열 발현을 유도하는 프로모터를 의미한다. "낮은 수준"의 발현은 약 1/10,000 전사 내지 약 1/100,000 전사 내지 약 1/500,000 전사 수준의 발현을 의미하는 것이다. 반대로, 강한 프로모터는 높은 수준, 또는 약 1/10 전사 내지 약 1/100 전사 내지 약 1/1,000 전사 수준의 코딩 서열 발현을 유도한다.

[0186] 본 발명의 프로모터는 천연 코딩 서열과 함께 사용되어 발현을 증가 또는 감소시킴으로써 형질전환 식물의 표현형을 변화시킬 수 있는 것으로 인식된다. 본원에 개시된 뉴클레오티드 서열(표 1 참조), 뿐만 아니라 이들의 변이체 및 단편은 임의의 식물의 유전자 조작에 유용하다. 조절 서열은, 원하는 표현형 반응을 달성하도록 발현이 제어되어야 하는 이종 뉴클레오티드 서열과 작동 가능하게 연결되는 경우 이러한 양태에서 유용하다. 용어 "작동 가능하게 연결"은 이종 뉴클레오티드 서열의 전사 또는 번역이 프로모터 서열의 영향을 받고 있음을 의미한다. 이러한 방식으로, 본 발명의 프로모터에 대한 뉴클레오티드 서열은 관심 식물에서의 발현을 위해, 보다 구체적으로는 식물의 생식 조직에서의 발현을 위해 이종의 관심 뉴클레오티드 서열과 함께 발현 카세트에 제공될 수 있다.

[0187] 본 발명의 일 양태에서, 발현 카세트는, 형태형성 유전자 및/또는 이종 뉴클레오티드 서열에 작동 가능하게 연결되는, 본 발명의 프로모터 뉴클레오티드 서열 또는 이들의 변이체 또는 단편 중 하나를 포함하는 전사 개시 영역을 포함한다. 이러한 발현 카세트는 조절 영역의 전사 조절하에 있는 뉴클레오티드 서열의 삽입을 위한 복수의 제한 부위를 구비할 수 있다. 발현 카세트는 3' 종결 영역뿐만 아니라 선택 가능한 마커 유전자를 추가로 포함할 수 있다.

[0188] 발현 카세트는 5'-3' 전사 방향으로, 전사 개시 영역(즉, 본 발명의 프로모터, 또는 이의 변이체 또는 단편),

번역 개시 영역, 이종의 관심 뉴클레오티드 서열, 번역 종결 영역, 및 선택적으로 숙주 유기체에서 기능하는 전사 종결 영역을 포함할 수 있다. 상기 양태의 조절 영역(즉, 프로모터, 전사 조절 영역, 및 번역 종결 영역) 및/또는 폴리뉴클레오티드는 숙주 세포에 대해 또는 서로에 대해 고유/유사한 것일 수 있다. 대안적으로, 상기 양태의 조절 영역 및/또는 폴리뉴클레오티드는 숙주 세포에 대해 또는 서로에 대해 이종성일 수 있다. 본원에 사용된 바와 같이, 서열과 관련하여 "이종성"은 외래종에서 유래된 서열이거나, 또는 동일한 종에서 유래된 경우 의도적인 인간의 개입에 의해 조성물 및/또는 게놈 유전자좌의 고유 형태로부터 실질적으로 변형된 서열이다. 예를 들어, 이종 폴리뉴클레오티드에 작동 가능하게 연결된 프로모터는 폴리뉴클레오티드가 유래된 종과 상이한 종에서 유래되거나, 또는 동일/유사한 종에서 유래된 경우 하나 또는 둘 모두는 원래의 형태 및/또는 게놈 유전자좌로부터 실질적으로 변형되거나 프로모터는 작동 가능하게 연결된 폴리뉴클레오티드에 대한 천연 프로모터가 아니다.

[0189] 종결 영역은 전사 개시 영역에 고유한 것이거나, 작동 가능하게 연결된 관심 DNA 서열에 고유한 것이거나, 식물 숙주에 고유한 것이거나, 또는 다른 공급원에서 유래된 것일 수 있다(즉, 프로모터, 발현되는 DNA 서열, 식물 숙주, 또는 이들의 임의의 조합에 대해 외래성 또는 이종성). 간편한 종결 영역은 옥토핀 신타제 및 노팔린 신타제 종결 영역과 같은 A. 투메파시엔스의 Ti-플라스미드로부터 이용 가능하다. 또한, 본원에 전문이 참조로 포함되는 문헌[Guerineau, *et al.*, (1991) *Mol. Gen. Genet.* 262:141-144; Proudfoot, (1991) *Cell* 64:671-674; Sanfacon, *et al.*, (1991) *Genes Dev.* 5:141-149; Mogen, *et al.*, (1990) *Plant Cell* 2:1261-1272; Munroe, *et al.*, (1990) *Gene* 91:151-158; Ballas, *et al.*, (1989) *Nucleic Acids Res.* 17:7891-7903; 및 Joshi, *et al.*, (1987) *Nucleic Acid Res.* 15:9627-9639] 참조.

[0190] 본 발명의 서열을 포함하는 발현 카세트는 또한, 유기체 내로 공동 형질전환될 적어도 하나의 추가 유전자 뉴클레오티드 서열, 이종 뉴클레오티드 서열, 이종의 관심 폴리뉴클레오티드, 또는 이종 폴리뉴클레오티드를 포함할 수 있다. 대안적으로, 추가의 뉴클레오티드 서열(들)은 다른 발현 카세트에 제공될 수 있다.

[0191] 적절한 경우, 본 발명의 조직-선호적 프로모터 서열의 제어하에 발현되는 뉴클레오티드 서열, 및 임의의 추가 뉴클레오티드 서열(들)은 형질전환 식물에서의 발현 증가를 위해 최적화될 수 있다. 즉, 이들 뉴클레오티드 서열은 개선된 발현을 위해 식물-선호적 코돈을 사용하여 합성될 수 있다. 숙주-선호적 코돈 사용의 논의에 대해서는, 예를 들어 본원에 전문이 참조로 포함되는 문헌[Campbell and Gowri, (1990) *Plant Physiol.* 92:1-11] 참조. 식물-선호적 유전자를 합성하기 위한 방법은 당업계에서 이용 가능하다. 예를 들어, 본원에 전문이 참조로 포함되는 미국 특허 번호 5,380,831, 5,436,391 및 문헌[Murray, *et al.*, (1989) *Nucleic Acids Res.* 17:477-498] 참조.

[0192] 추가의 서열 변형은 세포 숙주에서 유전자 발현을 향상시키는 것으로 알려져 있다. 이들은 가짜 폴리아데닐화 신호를 암호화하는 서열, 엑손-인트론 스플라이스 부위 신호, 전이인자 유사 반복서열, 및 유전자 발현에 유해할 수 있는 기타 이러한 잘 특성화된 서열의 제거를 포함한다. 이종 뉴클레오티드 서열의 G-C 함량은 숙주 세포에서 발현되는 공지된 유전자를 참조하여 계산되는 주어진 세포 숙주에 대한 평균 수준으로 조정될 수 있다. 가능한 경우, 서열은 예측된 헤어핀 2차 mRNA 구조를 피하도록 변형된다.

[0193] 발현 카세트는 5' 리더 서열을 추가로 포함할 수 있다. 이러한 리더 서열은 번역을 향상시키는 작용을 할 수 있다. 번역 리더는 당업계에 알려져 있으며, 피코르나바이러스 리더, 예를 들어 EMCV 리더(뇌심근염 5' 비코딩 영역)(Elroy-Stein, *et al.*, (1989) *Proc. Nat. Acad. Sci. USA* 86:6126-6130); 포티바이러스 리더, 예를 들어 TEV 리더(담배 에칭 바이러스)(Allison, *et al.*, (1986) *Virology* 154:9-20); MDMV 리더(옥수수 위축 모자이크 바이러스); 인간 면역글로불린 중쇄 결합 단백질(BiP)(Macejak, *et al.*, (1991) *Nature* 353:90-94); 알팔파 모자이크 바이러스의 외피 단백질 mRNA로부터의 미번역 리더(AMV RNA 4)(Jobling, *et al.*, (1987) *Nature* 325:622-625); 담배 모자이크 바이러스 리더(TMV)(Gallie, *et al.*, (1989) *Molecular Biology of RNA,* pages 237-256), 및 옥수수 백화 얼룩 바이러스 리더(MCMV)(Lommel, *et al.*, (1991) *Virology* 81:382-385)를 제한 없이 포함하며, 이들 문헌의 전문은 본원에 참조로 포함된다. 또한, 본원에 전문이 참조로 포함되는 문헌[Della-Cioppa, *et al.*, (1987) *Plant Physiology* 84:965-968] 참조. mRNA 안정성을 향상시키는 것으로 알려진 방법, 예를 들어 옥수수 유비퀴틴 인트론(Christensen and Quail, (1996) *Transgenic Res.* 5:213-218; Christensen, *et al.*, (1992) *Plant Molecular Biology* 18:675-689) 또는 옥수수 AdhI 인트론(Kyozuka, *et al.*, (1991) *Mol. Gen. Genet.* 228:40-48; Kyozuka, *et al.*, (1990) *Maydica* 35:353-357)과 같은 인트론 등이 또한 이용될 수 있으며, 이들 문헌의 전문은 본원에 참조로 포함된다.

[0194] 상기 양태의 DNA 구축물은 또한, 필요에 따라 인핸서(번역 또는 전사 인핸서)를 추가로 포함할 수 있다. 이러한

인핸서 영역은 당업자에게 잘 알려져 있으며, ATG 개시 코돈 및 인접 서열을 포함할 수 있다. 개시 코돈은 전체 서열의 번역을 보장하기 위해 코딩 서열의 리딩 프레임과 동일한 위상이어야 한다. 번역 제어 신호 및 개시 코돈은 천연 및 합성의 다양한 기원으로부터 유래될 수 있다. 번역 개시 영역은 전사 개시 영역의 공급원로부터, 또는 구조 유전자로부터 제공될 수 있다. 서열은 또한, 유전자를 발현시키도록 선택된 조절 요소로부터 유래될 수 있으며, mRNA의 번역을 증가시키도록 특이적으로 변형될 수 있다. 전사 수준을 높이기 위해 상기 양태의 프로모터 영역과 함께 인핸서가 이용될 수 있는 것으로 인식된다. 인핸서는 당업계에 알려져 있으며, SV40 인핸서 영역, 35S 인핸서 요소 등을 포함한다.

[0195] 발현 카세트 제조시, DNA 서열을 적절한 방향으로, 적절한 리딩 프레임에서 적절히 제공하기 위해 다양한 DNA 단편이 조작될 수 있다. 이를 위해, 어댑터 또는 링커를 사용하여 DNA 단편일 연결하거나, 간편한 제한 부위, 불필요한 DNA의 제거, 제한 부위의 제거 등을 제공하기 위한 다른 조작이 수반될 수 있다. 이를 위해, 시험관내 돌연변이 유도, 프라이머 복구, 제한, 어닐링, 재치환, 예를 들어 전이 및 전환이 수반될 수 있다.

[0196] 리포터 유전자 또는 선택 가능한 마커 유전자가 본 발명의 발현 카세트에 또한 포함될 수 있다. 당업계에 알려진 적합한 리포터 유전자의 예는, 예를 들어 본원에 전문이 참조로 포함되는 문헌[Jefferson, *et al.*, (1991), Plant Molecular Biology Manual, ed. Gelvin, *et al.*, (Kluwer Academic Publishers), pp. 1-33; DeWet, *et al.*, (1987) *Mol. Cell. Biol.* 7:725-737; Goff, *et al.*, (1990) *EMBO J.* 9:2517-2522; Kain, *et al.*, (1995) *Bio Techniques* 19:650-655 및 Chiu, *et al.*, (1996) *Current Biology* 6:325-330]에서 확인할 수 있다.

[0197] 형질전환 세포 또는 조직의 선별을 위한 선택 가능한 마커 유전자는 항생제 저항성 또는 제초제에 대한 저항성을 부여하는 유전자를 포함할 수 있다. 적합한 선택 가능한 마커 유전자의 예는 클로람페니콜에 대한 저항성을 암호화하는 유전자(Herrera Estrella, *et al.*, (1983) *EMBO J.* 2:987-992); 메토트렉세이트(Herrera Estrella, *et al.*, (1983) *Nature* 303:209-213; Meijer, *et al.*, (1991) *Plant Mol. Biol.* 16:807-820); 히그로마이신(Waldron, *et al.*, (1985) *Plant Mol. Biol.* 5:103-108 및 Zhijian, *et al.*, (1995) *Plant Science* 108:219-227); 스트렙토마이신(Jones, *et al.*, (1987) *Mol. Gen. Genet.* 210:86-91); 스펙티노마이신(Bretagne-Sagnard, *et al.*, (1996) *Transgenic Res.* 5:131-137); 블레오마이신(Hille, *et al.*, (1990) *Plant Mol. Biol.* 7:171-176); 설폰아미드(Guerineau, *et al.*, (1990) *Plant Mol. Biol.* 15:127-36); 브로목시닐(Stalker, *et al.*, (1988) *Science* 242:419-423); 글리포세이트(Shaw, *et al.*, (1986) *Science* 233:478-481 및 미국 특허 출원 일련번호 10/004,357 및 10/427,692); 포스피노트리신(DeBlock, *et al.*, (1987) *EMBO J.* 6:2513-2518)을 포함하나, 이에 한정되지 않으며, 이들 문헌의 전문은 본원에 참조로 포함된다.

[0198] 유전자이식 이벤트의 재생에 유용할 수 있는 다른 유전자는 GUS(베타-글루쿠로니다제; Jefferson, (1987) *Plant Mol. Biol. Rep.* 5:387), GFP(녹색 형광 단백질; Chalfie, *et al.*, (1994) *Science* 263:802), 루시퍼라제(Riggs, *et al.*, (1987) *Nucleic Acids Res.* 15(19):8115 및 Luehrsen, *et al.*, (1992) *Methods Enzymol.* 216:397-414) 및 안토시아닌 생성을 암호화하는 옥수수 유전자(Ludwig, *et al.*, (1990) *Science* 247:449)와 같은 예를 포함하나, 이에 한정되지 않으며, 이들 문헌의 전문은 본원에 참조로 포함된다.

[0199] 형태형성 유전자에 작동 가능하게 연결되고, 선택적으로 이종 뉴클레오티드 서열, 이종의 관심 폴리뉴클레오티드, 이종 폴리뉴클레오티드 뉴클레오티드, 또는 관심 서열에 추가로 작동 가능하게 연결되는 본 발명의 프로모터 서열을 포함하는 발현 카세트는 임의의 식물을 형질전환시키는 데 사용될 수 있다. 이러한 방식으로, 유전자 변형된 식물, 식물 세포, 식물 조직, 종자, 뿌리 등을 얻을 수 있다.

[0200] 본원에 사용된 바와 같이, "벡터"는 숙주 세포에 뉴클레오티드 구축물, 예를 들어 발현 카세트 또는 구축물을 도입하기 위한 플라스미드, 코스미드, 또는 박테리아 파지와 같은 DNA 분자를 지칭한다. 클로닝 벡터는 일반적으로, 외래 DNA 서열이 벡터의 본질적인 생물학적 기능의 손실 없이 확인 가능한 방식으로 삽입될 수 있는 하나 또는 소수의 제한 엔도뉴클레아제 인식 부위, 뿐만 아니라 클로닝 벡터로 형질전환된 세포의 동정 및 선별에 사용하기에 적합한 마커 유전자를 포함한다. 마커 유전자는 일반적으로, 테트라사이클린 저항성, 히그로마이신 저항성, 또는 암피실린 저항성을 제공하는 유전자를 포함한다.

[0201] 본 발명의 방법은 폴리펩티드 또는 폴리뉴클레오티드를 식물에 도입하는 것을 수반한다. 본원에 사용된 바와 같이, "도입"은 식물 세포의 내부로 서열이 접근하는 방식으로 식물에 폴리뉴클레오티드 또는 폴리펩티드를 제공하는 것을 의미한다. 본 발명의 방법은 폴리뉴클레오티드 또는 폴리펩티드가 식물의 적어도 하나의 세포의 내부로 접근하는 한, 식물에 서열을 도입하기 위한 특정 방법에 의존하지 않는다. 폴리뉴클레오티드 또는 폴리펩티드를 식물에 도입하는 방법은 당업계에 알려져 있으며, 안정적인 형질전환 방법, 일시적 형질전환 방법, 및 바이러스-매개 방법을 포함하나, 이에 한정되지 않는다.

[0202] "안정적인 형질전환"은 식물에 도입된 뉴클레오티드 구축물이 식물의 게놈에 통합되어 자손에 유전될 수 있는 형질전환이다. "일시적 형질전환"은 폴리뉴클레오티드가 식물에 도입되고 식물의 게놈에 통합되지 않거나 폴리펩티드가 식물에 도입되는 것을 의미한다.

[0203] 형질전환 프로토콜뿐만 아니라 뉴클레오티드 서열을 식물에 도입하기 위한 프로토콜은 형질전환 대상 식물 또는 식물 세포의 유형(즉, 외떡잎식물 또는 쌍떡잎식물)에 따라 달라질 수 있다. 뉴클레오티드 서열을 식물 세포에 도입하고 후속적으로 식물 게놈에 삽입하는 적합한 방법은 마이크로주입(Crossway, *et al.*, (1986) *Biotechniques* 4:320-334), 전기천공법(Riggs, *et al.*, (1986) *Proc. Natl. Acad. Sci. USA* 83:5602-5606), 아그로박테리움-매개 형질전환(Townsend, *et al.*, 미국 특허 번호 5,563,055 및 Zhao, *et al.*, 미국 특허 번호 5,981,840), 유전자 직접전달(Paszkowski, *et al.*, (1984) *EMBO J.* 3:2717-2722), 탄도 입자 가속(예를 들어, 미국 특허 번호 4,945,050; 5,879,918; 5,886,244; 5,932,782; 문헌[Tomes, *et al.*, (1995), Plant Cell, Tissue, and Organ Culture: Fundamental Methods, ed. Gamborg and Phillips (Springer-Verlag, Berlin); McCabe, *et al.*, (1988) *Biotechnology* 6:923-926)] 참조), 및 Lec1 형질전환(WO 00/28058)을 포함한다. 또한, 본원에 전문이 참조로 포함되는 문헌[Weissinger, *et al.*, (1988) *Ann. Rev. Genet.* 22:421-477; Sanford, *et al.*, (1987) *Particulate Science and Technology* 5:27-37(양파); Christou, *et al.*, (1988) *Plant Physiol.* 87:671-674(대두); McCabe, *et al.*, (1988) *Bio/Technology* 6:923-926(대두); Finer and McMullen, (1991) *In Vitro Cell Dev. Biol.* 27P:175-182(대두); Singh, *et al.*, (1998) *Theor. Appl. Genet.* 96:319-324(대두); Datta, *et al.*, (1990) *Biotechnology* 8:736-740(벼); Klein, *et al.*, (1988) *Proc. Natl. Acad. Sci. USA* 85:4305-4309(옥수수); Klein, *et al.*, (1988) *Biotechnology* 6:559-563(옥수수)]; 미국 특허 번호 5,240,855; 5,322,783 및 5,324,646; 문헌[Klein, *et al.*, (1988) *Plant Physiol.* 91:440-444(옥수수); Fromm, *et al.*, (1990) *Biotechnology* 8:833-839(옥수수); Hooykaas-Van Slogteren, *et al.*, (1984) *Nature* (London) 311:763-764]; 미국 특허 번호 5,736,369(곡물); 문헌[Bytebier, *et al.*, (1987) *Proc. Natl. Acad. Sci. USA* 84:5345-5349(백합과); De Wet, *et al.*, (1985), The Experimental Manipulation of Ovule Tissues, ed. Chapman, *et al.*, (Longman, New York), pp. 197-209(꽃가루); Kaeppler, *et al.*, (1990) *Plant Cell Reports* 9:415-418 및 Kaeppler, *et al.*, (1992) *Theor. Appl. Genet.* 84:560-566(휘스커-매개 형질전환); D'Halluin, *et al.*, (1992) *Plant Cell* 4:1495-1505(전기천공법); Li, *et al.*, (1993) *Plant Cell Reports* 12:250-255 및 Christou and Ford, (1995) *Annals of Botany* 75:407-413(벼); Osjoda, *et al.*, (1996) *Nature Biotechnology* 14:745-750(아그로박테리움 투메파시엔스를 통한 옥수수)] 참조. 빠른 식물 형질전환을 위한 방법 및 조성물은 본원에 전문이 참조로 포함되는 U.S. 2017/0121722에서 또한 확인된다. 식물 형질전환에 유용한 벡터는 본원에 전문이 참조로 포함되는 미국 특허 출원 일련번호 15/765,521에서 확인된다.

[0204] 특정 양태에서, 본 발명의 프로모터 서열을 포함하는 DNA 구축물은 다양한 일시적 형질전환 방법을 이용해 식물에 제공될 수 있다. 이러한 일시적 형질전환 방법은 바이러스 벡터 시스템, 및 DNA의 후속 방출을 배제하는 방식으로 폴리뉴클레오티드를 침전시키는 것을 포함하나, 이에 한정되지 않는다. 따라서, 입자 결합 DNA로부터 전사가 일어날 수 있지만, DNA가 방출되어 게놈에 통합되는 빈도는 크게 줄어든다. 이러한 방법은 폴리에틸이민(PEI; Sigma #P3143)으로 코팅된 입자의 사용을 포함한다.

[0205] 다른 양태에서, 본 발명의 폴리뉴클레오티드는 식물을 바이러스 또는 바이러스 핵산과 접촉시켜 식물에 도입될 수 있다. 일반적으로, 이러한 방법은 본 발명의 뉴클레오티드 구축물을 바이러스 DNA 또는 RNA 분자 내에 혼입시키는 것을 수반한다. 폴리뉴클레오티드를 식물에 도입하고, 바이러스 DNA 또는 RNA 분자를 포함하여 그 안에 암호화된 단백질을 발현시키는 방법은 당업계에 알려져 있다. 예를 들어, 본원에 전문이 참조로 포함되는 미국 특허 번호 5,889,191, 5,889,190, 5,866,785, 5,589,367, 5,316,931 및 문헌[Porta, *et al.*, (1996) *Molecular Biotechnology* 5:209-221] 참조.

[0206] 폴리뉴클레오티드를 식물 게놈의 특정 위치에 표적화 삽입하는 방법은 당업계에 알려져 있다. 일 양태에서, 폴리뉴클레오티드를 원하는 게놈 위치에 삽입하는 것은 부위-특이적 재조합 시스템을 이용하여 달성된다. 예를 들어, 본원에 전문이 참조로 포함되는 WO99/25821, WO99/25854, WO99/25840, WO99/25855, 및 WO99/25853 참조. 간략히 요약하면, 본 발명의 폴리뉴클레오티드는 2개의 동일하지 않은 재조합 부위로 플랭킹된 전달 카세트에 포함될 수 있다. 전달 카세트는 전달 카세트의 부위에 해당하는 2개의 동일하지 않은 재조합 부위로 플랭킹된 표적 부위가 게놈에 안정적으로 통합된 식물에 도입된다. 적절한 재조합효소가 제공되고 전달 카세트는 표적 부위에 통합된다. 이에 따라 관심 폴리뉴클레오티드는 식물 게놈의 특정 염색체 위치에 통합된다.

[0207] 형질전환된 세포는 통상적인 방식에 따라 식물로 성장될 수 있다. 예를 들어, 본원에 전문이 참조로 포함되는 문헌[McCormick, *et al.*, (1986) *Plant Cell Reports* 5:81-84] 참조. 이후, 이들 식물은 성장되고, 동일한 형

질전환 균주 또는 상이한 균주로 수분되어, 원하는 표현형 특성이 발현된 자손을 얻을 수 있다. 원하는 표현형 특성의 발현이 안정적으로 유지되고 유전되도록 하기 위해 2대 이상에 걸쳐 성장될 수 있고, 이후 원하는 표현형 특성의 발현이 달성되었음을 확실히 하기 위해 종자가 수확된다. 이러한 방식으로, 본 발명은 본 발명의 뉴클레오티드 구축물, 예를 들어 본 발명의 발현 카세트가 게놈에 안정적으로 통합된 형질전환 종자("유전자이식 종자"라고도 함)를 제공한다.

[0208] 식물 조직으로부터 식물을 재생하기 위한 다양한 방법이 있다. 특정 재생 방법은 출발 식물 조직 및 재생될 특정 식물 종에 따라 달라질 것이다. 단일의 식물 원형질 형질전환체 또는 다양한 형질전환 외식편으로부터의 식물의 재생, 발육, 및 배양은 당업계에 잘 알려져 있다(본원에 전문이 참조로 포함되는 문헌[Weissbach and Weissbach, (1988), Methods for Plant Molecular Biology, (Eds.), Academic Press, Inc., San Diego, Calif.]). 이러한 재생 및 성장 과정은 일반적으로, 형질전환 세포를 선별하고, 개별화된 세포를 통상적인 배발육기를 통해 발근 묘목기를 통해 배양하는 단계를 포함한다. 유전자이식 배아와 종자는 유사하게 재생된다. 이후, 생성된 유전자이식 발근된 싹을 토양과 같은 적절한 식물 성장 배지에 심는다. 바람직하게, 재생 식물은 동형접합 유전자이식 식물을 제공하기 위해 자가 수분된다. 그렇지 않으면, 재생 식물로부터 얻은 꽃가루를 작물학적으로 중요한 계통의 종자 식물과 교배한다. 반대로, 이러한 중요한 계통의 식물에서 나온 꽃가루는 재생 식물을 수분시키기 위해 사용된다. 원하는 폴리뉴클레오티드를 함유하는 상기 양태의 유전자이식 식물은 당업자에게 잘 알려진 방법을 이용해 배양된다.

[0209] 상기 양태는 식물 내에서 발현을 조절하는 화합물을 스크리닝하기 위한 조성물을 제공한다. 벡터, 세포, 및 식물은 본원에 개시된 조절 서열의 작용제 및 길항제를 위한 후보 분자를 스크리닝하는 데 사용될 수 있다. 예를 들어, 리포터 유전자가 조절 서열에 작동 가능하게 연결되어 식물에서 이식유전자로서 발현될 수 있다. 시험될 화합물을 첨가하고 리포터 유전자 발현을 측정하여 프로모터 활성에 대한 영향을 확인한다.

[0210] 일 양태에서, 개시된 방법 및 조성물은 체세포배로부터 유도된 식물의 게놈에서의 변형을 위한 특정 부위를 표적화하는 데 유용한 폴리뉴클레오티드를 증가된 효율과 속도로 체세포배에 도입하기 위해 사용될 수 있다. 개시된 방법 및 조성물로 도입될 수 있는 부위-특이적 변형은 부위-특이적 변형을 도입하기 위한 임의의 방법(유전자 복구 올리고뉴클레오티드의 사용(예를 들어, 미국 공보 2013/0019349), 또는 TALEN, 메가뉴클레아제, 징크 핑거 뉴클레아제, CRISPR-Cas 등과 같은 이중가닥 절단 기술의 사용을 포함하나, 이에 한정되지 않음)을 이용해 생성된 것들을 포함한다. 예를 들어, 개시된 방법 및 조성물은 식물 또는 식물 세포의 게놈에서 표적 서열의 게놈 변형을 위해, 식물 선별을 위해, 염기 또는 서열의 제거를 위해, 유전자 편집을 위해, 식물 또는 식물 세포의 게놈에 관심 폴리뉴클레오티드를 삽입하기 위해, 식물 세포 또는 식물에 CRISPR-Cas 시스템을 도입하는 데 사용될 수 있다. 따라서, 개시된 방법 및 조성물은 식물, 식물 세포 또는 종자의 게놈 내 표적 부위 및 관심 뉴클레오티드의 변형 또는 변경을 위한 효과적인 시스템을 제공하기 위해 CRISPR-Cas 시스템과 함께 사용될 수 있다. Cas 엔도뉴클레아제 유전자는 식물 최적화 Cas9 엔도뉴클레아제이며, 식물 최적화 Cas9 엔도뉴클레아제는 식물 게놈의 게놈 표적 서열에 결합하여 이중가닥 절단을 생성할 수 있다.

[0211] Cas 엔도뉴클레아제는 특정 표적 부위에서 이중가닥 절단을 인식하여 선택적으로 세포의 게놈에 도입하도록 가이드 뉴클레오티드에 의해 가이드된다. CRISPR-Cas 시스템은 식물, 식물 세포 또는 종자의 게놈 내 표적 부위의 변형을 위한 효과적인 시스템을 제공한다. 세포의 게놈 내 표적 부위의 변형 및 세포의 게놈 내 뉴클레오티드 서열의 편집을 위한 효과적인 시스템을 제공하기 위해 가이드 폴리뉴클레오티드/Cas 엔도뉴클레아제 시스템을 이용하는 방법 및 조성물이 또한 제공된다. 게놈 표적 부위가 확인되면, 다양한 관심 폴리뉴클레오티드를 함유하도록 표적 부위를 추가로 변형시키기 위해 다양한 방법이 사용될 수 있다. 개시된 조성물 및 방법은 세포의 게놈 내 뉴클레오티드 서열의 편집을 위한 CRISPR-Cas 시스템을 도입하는 데 사용될 수 있다. 편집될 뉴클레오티드 서열(관심 뉴클레오티드 서열)은 Cas 엔도뉴클레아제에 의해 인식되는 표적 부위 내에 또는 밖에 위치할 수 있다.

[0212] CRISPR 유전자좌(일정한 간격을 두고 규칙적으로 분포하는 짧은 회문 반복서열)(SPIDR(SPacer Interspersed Direct Repeats)로도 알려져 있음)는 최근에 기술된 DNA 유전자좌의 계열을 구성한다. CRISPR 유전자좌는 부분적으로 회문구조인, 짧고 고도로 보존된 DNA 반복서열(일반적으로 24 내지 40 bp, 1 내지 140회 반복되며, CRISPR 반복서열이라고도 함)로 이루어진다. (일반적으로 종에 특이적인) 반복 서열이 일정한 길이(CRISPR 유전자좌에 따라 일반적으로 20 내지 58 bp)의 가변 서열을 사이에 두고 분포된다(2007년 3월 1일 공개된 WO2007/025097).

[0213] CRISPR 유전자좌는 대장균에서 처음 인식되었다(Ishino *et al.* (1987) J. Bacterial. 169:5429-5433; Nakata

et al. (1989) J. Bacterial. 171 :3553-3556). 유사한 산재형 짧은 서열 반복이 문헌[*Haloferax mediterranei*, *Streptococcus pyogenes*, *Anabaena*, and *Mycobacterium tuberculosis* (Groenen *et al.* (1993) Mol. Microbiol. 10:1057-1065; Hoe *et al.* (1999) Emerg. Infect. Dis. 5:254- 263; Masepohl *et al.* (1996) Biochim. Biophys. Acta 1307:26-30; Mojica *et al.* (1995) Mol. Microbiol. 17:85-93)]에서 확인되었다. CRISPR 유전자좌는 다른 SSR과는 반복서열의 구조가 상이해, 일정한 간격을 두고 규칙적으로 분포하는 짧은 반복서열(SRSR)로 명명되었다(Janssen *et al.* (2002) OMICS J. Integ. Biol. 6:23-33; Mojica *et al.* (2000) Mol. Microbiol. 36:244-246). 반복서열은 클러스터에서 발생하는 짧은 요소로서, 항상 일정한 길이의 가변 서열을 사이에 두고 규칙적으로 분포한다(Mojica *et al.* (2000) Mol. Microbiol. 36:244-246).

[0214] Cas 유전자는, 일반적으로 플랭킹 CRISPR 유전자좌에 커플링되거나 결합되거나 가까이 있거나, 또는 그 부근에 있는 유전자를 포함한다. 용어 "Cas 유전자" 및 "CRISPR-결합 (Cas) 유전자"는 본원에서 상호교환적으로 사용된다. Cas 단백질 계열에 대한 포괄적인 리뷰는 문헌[Haft *et al.* (2005) Computational Biology, PLoS Comput Biol 1 (6): e60. doi:10.1371/journal.pcbi.0010060]에 제공된다.

[0215] 초기에 기술된 4개의 유전자 계열 외에, 추가 41개의 CRISPR-결합 (Cas) 유전자 계열이, 본원에 참조로 포함되는 WO/2015/026883에 기술되었다. 이 문헌은 CRISPR 시스템이 상이한 반복 패턴, 유전자 세트, 및 종 범위를 갖는 상이한 부류에 속함을 나타낸다. 주어진 CRISPR 유전자좌에서 Cas 유전자의 수는 종에 따라 다를 수 있다. Cas 엔도뉴클레아제는 Cas 유전자에 의해 암호화된 Cas 단백질과 관련되며, Cas 단백질은 DNA 표적 서열에 이중가닥 절단을 도입할 수 있다. Cas 엔도뉴클레아제는 특정 표적 부위에서 이중가닥 절단을 인식하여 선택적으로 세포의 게놈에 도입하도록 가이드 폴리뉴클레오티드에 의해 가이드된다. 본원에 사용된 용어 "가이드 폴리뉴클레오티드/Cas 엔도뉴클레아제 시스템"은 DNA 표적 서열에 이중가닥 절단을 도입할 수 있는 Cas 엔도뉴클레아제와 가이드 폴리뉴클레오티드의 복합체를 포함한다. Cas 엔도뉴클레아제는 게놈 표적 부위에 아주 근접하여 DNA 듀플렉스를 풀고, 가이드 뉴클레오티드가 표적 서열을 인식하면(단, 정확한 프로토스페이서 인접 모티프(PAM)의 방향이 대략 표적 서열의 3' 말단을 향하는 경우에만) 두 DNA 가닥을 절단한다(2015년 2월 26일 공개된 WO/2015/02688의 도 2A 및 도 2B 참조).

[0216] 일 양태에서, Cas 엔도뉴클레아제 유전자는 Cas9 엔도뉴클레아제(예컨대, 이에 한정되는 것은 아니지만, 본원에 참조로 포함되는 WO2007/025097(2007년 3월 1일 공개)의 서열 번호 462, 474, 489, 494, 499, 505, 및 518에 나열된 Cas9 유전자)이다. 다른 양태에서, Cas 엔도뉴클레아제 유전자는 식물, 옥수수 또는 대두 최적화 Cas9 엔도뉴클레아제(예컨대, 이에 한정되는 것은 아니지만 WO/2015/026883의 도 1A에 표시된 것들)이다. 다른 양태에서, Cas 엔도뉴클레아제 유전자는 Cas 코돈 영역 상류의 SV40 핵 표적화 신호 및 Cas 코돈 영역 하류의 2부분 VirD2 핵 국재화 신호(Tinland *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:7442-6)에 작동 가능하게 연결된다.

[0217] 일 양태에서, Cas 엔도뉴클레아제 유전자는 WO/2015/026883의 서열 번호 1, 124, 212, 213, 214, 215, 216, 193 또는 서열 번호 5의 뉴클레오티드 2037-6329, 또는 이들의 임의의 기능적 단편 또는 변이체의 Cas9 엔도뉴클레아제 유전자이다.

[0218] Cas 엔도뉴클레아제와 관련하여, 용어 "기능적 단편", "기능적으로 동등한 단편", 및 "기능적 동등 단편"은 본원에서 상호교환적으로 사용된다. 이들 용어는 이중가닥 절단을 생성하는 능력이 유지된, 본 발명의 Cas 엔도뉴클레아제 서열의 일부 또는 하위서열을 지칭한다.

[0219] Cas 엔도뉴클레아제와 관련하여, 용어 "기능적 변이체", "기능적으로 동등한 변이체", 및 "기능적 동등 변이체"는 본원에서 상호교환적으로 사용된다. 이들 용어는 이중가닥 절단을 생성하는 능력이 유지된, 본 발명의 Cas 엔도뉴클레아제의 변이체를 지칭한다. 단편 및 변이체는 부위-특이적 돌연변이 유도 및 합성 제조와 같은 방법을 통해 얻을 수 있다.

[0220] 일 양태에서, Cas 엔도뉴클레아제 유전자는 원칙적으로 표적화될 수 있는 N(12~30)NGG 형태의 임의의 게놈 서열을 인식할 수 있는 식물 코돈 최적화 *Streptococcus pyogenes* Cas9 유전자이다.

[0221] 엔도뉴클레아제는 폴리뉴클레오티드 사슬 내 포스포디에스테르 결합을 절단하는 효소로서, 염기를 손상시키지 않고 특정 부위에서 DNA를 절단하는 제한 엔도뉴클레아제를 포함한다. 제한 엔도뉴클레아제는 I형, II형, III형, 및 IV형 엔도뉴클레아제를 포함하고, 이들은 하위유형을 추가로 포함한다. I형 및 III형 시스템에서, 메틸라제 활성과 제한 활성 모두 단일 복합체에 포함된다. 엔도뉴클레아제는 호밍 엔도뉴클레아제(HEase)로도 알려진 메가뉴클레아제도 포함하는데, 이는 제한 엔도뉴클레아제처럼 특정 인식 부위에서 결합하고 절단하지만,

메가뉴클레아제에 대한 인식 부위는 약 18 bp 이상으로 일반적으로 더 길다(2012년 3월 22일 출원된 특허 출원 PCT/US 12/30061). 메가뉴클레아제는 보존된 서열 모티프에 기초하여 4개의 계열로 분류되었는데, 그 계열은 LAGLIDADG, GIY-YIG, H-N-H, 및 His-Cys 박스 계열이다. 이들 모티프는 금속 이온의 배위 및 포스포디에스테르 결합의 가수분해에 참여한다. 메가뉴클레아제는 긴 인식 부위, 및 이의 DNA 기질에서 일부 서열 다형성을 용인하는 것으로 유명하다. 메가뉴클레아제에 대한 명명 규칙은 다른 제한 엔도뉴클레아제에 대한 규칙과 유사하다. 메가뉴클레아제는 또한, 독립형 ORF, 인트론, 및 인테인에 의해 각각 암호화되는 효소에 대한 접두사 F-, I-, 또는 PI-를 특징으로 한다. 재조합 프로세스에서 하나의 단계는 인식 부위 또는 그 근처에서의 폴리뉴클레오티드 절단을 수반한다. 이 절단 활성은 이중가닥 절단을 생성하는 데 이용될 수 있다. 부위-특이적 재조합효소 및 그 인식 부위에 대한 리뷰에 대해서는 문헌[Sauer (1994) Curr Op Biotechnol 5:521 -7; 및 Sadowski (1993) FASEB 7:760-7] 참조. 일부 예에서, 재조합효소는 인테그라제 또는 레솔바제 계열에 속한다. TAL 이펙터 뉴클레아제는 식물 또는 다른 생물의 게놈 내 특정 표적 서열에서 이중가닥 절단을 만들기 위해 사용될 수 있는 새로운 부류의 서열-특이적 뉴클레아제이다(Miller, *et al.* (2011) Nature Biotechnology 29:143-148). 징크 핑거 뉴클레아제(ZFN)는 징크 핑거 DNA 결합 도메인 및 이중가닥 절단 유도제 도메인으로 이루어진 조작된 이중가닥 절단 유도제이다. 인식 부위 특이성은 징크 핑커 도메인에 의해 부여되며, 이는 일반적으로 2, 3, 또는 4개의 징크 핑거를 포함하고, 예를 들어, C2H2 구조를 갖지만, 다른 징크 핑거 구조는 알려져 있고 유전자 조작되었다. 징크 핑거 도메인은 선택된 폴리뉴클레오티드 인식 서열에 특이적으로 결합하는 폴리펩티드를 설계하는 데 쉽게 이용될 수 있다. ZFN은 비특이적 엔도뉴클레아제 도메인, 예를 들어, FokI와 같은 Ms형 엔도뉴클레아제로부터의 뉴클레아제 도메인에 연결된 조작된 DNA-결합 징크 핑거 도메인을 포함한다. 전사 활성물질 도메인, 전사 억제물질 도메인, 및 메틸라제를 포함하여, 추가 기능이 징크 핑거 결합 도메인에 융합될 수 있다. 일부 예에서, 절단 활성을 위해 뉴클레아제 도메인의 이합체화가 요구된다. 각각의 징크 핑거는 표적 DNA에서 3개의 연속 염기쌍을 인식한다. 예를 들어, 뉴클레아제의 이합체화 요건하에, 3-핑거 도메인은 9개 연속 뉴클레오티드의 서열을 인식하고, 18-뉴클레오티드 인식 서열을 결합시키는 데 두 세트의 징크 핑거 트리플렛이 사용된다.

[0222] 박테리아 및 고세균은, 짧은 RNA를 이용해 외래 핵산의 분해를 유도하는, 일정한 간격을 두고 규칙적으로 분포하는 짧은 회문 반복서열(CRISPR)/CRISPR-결합 (Cas) 시스템이라고 하는 적응 면역 방어를 진화시켰다(2007년 3월 1일 공개된 WO2007/025097). 박테리아로부터의 II형 CRISPR/Cas 시스템은 crRNA 및 tracrRNA를 사용하여 Cas 엔도뉴클레아제를 DNA 표적으로 가이드한다. crRNA(CRISPR RNA)는 이중가닥 DNA 표적의 한 가닥에 상보적인 영역 및 Cas 엔도뉴클레아제가 DNA 표적을 절단하도록 유도하는 RNA 듀플렉스를 형성하는 tracrRNA(트랜스-활성화 CRISPR RNA)와의 염기쌍을 포함한다.

[0223] 본원에 사용된 용어 "가이드 뉴클레오티드"는 2개의 RNA 분자, 가변 표적화 도메인을 포함하는 crRNA(CRISPR RNA), 및 tracrRNA의 합성 융합체에 관한 것이다. 일 양태에서, 가이드 뉴클레오티드는 12 내지 30개 뉴클레오티드 서열의 가변 표적화 도메인, 및 Cas 엔도뉴클레아제와 상호작용할 수 있는 RNA 단편을 포함한다.

[0224] 본원에 사용된 용어 "가이드 폴리뉴클레오티드"는, Cas 엔도뉴클레아제와 복합체를 형성할 수 있고 Cas 엔도뉴클레아제가 DNA 표적 부위를 인식하고 선택적으로 절단할 수 있게 하는 폴리뉴클레오티드 서열에 관한 것이다. 가이드 폴리뉴클레오티드는 단일 분자 또는 이중 분자일 수 있다. 가이드 폴리뉴클레오티드 서열은 RNA 서열, DNA 서열, 또는 이들의 조합(RNA-DNA 조합 서열)일 수 있다. 선택적으로, 가이드 폴리뉴클레오티드는 적어도 하나의 뉴클레오티드, 포스포디에스테르 결합 또는 연결 변형, 예컨대 고정 핵산(LNA), 5-메틸 dC, 2,6-디아미노퓨린, 2'-플루오로 A, 2'-플루오로 U, 2'-O-메틸 RNA, 포스포로티오에이트 결합, 콜레스테롤 분자에 대한 연결, 폴리에틸렌 글리콜 분자에 대한 연결, 스페이서 18(헥사에틸렌 글리콜 사슬) 분자에 대한 연결, 또는 고리화를 초래하는 5'에서 3'으로의 공유 연결을 포함할 수 있으나, 이에 한정되지 않는다. 단독으로 리보핵산을 포함하는 가이드 폴리뉴클레오티드는 "가이드 뉴클레오티드"라고도 지칭된다.

[0225] 가이드 폴리뉴클레오티드는 표적 DNA의 뉴클레오티드 서열에 상보적인 제1 뉴클레오티드 서열(가변 표적화 도메인 또는 VT 도메인으로 지칭됨) 및 Cas 엔도뉴클레아제 폴리펩티드와 상호작용하는 제2 뉴클레오티드 서열 도메인(Cas 엔도뉴클레아제 인식 도메인 또는 CER 도메인으로 지칭됨)을 포함하는 이중 분자(듀플렉스 가이드 폴리뉴클레오티드라고도 지칭됨)일 수 있다. 이중 분자 가이드 폴리뉴클레오티드의 CER 도메인은 상보성 영역을 따라 혼성화된 2개의 별개의 분자를 포함한다. 2개의 별개의 분자는 RNA, DNA, 및/또는 RNA-DNA- 조합 서열일 수 있다. 일 양태에서, CER 도메인에 연결된 VT 도메인을 포함하는 듀플렉스 가이드 폴리뉴클레오티드의 제1 분자는 "crDNA"(연속된 DNA 뉴클레오티드들로 구성되는 경우) 또는 "crRNA"(연속된 RNA 뉴클레오티드들로 구성되는 경우), 또는 "crDNA-RNA"(DNA와 RNA 뉴클레오티드의 조합으로 구성되는 경우)로 지칭된다. cr뉴클레오티드는 박

테리아 및 고세균에서 자연적으로 발생하는 cRNA의 단편을 포함할 수 있다. 일 양태에서, 박테리아 및 고세균류에서 자연적으로 발생하고 본원에 개시된 cr뉴클레오티드에 존재하는 cRNA의 단편의 크기는 2, 3, 4, 5, 6, 7, 8, 9,10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20개 이상의 뉴클레오티드 범위일 수 있지만, 이들 범위로 제한되는 것은 아니다.

[0226] 일 양태에서, CER 도메인을 포함하는 듀플렉스 가이드 폴리뉴클레오티드의 제2 분자는 "tracrRNA"(연속된 RNA 뉴클레오티드들로 구성되는 경우) 또는 "tracrDNA"(연속된 DNA 뉴클레오티드들로 구성되는 경우) 또는 "tracrDNA-RNA"(DNA와 RNA 뉴클레오티드의 조합으로 구성되는 경우)로 지칭된다. 일 양태에서, RNA Cas9 엔도뉴클레아제 복합체를 가이드하는 RNA는 듀플렉스 crRNA-tracrRNA를 포함하는 듀플렉스화된 RNA이다.

[0227] 또한, 가이드 폴리뉴클레오티드는 표적 DNA의 뉴클레오티드 서열에 상보적인 (가변 표적화 도메인 또는 VT 도메인으로 지칭되는) 제1 뉴클레오티드 서열 도메인 및 Cas 엔도뉴클레아제 폴리펩티드와 상호작용하는 (Cas 엔도뉴클레아제 인식 도메인 또는 CER 도메인으로 지칭되는) 제2 뉴클레오티드 도메인을 포함하는 단일 분자일 수 있다. "도메인"은 RNA, DNA, 및/또는 RNA-DNA 조합 서열일 수 있는 뉴클레오티드들이 연속되어 있는 것을 의미한다. 단일 가이드 폴리뉴클레오티드의 VT 도메인 및/또는 CER 도메인은 RNA 서열, DNA 서열, 또는 RNA-DNA-조합 서열을 포함할 수 있다. 일 양태에서, 단일 가이드 폴리뉴클레오티드는 (CER 도메인을 포함하는) tracr뉴클레오티드에 연결된 (CER 도메인에 연결된 VT 도메인을 포함하는) cr뉴클레오티드를 포함하며, 이러한 연결은 RNA 서열 DNA 서열, 또는 RNA- DNA 조합 서열을 포함하는 뉴클레오티드 서열이다. cr뉴클레오티드 및 tracr뉴클레오티드로부터의 서열들로 구성되는 단일 가이드 폴리뉴클레오티드는 (연속된 RNA 뉴클레오티드들로 구성되는 경우) "단일 가이드 뉴클레오티드" 또는 (연속된 DNA 뉴클레오티드들로 구성되는 경우) "단일 가이드 DNA" 또는 (RNA와 DNA 뉴클레오티드의 조합으로 구성되는 경우) "단일 가이드 뉴클레오티드-DNA"로 지칭될 수 있다. 본 발명의 일 양태에서, 단일 가이드 뉴클레오티드는 II형 Cas 엔도뉴클레아제와 복합체를 형성할 수 있는 II형 CRISPR/Cas 시스템의 cRNA 또는 cRNA 단편 및 tracrRNA 또는 tracrRNA 단편을 포함하고, 여기서 가이드 뉴클레오티드 Cas 엔도뉴클레아제 복합체는 Cas 엔도뉴클레아제를 식물 게놈 표적 부위로 유도하여, Cas 엔도뉴클레아제가 게놈 표적 부위에 이중가닥 절단을 도입할 수 있게 한다. 듀플렉스 가이드 폴리뉴클레오티드 대비 단일 가이드 폴리뉴클레오티드를 사용하는 일 양태는, 단일 가이드 폴리뉴클레오티드를 발현시키기 위해 하나의 발현 카세트만 제조될 필요가 있다는 것이다.

[0228] 용어 "가변 표적화 도메인" 또는 "VT 도메인"은 본원에서 상호교환적으로 사용되며, 이중가닥 DNA 표적 부위의 한 가닥(뉴클레오티드 서열)에 상보적인 뉴클레오티드 서열을 포함한다. 제1 뉴클레오티드 서열 도메인(VT 도메인)과 표적 서열 간의 상보성 백분율은 적어도 50%, 51 %, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61 %, 62%, 63%, 63%, 65%, 66%, 67%, 68%, 69%, 70%, 71 %, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81 %, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 또는 100%일 수 있다. 가변 표적화 도메인은 적어도 12개, 13개, 14개, 15개, 16개, 17개, 18개, 19개, 20개, 21개, 22개, 23개, 24개, 25개, 26개, 27개, 28개, 29개 또는 30개의 뉴클레오티드 길이일 수 있다. 일 양태에서, 가변 표적화 도메인은 연속된 12개 내지 30개의 뉴클레오티드를 포함한다. 가변 표적화 도메인은 DNA 서열, RNA 서열, 변형된 DNA 서열, 변형된 RNA 서열, 또는 이들의 임의의 조합으로 구성될 수 있다.

[0229] 가이드 폴리뉴클레오티드의 "Cas 엔도뉴클레아제 인식 도메인" 또는 "CER 도메인"이란 용어는 본원에서 상호교환적으로 사용되며, Cas 엔도뉴클레아제 폴리펩티드와 상호작용하는 뉴클레오티드 서열(예컨대, 가이드 폴리뉴클레오티드의 제2 뉴클레오티드 서열 도메인)을 포함한다. CER 도메인은 DNA 서열, RNA 서열, 변형된 DNA 서열, 변형된 RNA 서열(예컨대, 본원에 기술된 변형 참조), 또는 이들의 임의의 조합으로 구성될 수 있다.

[0230] 단일 가이드 폴리뉴클레오티드의 cr뉴클레오티드와 tracr뉴클레오티드를 연결하는 뉴클레오티드 서열은 RNA 서열, DNA 서열, 또는 RNA-DNA 조합 서열을 포함할 수 있다. 일 양태에서, 단일 가이드 폴리뉴클레오티드의 cr뉴클레오티드와 tracr뉴클레오티드를 연결하는 뉴클레오티드 서열은 적어도 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 또는 100개의 뉴클레오티드 길이일 수 있다. 다른 양태에서, 단일 가이드 폴리뉴클레오티드의 cr뉴클레오티드와 tracr뉴클레오티드를 연결하는 뉴클레오티드 서열은 테트라루프 서열, 예컨대, GAAA 테트라루프 서열을 포함할 수 있지만, 이에 한정되는 것은 아니다.

[0231] 가이드 폴리뉴클레오티드, VT 도메인 및/또는 CER 도메인의 뉴클레오티드 서열 변형은 5' 캡, 3' 폴리아데닐화

테일, 리보스위치 서열, 안정성 조절 서열, dsRNA 듀플렉스를 형성하는 서열, 가이드 폴리뉴클레오티드를 세포 내 위치에 표적화하는 변형 또는 서열, 추적을 제공하는 변형 또는 서열, 단백질을 위한 결합 부위를 제공하는 변형 또는 서열, 잠김 핵산(Locked Nucleic Acid, LNA), 5-메틸 dC 뉴클레오티드, 2,6-디아미노퓨린 뉴클레오티드, 2'-플루오로 A 뉴클레오티드, 2'-플루오로 U 뉴클레오티드; 2'-O-메틸 RNA 뉴클레오티드, 포스포로티오에이트 결합, 콜레스테롤 분자에 대한 연결, 폴리에틸렌 글리콜 분자에 대한 연결, 스페이서 18 분자에 대한 연결, 5'에서 3'으로의 공유 연결, 또는 이들의 임의의 조합으로 이루어진 군으로부터 선택될 수 있지만, 이에 한정되는 것은 아니다. 이러한 변형은 적어도 하나의 추가적인 유익한 특징을 가져올 수 있고, 추가적인 유익한 특징은 변형 또는 조절된 안정성, 세포내 표적화, 추적, 형광 표지, 단백질 또는 단백질 복합체에 대한 결합 부위, 상보적인 표적 서열에 대한 변형된 결합 친화도, 세포 분해에 대한 변형된 저항성, 및 증가된 세포 투과성의 군으로부터 선택된다.

[0232] 일 양태에서, 가이드 뉴클레오티드 및 Cas 엔도뉴클레아제는 Cas 엔도뉴클레아제가 DNA 표적 부위에 이중가닥 절단을 도입할 수 있게 하는 복합체를 형성할 수 있다.

[0233] 본 발명의 일 양태에서, 가변 표적화 도메인은 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 또는 30개의 뉴클레오티드 길이이다.

[0234] 본 발명의 일 양태에서, 가이드 뉴클레오티드는 II형 Cas 엔도뉴클레아제와 복합체를 형성할 수 있는 II형 CRISPR/Cas 시스템의 cRNA(또는 cRNA 단편) 및 tracrRNA(또는 tracrRNA 단편)을 포함하고, 여기서 가이드 뉴클레오티드 Cas 엔도뉴클레아제 복합체는 Cas 엔도뉴클레아제를 식물 게놈 표적 부위로 유도하여, Cas 엔도뉴클레아제가 게놈 표적 부위에 이중가닥 절단을 도입할 수 있게 한다. 가이드 뉴클레오티드는 당업계에 알려진 임의의 방법(예컨대, 이에 한정되는 것은 아니지만 유전자총 또는 국소 처리법)을 이용해 직접 식물 또는 식물 세포에 도입될 수 있다.

[0235] 일 양태에서, 가이드 뉴클레오티드는 식물 세포에서 가이드 뉴클레오티드를 전사할 수 있는 식물 특이적 프로모터에 작동 가능하게 연결되는 해당 가이드 DNA 서열을 포함하는 재조합 DNA 분자를 도입함으로써 간접적으로 도입될 수 있다. 용어 "해당 가이드 DNA"는, RNA 분자와 동일하지만 RNA 분자의 각각의 "U"가 "T"로 치환된 DNA 분자를 포함한다.

[0236] 일 양태에서, 가이드 뉴클레오티드는 유전자총을 통해, 또는 식물 U6 중합효소 III 프로모터에 작동 가능하게 연결되는 해당 가이드 DNA를 포함하는 재조합 DNA 구축물의 아그로박테리움 형질전환을 위한 개시된 방법 및 조성물을 이용해 도입된다.

[0237] 일 양태에서, RNA Cas9 엔도뉴클레아제 복합체를 가이드하는 RNA는 듀플렉스 crRNA-tracrRNA를 포함하는 듀플렉스화된 RNA이다. 듀플렉스화된 crRNA-tracrRNA 대비 가이드 뉴클레오티드를 사용하는 한 가지 장점은 융합된 가이드 뉴클레오티드를 발현시키기 위해 발현 카세트만 제조될 필요가 있다는 것이다.

[0238] 용어 "표적 부위", "표적 서열", "표적 DNA", "표적 유전자좌", "게놈 표적 부위", "게놈 표적 서열", 및 "게놈 표적 유전자좌"는 본원에서 상호교환적으로 사용되며, Cas 엔도뉴클레아제에 의해 식물 세포 게놈에서 이중가닥 절단이 유도되는 식물 세포의 게놈(엽록체 및 미토콘드리아의 DNA 포함) 내 폴리뉴클레오티드 서열을 지칭한다. 표적 부위는 식물 게놈 내의 내인성 부위일 수 있거나, 또는 대안적으로 표적 부위가 식물에 이종성이어서 게놈에서 자연적으로 발생하지 않을 수 있거나, 또는 표적 부위가 자연적으로 발생하는 경우에 비해 이종 게놈 위치에서 확인될 수 있다.

[0239] 본원에 사용된 용어 "내인성 표적 서열" 및 "고유 표적 서열"은, 식물의 게놈에 내인성이거나 고유하고 식물의 게놈 내 표적 서열의 내인성 또는 고유 위치에 있는 표적 서열을 지칭하기 위해 본원에서 상호교환적으로 사용된다. 일 양태에서, 표적 부위는 LIG3-4 엔도뉴클레아제(미국 특허 공보 2009-0133152 A1(2009년 5월 21일 공개)) 또는 MS26++ 메가뉴클레아제(2012년 6월 19일 출원된 미국 특허 출원 13/526912)와 같은 이중가닥 절단 유도제에 의해 특이적으로 인식 및/또는 결합되는 DNA 인식 부위 또는 표적 부위와 유사할 수 있다.

[0240] "인공 표적 부위" 또는 "인공 표적 서열"은 본원에서 상호교환적으로 사용되며, 식물의 게놈에 도입된 표적 서열을 지칭한다. 이러한 인공 표적 서열은 식물의 게놈 내 내인성 또는 고유 표적 서열과 서열이 동일할 수 있지만, 식물의 게놈에서 상이한 위치(즉, 비내인성 또는 비고유 위치)에 위치할 수 있다.

[0241] "변경된 표적 부위", "변경된 표적 서열", "변형된 표적 부위", 및 "변형된 표적 서열"은 본원에서 상호교환적으로 사용되며, 변경되지 않은 표적 서열에 비해 적어도 하나의 변경을 포함하는 본원에 개시된 표적 서열을 지칭한다. 이러한 "변경"은, 예를 들어 (i) 적어도 하나의 뉴클레오티드의 치환, (ii) 적어도 하나의 뉴클레오티

드의 결실, (iii) 적어도 하나의 뉴클레오티드의 삽입, 또는 (iv) (i) 내지 (iii)의 임의의 조합을 포함한다.

[0242] 서열 번호 1~189의 요약을 표 1에 나타내었다.

표 1

서열 번호 1~189 의 요약

서열 번호	폴리뉴클레오티드 (DNA) 또는 폴리펩티드 (PRT)	명칭	설명
1	DNA	GM-HBSTART3	*Glycine max* HBSTART3 (호메오도메인 StAR-관련 지질 운반 3) 프로모터 서열
2	DNA	GM-HBSTART3 (TRUNCATED)	*Glycine max* HBSTART3 (호메오도메인 StAR-관련 지질 운반 3) 프로모터 서열 (TRUNCATED)
3	DNA	AT-ML1	*Arabidopsis thaliana* ML1(MERISTEM LAYER1) 프로모터 서열
4	DNA	GM-ML1-유사	*Glycine max* ML1-유사(MERISTEM LAYER1-유사) 프로모터 서열
5	DNA	GM-ML1-유사 (TRUNCATED)	*Glycine max* ML1-유사(MERISTEM LAYER1-유사) 프로모터 서열 (TRUNCATED)
6	DNA	ZM-HBSTART3	*Zea mays* HBSTART3 (호메오도메인 StAR-관련 지질 운반 3) 프로모터 서열
7	DNA	OS-HBSTART3	*Oryza sativa* HBSTART3 (호메오도메인 StAR-관련 지질 운반 3) 프로모터 서열
8	DNA	AT-PDF1	*Arabidopsis thaliana* PDF1 (PROTODERMAL FACTOR1) 프로모터 서열
9	DNA	GM-PDF1	*Glycine max* PDF1 (PROTODERMAL FACTOR1) 프로모터 서열
10	DNA	GM-PDF1 (TRUNCATED)	*Glycine max* PDF1 (PROTODERMAL FACTOR1) 프로모터 서열 (TRUNCATED)
11	DNA	SB-PDF1	*Sorghum bicolor* PDF1 (PROTODERMAL FACTOR1) 프로모터 서열
12	DNA	OS-PDF1	*Oryza sativa* PDF1 (PROTODERMAL FACTOR1) 프로모터 서열
13	DNA	OS-PDF1 (TRUNCATED)	*Oryza sativa* PDF1 (PROTODERMAL FACTOR1) 프로모터 서열 (TRUNCATED)

[0243]

14	DNA	PT-PDF1	*Populus trichocarpa* PDF1 (PROTODERMAL FACTOR1) 프로모터 서열
15	DNA	PT-PDF1 (TRUNCATED)	*Populus trichocarpa* PDF1 (PROTODERMAL FACTOR1) 프로모터 서열(TRUNCATED)
16	DNA	SI-PDF1	*Setaria italica* PDF1 (PROTODERMAL FACTOR1) 프로모터 서열
17	DNA	SI-PDF1 (TRUNCATED)	*Setaria italica* PDF1(PROTODERMAL FACTOR1) 프로모터 서열(TRUNCATED)
18	DNA	AT-PDF2	*Arabidopsis thaliana* PDF2 (PROTODERMAL FACTOR2) 프로모터 서열
19	DNA	GM-PDF2	*Glycine max* PDF2 (PROTODERMAL FACTOR2) 프로모터 서열
20	DNA	GM-PDF2 (TRUNCATED)	*Glycine max* PDF2 (PROTODERMAL FACTOR2) 프로모터 서열(TRUNCATED)
21	DNA	ZM-GL1	*Zea mays* GL1 (GLABROUS1) 프로모터 서열
22	DNA	AT-PDF2a	*Arabidopsis thaliana* PDF2a (PROTODERMAL FACTOR2a) 프로모터 서열
23	DNA	AT-PDF2a (TRUNCATED)	*Arabidopsis thaliana* PDF2a (PROTODERMAL FACTOR2a) 프로모터 서열(TRUNCATED)
24	DNA	GM-PDF2a	*Glycine max* PDF2a (PROTODERMAL FACTOR2a) 프로모터 서열
25	DNA	GM-PDF2a (TRUNCATED)	*Glycine max* PDF2a (PROTODERMAL FACTOR2a) 프로모터 서열(TRUNCATED)
26	DNA	OS-PDF2	*Oryza sativa* PDF2 (PROTODERMAL FACTOR2) 프로모터 서열
27	DNA	OS-PDF2 (TRUNCATED)	*Oryza sativa* PDF2 (PROTODERMAL FACTOR2) 프로모터 서열(TRUNCATED)
28	DNA	PT-PDF2	*Populus trichocarpa* PDF2 (PROTODERMAL FACTOR2) 프로모터 서열
29	DNA	PT-PDF2 (TRUNCATED)	*Populus trichocarpa* PDF2 (PROTODERMAL FACTOR2) 프로모터 서열(TRUNCATED)

[0244]

[0245]

30	DNA	VV-PDF2	*Vitis vinifera* PDF2 (PROTODERMAL FACTOR2) 프로모터 서열
31	DNA	VV-PDF2 (TRUNCATED)	*Vitis vinifera* PDF2(PROTODERMAL FACTOR2) 프로모터 서열(TRUNCATED)
32	DNA	ZM-PDF2	*Zea mays* PDF2 (PROTODERMAL FACTOR2) 프로모터 서열
33	DNA	SI-PDF2	*Setaria italica* PDF2 (PROTODERMAL FACTOR2) 프로모터 서열
34	DNA	SI-PDF2 (TRUNCATED)	*Setaria italica* PDF2 (PROTODERMAL FACTOR2) 프로모터 서열(TRUNCATED)
35	DNA	VV-PDF2a	*Vitis vinifera* PDF2a (PROTODERMAL FACTOR2a) 프로모터 서열
36	DNA	PT-PDF2a	*Populus trichocarpa* PDF2a (PROTODERMAL FACTOR2a) 프로모터 서열
37	DNA	PT-PDF2a (TRUNCATED)	*Populus trichocarpa* PDF2a (PROTODERMAL FACTOR2a) 프로모터 서열(TRUNCATED)
38	DNA	MT-PDF2	*Medicago trunculata* PDF2 (PROTODERMAL FACTOR2) 프로모터 서열
39	DNA	MT-PDF2 (TRUNCATED)	*Medicago trunculata* PDF2 (PROTODERMAL FACTOR2) 프로모터 서열(TRUNCATED)
40	DNA	AT-HDG2	*Arabidopsis thaliana* HDG2 (호메오도메인 GLABROUS2) 프로모터 서열
41	DNA	GM-HDG2	*Glycine max* HDG2 (호메오도메인 GLABROUS2) 프로모터 서열
42	DNA	GM-HDG2 (TRUNCATED)	*Glycine max* HDG2 (호메오도메인 GLABROUS2) 프로모터 서열(TRUNCATED)
43	DNA	SB-HDG2	*Sorghum bicolor* HDG2 (호메오도메인 GLABROUS2) 프로모터 서열
44	DNA	SB-HDG2 (TRUNCATED)	*Sorghum bicolor* HDG2 (호메오도메인 GLABROUS2) 프로모터 서열(TRUNCATED)
45	DNA	AT-CER6	*Arabidopsis thaliana* CER6 (ECERIFERUM6) 프로모터 서열

46	DNA	AT-CER60	*Arabidopsis thaliana* CER60 (ECERIFERUM60) 프로모터 서열
47	DNA	AT-CER60 (TRUNCATED)	*Arabidopsis thaliana* CER60 (ECERIFERUM60) 프로모터 서열(TRUNCATED)
48	DNA	GM-CER6	*Glycine max* CER6 (ECERIFERUM6) 프로모터 서열
49	DNA	GM-CER6 (TRUNCATED)	*Glycine max* CER6 (ECERIFERUM6) 프로모터 서열(TRUNCATED)
50	DNA	PT-CER6	*Populus trichocarpa* CER6 (ECERIFERUM6) 프로모터 서열
51	DNA	PT-CER6 (TRUNCATED)	*Populus trichocarpa* CER6 (ECERIFERUM6) 프로모터 서열(TRUNCATED)
52	DNA	VV-CER6	*Vitis vinifera* CER6 (ECERIFERUM6) 프로모터 서열
53	DNA	VV-CER6 (TRUNCATED)	*Vitis vinifera* CER6 (ECERIFERUM6) 프로모터 서열(TRUNCATED)
54	DNA	SB-CER6	*Sorghum bicolor* CER6 (ECERIFERUM6) 프로모터 서열
55	DNA	ZM-CER6	*Zea mays* CER6 (ECERIFERUM6) 프로모터 서열
56	DNA	SI-CER6	*Setaria italica* CER6 (ECERIFERUM6) 프로모터 서열
57	DNA	SI-CER6 (TRUNCATED)	*Setaria italica* CER6 (ECERIFERUM6) 프로모터 서열(TRUNCATED)
58	DNA	OS-CER6	*Oryza sativa* CER6(ECERIFERUM6) 프로모터 서열
59	DNA	OS-CER6 (TRUNCATED)	*Oryza sativa* CER6 (ECERIFERUM6) 프로모터 서열(TRUNCATED)
60	DNA	AT-WUS	*Arabidopsis thaliana* WUS 코딩 서열
61	PRT	AT-WUS	*Arabidopsis thaliana* WUS 단백질 서열
62	DNA	LJ-WUS	*Lotus japonicus* WUS 코딩 서열
63	PRT	LJ-WUS	*Lotus japonicus* WUS 단백질 서열
64	DNA	GM-WUS	*Glycine max* WUS 코딩 서열
65	PRT	GM-WUS	*Glycine max* WUS 단백질 서열
66	DNA	CS-WUS	*Camelina sativa* WUS 코딩 서열
67	PRT	CS-WUS	*Camelina sativa* WUS 단백질 서열
68	DNA	CR-WUS	*Capsella rubella* WUS 코딩 서열
69	PRT	CR-WUS	*Capsella rubella* WUS 단백질 서열

[0246]

70	DNA	AA-WUS	*Arabis alpina* WUS 코딩 서열
71	PRT	AA-WUS	*Arabis alpina* WUS 단백질 서열
72	DNA	RS-WUS	*Raphanus sativus* WUS 코딩 서열
73	PRT	RS-WUS	*Raphanus sativus* WUS 단백질 서열
74	DNA	BN-WUS	*Brassica napus* WUS 코딩 서열
75	PRT	BN-WUS	*Brassica napus* WUS 단백질 서열
76	DNA	BO-WUS	*Brassica oleracea* 변종 oleracea WUS 코딩 서열
77	PRT	BO-WUS	*Brassica oleracea* 변종 oleracea WUS 단백질 서열
78	DNA	HA-WUS	*Helianthus annuus* WUS 코딩 서열
79	PRT	HA-WUS	*Helianthus annuus* WUS 단백질 서열
80	DNA	PT-WUS	*Populus trichocarpa* WUS 코딩 서열
81	PRT	PT-WUS	*Populus trichocarpa* WUS 단백질 서열
82	DNA	VV-WUS	*Vitis vinifera* WUS 코딩 서열
83	PRT	VV-WUS	*Vitis vinifera* WUS 단백질 서열
84	DNA	AT-WUS	*Arabidopsis thaliana* WUS 코딩 서열(대두 최적화)
85	PRT	AT-WUS	*Arabidopsis thaliana* WUS 단백질 서열
86	DNA	LJ-WUS	*Lotus japonicus* WUS 코딩 서열(대두 최적화)
87	PRT	LJ-WUS	*Lotus japonicus* WUS 단백질 서열
88	DNA	MT-WUS	*Medicago trunculata* WUS 코딩 서열(대두 최적화)
89	PRT	MT-WUS	*Medicago trunculata* WUS 단백질 서열
90	DNA	PY-WUS	*Petunia hybrida* WUS 코딩 서열(대두 최적화)
91	PRT	PY-WUS	*Petunia hybrida* WUS 단백질 서열
92	DNA	PV-WUS	*Phaseolus vulgaris* WUS 코딩 서열(대두 최적화)
93	PRT	PV-WUS	*Phaseolus vulgaris* WUS 단백질 서열
94	DNA	ZM-WUS1	*Zea mays* WUS1 코딩 서열
95	PRT	ZM-WUS1	*Zea mays* WUS1 단백질 서열
96	DNA	ZM-WUS2	*Zea mays* WUS2 코딩 서열
97	PRT	ZM-WUS2	*Zea mays* WUS2 단백질 서열
98	DNA	ZM-WUS3	*Zea mays* WUS3 코딩 서열
99	PRT	ZM-WUS3	*Zea mays* WUS3 단백질 서열
100	DNA	ZM-WOX2A	*Zea mays* WOX2A 코딩 서열
101	PRT	ZM-WOX2A	*Zea mays* WOX2A 단백질 서열
102	DNA	ZM-WOX4	*Zea mays* WOX4 코딩 서열

[0247]

103	PRT	ZM-WOX4	*Zea mays* WOX4 단백질 서열
104	DNA	ZM-WOX5A	*Zea mays* WOX5A 코딩 서열
105	PRT	ZM-WOX5A	*Zea mays* WOX5A 단백질 서열
106	DNA	ZM-WOX9	*Zea mays* WOX9 코딩 서열
107	PRT	ZM-WOX9	*Zea mays* WOX9 단백질 서열
108	DNA	GM-HBSTART2	*Glycine max* HBSTART2 (호메오도메인 StAR-관련 지질 운반 2) 프로모터 서열
109	DNA	GM-MATE1	*Glycine max* MATE1 (다중 항균 분출 단백질 1) 프로모터 서열
110	DNA	GM-NED1	*Glycine max* NED1 (NAD 의존성 에피머라제/탈수효소 1) 프로모터 서열
111	DNA	PHP80728	T-DNA(RB 내지 LB)(RB + GM-UBQ PRO::GM-UBQ INTRON1::TAG-RFP::UBQ3 TERM + GM-SAMS PRO::GM-SAMS INTRON1::GM-HRA::GM-ALS TERM + LB)를 포함하는 합성 구축물
112	DNA	PHP80730	T-DNA(RB 내지 LB)(RB + GM-LTP3 PRO::AT-WUS::UBQ14 TERM + GM-UBQ PRO::GM-UBQ INTRON1::TAG-RFP::UBQ3 TERM + GM-SAMS PRO::GM-SAMS INTRON1::GM-HRA::GM-ALS TERM + LB)를 포함하는 합성 구축물
113	DNA	PHP80734	T-DNA(RB 내지 LB)(RB + GM-HBSTART2 PRO::AT-WUS::UBQ14 TERM + GM-UBQ PRO::GM-UBQ INTRON1::TAG-RFP::UBQ3 TERM + GM-SAMS PRO::GM-SAMS INTRON1::GM-HRA::GM-ALS TERM + LB)를 포함하는 합성 구축물
114	DNA	PHP80736	T-DNA(RB 내지 LB)(RB + GM-MATE1 PRO::AT-WUS::UBQ14 TERM + GM-UBQ PRO::GM-UBQ INTRON1::TAG-RFP::UBQ3 TERM + GM-SAMS PRO::GM-SAMS INTRON1::GM-HRA::GM-ALS TERM + LB)를 포함하는 합성 구축물

[0248]

115	DNA	PHP81060	T-DNA(RB 내지 LB)(RB + GM-NED1 PRO::AT-WUS::UBQ14 TERM + GM-UBQ PRO::GM-UBQ INTRON1::TAG-RFP::UBQ3 TERM + GM-SAMS PRO::GM-SAMS INTRON1::GM-HRA::GM-ALS TERM + LB)를 포함하는 합성 구축물
116	DNA	PHP81343	T-DNA(RB 내지 LB)(RB + GM-HBSTART3 PRO::AT-WUS::UBQ14 TERM + GM-UBQ PRO::GM-UBQ INTRON1::TAG-RFP::UBQ3 TERM + GM-SAMS PRO::GM-SAMS INTRON1::GM-HRA::GM-ALS TERM + LB)를 포함하는 합성 구축물
117	DNA	QC318	GM-EF1A PRO::GM-EF1A INTRON1::ZS-YELLOW::NOS TERM + GM-SAMS PRO::GM-SAMS INTRON1::GM-ALS::GM-ALS TERM
118	DNA	pVER9662	AT-UBIQ10 PRO::AT-UBIQ10 INTRON1::AT-WUS::UBQ14 TERM
119	DNA	UBIGMWUS	AT-UBIQ10 PRO::AT-UBIQ10 INTRON1::GM-WUS:: UBQ3 TERM
120	DNA	UBIMTWUS	AT-UBIQ10 PRO::AT-UBIQ10 INTRON1::MT-WUS:: UBQ3 TERM
121	DNA	UBILJWUS	AT-UBIQ10 PRO::AT-UBIQ10 INTRON1::LJ-WUS:: UBQ3 TERM
122	DNA	UBIPVWUS	AT-UBIQ10 PRO::AT-UBIQ10 INTRON1::PV-WUS:: UBQ3 TERM
123	DNA	UBIPYWUS	AT-UBIQ10 PRO::AT-UBIQ10 INTRON1::PY-WUS:: UBQ3 TERM
124	DNA	GM-LTP3	*Glycine max* LTP3(지질 운반 단백질 3) 프로모터 서열
125	DNA	AT-ML1 (TRUNCATED)	*Arabidopsis thaliana* ML1 (MERISTEM LAYER1) 프로모터 서열(TRUNCATED)
126	DNA	AT-CER6 (TRUNCATED1)	*Arabidopsis thaliana* CER6 (ECERIFERUM6) 프로모터 서열(TRUNCATED)
127	DNA	GG- WUS	*Gnetum gnemon* WUS 코딩 서열
128	PRT	GG- WUS	*Gnetum gnemon* WUS 단백질 서열
129	DNA	MD-WUS	*Malus domestica* WUS 코딩 서열
130	PRT	MD-WUS	*Malus domestica* WUS 단백질 서열
131	DNA	ME-WUS	*Manihot esculenta* WUS 코딩 서열
132	PRT	ME-WUS	*Manihot esculenta* WUS 단백질 서열

[0249]

133	DNA	KF-WUS	*Kalanchoe fedtschenkoi* WUS 코딩 서열
134	PRT	KF-WUS	*Kalanchoe fedtschenkoi* WUS 단백질 서열
135	DNA	GH-WUS	*Gossypium hirsutum* WUS 코딩 서열
136	PRT	GH-WUS	*Gossypium hirsutum* WUS 단백질 서열
137	DNA	ZOSMA-WUS	*Zostera marina* WUS 코딩 서열
138	PRT	ZOSMA-WUS	*Zostera marina* WUS 단백질 서열
139	DNA	AMBTR-WUS	*Amborella trichopoda* WUS 코딩 서열
140	PRT	AMBTR-WUS	*Amborella trichopoda* WUS 단백질 서열
141	DNA	AC-WUS	*Aquilegia coerulea* WUS 코딩 서열
142	PRT	AC-WUS	*Aquilegia coerulea* WUS 단백질 서열
143	DNA	AH-WUS	*Amaranthus hypochondriacus* WUS 코딩 서열
144	PRT	AH-WUS	*Amaranthus hypochondriacus* WUS 단백질 서열
145	DNA	CUCSA-WUS	*Cucumis sativus* WUS 코딩 서열
146	PRT	CUCSA -WUS	*Cucumis sativus* WUS 단백질 서열
147	DNA	PINTA-WUS	*Pinus taeda* WUS 코딩 서열
148	PRT	PINTA-WUS	*Pinus taeda* WUS 단백질 서열
149	DNA	AT-PDF1 (TRUNCATED)	*Arabidopsis thaliana* PDF1 (PROTODERMAL FACTOR1) 프로모터 서열(TRUNCATED)
150	DNA	AT-PDF2 (TRUNCATED)	*Arabidopsis thaliana* PDF1 (PROTODERMAL FACTOR1) 프로모터 서열(TRUNCATED)
151	DNA	AT-HDG2 (TRUNCATED)	*Arabidopsis thaliana* HDG2 (호메오도메인 GLABROUS2) 프로모터 서열(TRUNCATED)
152	DNA	AT-ANL2	*Arabidopsis thaliana* Anthocyanless2 프로모터
153	DNA	RV021090	T-DNA(RB 내지 LB): RB + CAMV35S PRO::HA-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물

[0250]

154	DNA	RV026522	GNEGN-WUS(GG - *Gnetum gnemon*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO:: GG-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
155	DNA	RV026526	VITVI-WUS(VV - *Vitis vinifera*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO::VV-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
156	DNA	RV026534	PETHY-WUS(PH - *Petunia hybrida*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO::PH-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
157	DNA	RV026531	MALDO-WUS(MD - *Malus domestica*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO::MD-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10

[0251]

			5UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
158	DNA	RV026524	MANES-WUS(ME - *Manihot esculenta*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO::ME-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
159	DNA	RV026523	KALFE-WUS(KF - *Kalanchoe fedtschenkoi*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO::KF-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
160	DNA	RV026530	GOSHI-WUS(GH - *Gossypium hirsutum*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO::GH-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT- UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
161	DNA	RV026527	ZOSMA-WUS(*Zostera marina*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO::ZOSMA-WUS::OS-

[0252]

			T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
162	DNA	RV026529	AMBTR-WUS(*Amborella trichopoda*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO::AMBTR-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
163	DNA	RV026528	AQUCO-WUS(*Aquilegia coerulea*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO::AC-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
164	DNA	RV026520	POPTR-WUS(*Populus trichocarpa*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO::PT-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5; UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물

[0253]

165	DNA	RV026533	AMAHY-WUS(*Amaranthus hypochondriacus*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO::AH-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
166	DNA	RV026521	CUCSA-WUS(*Cucumis sativus*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO::CS-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
167	DNA	RV026525	PINTA-WUS(*Pinus taeda*)를 갖는 T-DNA T-DNA(RB 내지 LB): RB + CAMV35S PRO::PINTA-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
168	DNA	RV022814	WUS 카세트가 없는 T-DNA T-DNA(RB 내지 LB): RB + CCDB + GM-UBQ PRO-V1::GM-UBQ 5' UTR::GM-UBQ INTRON1::CTP::SPCN::UBQ14 TERM + GM-EF1A2 PRO:: GM-EF1A2 5' UTR:: GM-EF1A2 INTRON1:: GM-EF1A2 5' UTR::DS-

[0254]

[0255]

			RED2::UBQ3 TERM + LB 를 포함하는 합성 구축물
169	DNA	RV027344	AT-PDF2 PRO 를 갖는 T-DNA T-DNA(RB 내지 LB): RB + AT-PDF2 PRO::HA-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
170	DNA	RV026532	PRO TEST NEG-NO WUS 에 대한 T-DNA T-DNA(RB 내지 LB): RB + GM-UBQ PRO-V1::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + GM-UBQ PRO-V1::GM-UBQ 5' UTR::GM-UBQ INTRON1::CTP::SPCN::UBQ14 TERM + GM-EF1A2 PRO:: GM-EF1A2 5' UTR:: GM-EF1A2 INTRON1:: GM-EF1A2 5' UTR::DS-RED2::UBQ3 TERM + LB 를 포함하는 합성 구축물 GM-EF1A2 5' UTR:: GM-EF1A2 INTRON1:: GM-EF1A2 5' UTR::DS-RED2::UBQ3 TERM + LB
171	DNA	RV027337	AT-ANL2 PRO 를 갖는 T-DNA T-DNA(RB 내지 LB): RB + AT-ANL2 PRO::HA-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
172	DNA	RV027338	AT-CER6 PRO 를 갖는 T-DNA T-DNA(RB 내지 LB): RB + AT-CER6 PRO::HA-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5'

			UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
173	DNA	RV027340	AT-HDG2 PRO 를 갖는 T-DNA T-DNA(RB 내지 LB): RB + AT-HDG2 PRO::HA-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
174	DNA	RV027342	AT-ML1 PRO 를 갖는 T-DNA T-DNA(RB 내지 LB): RB + AT-ML1 PRO::HA-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
175	DNA	RV027343	AT-PDF1 PRO 를 갖는 T-DNA T-DNA(RB 내지 LB): RB + AT-PDF1 PRO::HA-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5' UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5' UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 를 포함하는 합성 구축물
176	DNA	PCR 표적 1	합성 서열 PCR 표적 1
177	DNA	PCR 표적 2	합성 서열 PCR 표적 2
178	DNA	PCR 표적 3	합성 서열 PCR 표적 3
179	DNA	PCR 표적 4	합성 서열 PCR 표적 4

[0256]

180	DNA	PCR 표적 5	합성 서열 PCR 표적 5
181	DNA	PCR 표적 6	합성 서열 PCR 표적 6
182	DNA	PCR 표적 7	합성 서열 PCR 표적 7
183	DNA	PCR 표적 8	합성 서열 PCR 표적 8
184	DNA	PCR 표적 9	합성 서열 PCR 표적 9
185	DNA	PCR 표적 10	합성 서열 PCR 표적 10
186	DNA	PCR 표적 11	합성 서열 PCR 표적 11
187	DNA	PCR 표적 12	합성 서열 PCR 표적 12
188	DNA	PCR 표적 13	합성 서열 PCR 표적 13
189	DNA	AT-CER6 (TRUNCATED2)	Arabidopsis thaliana CER6 (ECERIFERUM6) 프로모터 서열 (TRUNCATED2)

[0257]

[0258] 하기 실시예는 제한이 아닌 예시로서 제공된다.

[0259] **실시예**

[0260] 본 발명의 양태를 하기 실시예에서 추가로 정의하며, 달리 명시하지 않는 한, 부 및 백분율은 중량 기준이고 온도는 섭씨 온도이다. 이 실시예들은 본 발명의 양태들을 나타내지만 단지 예시로서 제공된다. 상기 설명 및 이 실시예들로부터, 당업자는 본 발명의 양태의 본질적 특징들을 확인할 수 있으며, 본 발명의 사상 및 범위를 벗어나지 않고 다양한 용도와 조건에 맞게 본 발명을 다양하게 변경하고 수정할 수 있다. 따라서, 본원에 나타내

고 설명한 것 외에 다양한 변형이 상기 설명으로부터 당업자에게 명백할 것이다. 이러한 변형도 첨부된 청구범위의 범주 내에 속하는 것으로 의도된다.

[0261] **실시예 1. 식물 재료 및 배지 조성**

[0262] 현탁 배양물, 미숙 자엽, 성숙 자엽, 분할 종자, 배축, 하배축, 상배축, 및 잎을 포함하여 다양한 조직 또는 외식편 유형이 본 방법에 사용될 수 있다. 대두 형질전환, 조직 배양, 및 재생에 사용된 다양한 배지의 조성을 표 2에 요약하였다. 이 표에서, 배지 M1이 형질전환의 출발 물질인 경우, 이는 현탁 배양물의 개시를 위해 사용된다. 배지 M2 및 M3는 상기 열거된 전범위의 외식편에 대한 아그로박테리움 형질전환에 유용한 일반적인 공동 배양 배지를 나타낸다. 배지 M4는 (적절한 선별제의 존재하에) 선별에 유용하고, M5는 체세포배 성숙에 사용되고, 배지 M6는 T0 묘목을 제조하기 위해 발아에 사용된다.

표 2

배양 배지 M1~M5 의 조성

[0263]

	M1	M2	M3	M4	M5	M6
B5 비타민과 MS 염 (PhytoTech M404)	4.44 g/L			4.4 g/L	4.44 g/L	
Gamborg B-5 기본 배지 (PhytoTech G398)						3.21 g/L
변형된 MS 염 (PhytoTech M571)		2.68 g/L	2.68 g/L			
B5 비타민 (1000X) (PhytoTech G249)		1 ml	1 ml	1 ml		
2,4-D 원액 10 mg/ml	4 ml	1 ml	1 ml	4 ml		
KNO_3		1.64 g/L	1.64 g/L			
$(NH_4)_2SO_4$		0.463 g/L	0.463 g/L			
아스파라긴		1 g/L	1 g/L			
수크로스		68.5 g/L	85.6 g/L			20 g/L
글루코스	31.5 g/L	36 g/L	49.6 g/L	31.5 g/l		
말토스					60 g/L	
$MgCl_2.6H_2O$					0.75 g/L	
활성탄 (PhytoTech C325)					5 g/L	
카제인 가수분해물 (PhytoTech C184)		1 g/L	1 g/L			
pH	7.0	5.4	5.4	7.0	5.7	5.7
아세토시린곤		300 μM	300 μM			
TC 한천	4 g/L					5 g/L
겔라이트 (식물 배지 Cat# 714246)				2 g/l	2 g/L	

[0264] 1~5일의 공동 배양 후, 조직을 1주일(재생 기간) 동안 선별 없이 M3 배지에서 배양한 후, 선별로 옮긴다. 선별에 있어서, 안정한 형질전환체의 선별을 위해 항생제 또는 제초제를 M3 배지에 첨가한다. 아그로박테리움에 대한 역선택을 시작하기 위해, 300 mg/l의 Timentin®(칼륨 클라불란산과 혼합된 멸균 티카실린 디소듐, PlantMedia, Dublin, OH, USA)을 또한 첨가하고, 선별제와 Timentin®을 모두 선별 기간(총 8주 이내)에 걸쳐 배지에 유지한다. 선별 배지를 매주 교체한다. 선별 배지에서 6~8주 후, 형질전환된 조직은 탈색된(또는 괴사성) 덜 건강한 백그라운드 조직에 반해 녹색 조직으로 가시화된다. 이들 조직 조각을 추가 4~8주 동안 배양한다.

[0265] 이후, 녹색의 건강한 체세포배를 100 mg/L의 Timentin®을 함유하는 M5 배지로 옮긴다. M5 배지에서 총 4주의 성숙 후, 성숙한 체세포배를 멸균된 빈 페트리 접시(Micropore™ 테이프(3M Health Care, St. Paul, MN, USA)로 밀봉됨) 또는 플라스틱 박스(섬유 테이프 없음)에 넣고 4~7일 동안 실온에 둔다.

[0266] 건조된 배아를 M6 배지에 심고, 26℃에서 60~100 μE/m^2/s의 광 강도와 18시간의 광주기로 발아시킨다. 발아 배지에서 4~6주 후, 묘목을 습기 있는 Jiffy-7 피트 펠릿(Jiffy Products Ltd, Shippagan, Canada)에 옮기고, 다음의 조건에서 Percival 인큐베이터에 적응될 때까지 투명 플라스틱 트레이 상자에 넣어 보관한다: 60~100 μE/m^2/s, 26℃/24℃의 낮/밤 온도에서 16시간의 광주기. 마지막으로, 경화된 묘목을 습기 있는 SunGro 702가 들어있는 2 갤런의 화분에 심고, 온실에서 씨앗을 맺고 성숙하게 성장시킨다.

[0267] **실시예 2. 대두의 형질전환**

[0268] 대두에 대해 유전자총에 대한 표준 프로토콜(Finer and McMullen, 1991, In Vitro Cell Dev. Biol. - Plant 27:175-182), 아그로박테리움-매개 형질전환(본원에 전문이 참조로 포함되는 문헌[Jia et al., 2015, Int J. Mol. Sci. 16:18552-18543]; US20170121722), 또는 오크로박트룸-매개 형질전환(본원에 전문이 참조로 포함되는 US20180216123)이 본 발명의 방법과 함께 사용될 수 있다.

[0269] **실시예 3. WUS 발현을 제어하는 GM-LTP3 프로모터가 대두 형질전환을 향상시킴**

[0270] 발현 카세트 GM-LTP3 PRO::AT-WUS::UBQ14 TERM + GM-UBQ PRO::GM-UBQ INTRON1::TAG-RFP::UBQ3 TERM + GM-SAMS PRO::GM-SAMS INTRON1::GM-HRA::GM-ALS TERM(PHP80730; 서열 번호 112)을 갖는 T-DNA를 함유하는 아그로박테리움 균주 AGL1을 사용하여 Pioneer의 대두 품종 PHY21을 형질전환시켰다. 아그로박테리움 감염이 시작되고 4일 후, 조직을 멸균 배양 배지로 세척하여 과잉의 박테리아를 제거하였다. 9일 후 조직을 체세포배 성숙 배지로 옮기고, 이어 22일 후 유전자이식 체세포배를 건조 준비시켰다. 이 시점에, 잘 형성되고 성숙한 체세포배는 RFP 필터 세트를 구비한 형광 입체 현미경하에서 적색 형광을 발하였다. 발육된 체세포배는 기능적이었고, 온실에서 발아되어 건강한 식물을 생성하였다. 체세포배를 생성하고 발아시켜 식물을 형성하는 이 빠른 방법은 아그로박테리움 감염으로부터 유전자이식 T0 식물을 온실로 옮기는 데 소요되는 일반적인 기간을 4개월(종래의 대두 형질전환의 경우)에서 2개월로 단축시켰다.

[0271] **실시예 4. WUS 발현을 제어하는 GM-HBSTART3 프로모터가 대두 형질전환 성숙 효율을 향상시킴**

[0272] 형질전환이 어려운 대두 품종에서, 대두의 형질전환 과정 중에 형성되는 유전자이식 체세포배의 대다수가 제대로 성숙하지 못하는 것이 일반적으로 관찰된다. 체세포배가 성숙하지 못하면 궁극적으로 온실에서 생존할 유전자이식 묘목을 형성하기 위한 발아의 빈도가 크게 감소한다. 대두 형질전환 성숙 효율에 미치는 영향을 확인하기 위해, WUS 발현을 유도하는 프로모터(GM-HBSTART3(서열 번호 1), GM-LTP3(서열 번호 124), GM-HBSTART2(서열 번호 108), GM-MATE1(서열 번호 109), GM-NED1(서열 번호 110))를 시험하였다. WUS 발현(PHP81343; 서열 번호 116)을 유도하는 GM-HBSTART3 프로모터(서열 번호 1)는 체세포배 성숙 효율을 크게 향상시켜, TAG-RFP 대조군(PHP80728; 서열 번호 111)에서의 8% 미만의 중앙값으로부터 50% 초과로 성숙 효율을 증가시켰다. 도 1 참조. 이는 유전자이식 T0 식물을 형성하도록 발아할 수 있는 유전자이식 성숙 체세포배의 빈도 증가로 직접 표현된다. 도 3a 및 3b 참조.

[0273] 다른 프로모터를 사용하여 WUS의 발현을 유도한 결과, GM-HBSTART2(PHP80734; 서열 번호 113), GM-LTP3(PHP80730; 서열 번호 112), GM-MATE1 (PHP80736; 서열 번호 114), 또는 GM-NED1(PHP81060; 서열 번호 115) 프로모터 각각에 대해 중앙값이 13%, 33%, 13%, 및 15%인 중간의 성숙 효율을 나타냈다. 도 1 참조. TAG-RFP 대조군 처리(PHP80728; 서열 번호 111)에서, 아그로박테리움 감염 후 5~6주차에 체세포배는 작고 발육부전을 보인 반면, GM-LTP3 PRO::WUS(PHP80730; 서열 번호 112) 및 GM-HBSTART3 PRO::WUS(PHP81343; 서열 번호 116) 처리에서의 체세포배는 각각 약 2배 내지 5배 더 컸다. 도 2의 A 내지 F 참조.

[0274] **실시예 5. 형태형성 유전자의 발현을 제어하는 GM-HBSTART3 또는 GM-LTP3 프로모터가 형질전환을 향상시킴**

[0275] 본원에 개시된 GM-HBSTART3 프로모터(서열 번호 1), GM-LTP3 프로모터(서열 번호 124) 또는 임의의 다른 프로모터를 사용하여 형광 마커를 포함하는 발현 카세트에서 애기장대 LEC1, LEC2, KN1, STM, 또는 LEC1-유사(Kwong et al., (2003) The Plant Cell, Vol. 15, 5-18) 유전자의 발현을 유도하면, 체세포배 형성의 빈도 및 유전자이식T0 식물의 재생을 증가시키는 것으로 확인된다. 아그로박테리움 균주 LBA4404를 사용하여, Pioneer의 대두 품종 PHY21의 미숙 자엽, 분할 종자, 단리된 배축, 성숙 자엽 마디, 하배축, 상배축, 또는 잎 조직을 포함한 다양한 외식편 유형을 형질전환시킨다. 아그로박테리움 감염이 시작되고 4일 후, 조직을 멸균 배양 배지로 세척하여 과잉의 박테리아를 제거한다. 약 9일 후 조직을 체세포배 성숙 배지로 옮기고, 이어 약 22일 후 유전자이식 체세포배를 건조 준비시킬 것으로 예상된다. 이 시점에, 잘 형성되고 성숙한 체세포배는 적절한 필터 세트를 구비한 형광 입체 현미경하에서 형광을 발한다. 발육된 체세포배는 기능적이고, 온실에서 발아되어 건강한 식물을

생성한다. 체세포배를 생성하고 발아시켜 식물을 형성하는 이 빠른 방법은 아그로박테리움 감염으로부터 유전자이식 T0 식물을 온실로 옮기는 데 소요되는 일반적인 기간을 4개월(종래의 대두 형질전환의 경우)에서 약 2~3개월로 단축시킬 것으로 예상된다.

[0276] **실시예 6. 아그로박테리움 IPT 유전자의 발현을 제어하기 위한 GM-HBSTART3 또는 GM-LTP3 프로모터의 사용이 직접적인 싹 형성을 자극하고 형질전환을 향상시킴**

[0277] 본원에 개시된 GM-HBSTART3 프로모터(서열 번호 1), GM-LTP3 프로모터(서열 번호 124) 또는 임의의 다른 프로모터를 사용하여 형광 마커를 포함하는 발현 카세트에서 아그로박테리움 IPT 유전자의 발현을 유도하면, 다경(multiple shoot) 형성의 빈도 및 유전자이식T0 식물의 재생을 증가시키는 것으로 확인된다. 아그로박테리움 균주 LBA4404를 사용하여, Pioneer의 대두 품종 PHY21의 미숙 자엽, 분할 종자, 단리된 배축, 성숙 자엽 마디, 하배축, 상배축, 또는 잎 조직을 포함한 다양한 외식편 유형을 형질전환시킨다. 아그로박테리움 감염이 시작되고 4일 후, 조직을 멸균 배양 배지로 세척하여 과잉의 박테리아를 제거하고, 자경 증식을 촉진시키는 배지로 옮긴다. 9일 후 조직을 싹 발육에 유리한 배지로 옮기고, 이어 22일 후 유전자이식 싹을 발근을 촉진시키는 배지로 옮길 것으로 예상된다. 이 시점에, 초기 묘목은 적절한 필터 세트를 구비한 형광 입체 현미경하에서 형광을 발한다. 기능성 묘목이 빠르게 발육되고, 온실에서 계속 성장하여 건강한 식물을 생성한다. 유전자이식 식물을 직접 형성하는 이 빠른 방법은 아그로박테리움 감염으로부터 유전자이식 T0 식물을 온실로 옮기는 데 소요되는 일반적인 기간을 4개월(종래의 대두 형질전환의 경우)에서 약 2~3개월로 단축시킬 것으로 예상된다.

[0278] **실시예 7. 애기장대 모노프테로스-델타 유전자의 발현을 제어하는 GM-HBSTART3 또는 GM-LTP3 프로모터가 직접적인 싹 형성을 자극하고 형질전환을 향상시킴**

[0279] 본원에 개시된 GM-HBSTART3 프로모터(서열 번호 1), GM-LTP3 프로모터(서열 번호 124) 또는 임의의 다른 프로모터를 사용하여 형광 마커를 포함하는 발현 카세트에서 애기장대 모노프테로스-델타 유전자의 발현을 유도하면, 다경 형성의 빈도 및 유전자이식T0 식물의 재생을 증가시키는 것으로 확인된다. 아그로박테리움 균주 LBA4404를 사용하여, Pioneer의 대두 품종 PHY21의 미숙 자엽, 분할 종자, 단리된 배축, 성숙 자엽 마디, 하배축, 상배축, 또는 잎 조직을 포함한 다양한 외식편 유형을 형질전환시킨다. 아그로박테리움 감염이 시작되고 4일 후, 조직을 멸균 배양 배지로 세척하여 과잉의 박테리아를 제거하고, 자경 증식을 촉진시키는 배지로 옮긴다. 9일 후 조직을 싹 발육에 유리한 배지로 옮기고, 이어 22일 후 유전자이식 싹을 발근을 촉진시키는 배지로 옮길 것으로 예상된다. 이 시점에, 초기 묘목은 적절한 필터 세트를 구비한 형광 입체 현미경하에서 형광을 발한다. 기능성 묘목이 빠르게 발육되고, 온실에서 계속 성장하여 건강한 식물을 생성한다. 유전자이식 식물을 직접 형성하는 이 빠른 방법은 아그로박테리움 감염으로부터 유전자이식 T0 식물을 온실로 옮기는 데 소요되는 일반적인 기간을 4개월(종래의 대두 형질전환의 경우)에서 약 2~3개월로 단축시킬 것으로 예상된다.

[0280] **실시예 8. 성장 강화 유전자의 발현을 제어하는 GM-HBSTART3 또는 GM-LTP3 프로모터가 형질전환을 향상시킴**

[0281] 본원에 개시된 GM-HBSTART3 프로모터(서열 번호 1), GM-LTP3 프로모터(서열 번호 124) 또는 임의의 다른 프로모터를 사용하여 형광 마커를 포함하는 발현 카세트에서 아그로박테리움 AV-6B 유전자, 아그로박테리움 IAA-h 유전자, 아그로박테리움 IAA-m 유전자, 애기장대 SERK 또는 애기장대 AGL15 유전자의 발현을 유도하면, 체세포배 형성의 빈도 및 유전자이식T0 식물의 재생을 증가시키는 것으로 확인된다. 아그로박테리움 균주 LBA4404를 사용하여, Pioneer의 대두 품종 PHY21의 미숙 자엽, 분할 종자, 단리된 배축, 성숙 자엽 마디, 하배축, 상배축, 또는 잎 조직을 포함한 다양한 외식편 유형을 형질전환시킨다. 아그로박테리움 감염이 시작되고 4일 후, 조직을 멸균 배양 배지로 세척하여 과잉의 박테리아를 제거한다. 9일 후 조직을 체세포배 성숙 배지로 옮기고, 이어 22일 후 유전자이식 체세포배를 건조 준비시킬 것으로 예상된다. 이 시점에, 잘 형성되고 성숙한 체세포배는 적절한 필터 세트를 구비한 형광 입체 현미경하에서 형광을 발한다. 발육된 체세포배는 기능적이고, 온실에서 발아되어 건강한 식물을 생성한다. 체세포배를 생성하고 발아시켜 식물을 형성하는 이 빠른 방법은 아그로박테리움 감염으로부터 유전자이식 T0 식물을 온실로 옮기는 데 소요되는 일반적인 기간을 4개월(종래의 대두 형질전환의 경우)에서 약 2~3개월로 단축시킬 것으로 예상된다.

[0282] **실시예 9. WUS의 발현을 유도하기 위해 GM-HBSTART3 프로모터와 함께 바이러스 인핸서 요소를 사용한 결과 대두 형질전환이 더 향상됨**

[0283] 본원에 개시된 GM-HBSTART3 프로모터(서열 번호 1), GM-LTP3 프로모터(서열 번호 124) 또는 임의의 다른 프로모터를 단독으로 사용하는 것에 비해, 본원에 개시된 GM-HBSTART3 프로모터(서열 번호 1), GM-LTP3 프로모터(서열 번호 124) 또는 임의의 다른 프로모터에 인접한 35S 인핸서와 같은 바이러스 인핸서 요소를 사용하여 형광 마커

를 포함하는 발현 카세트에서 WUS의 발현을 유도하면, 체세포배 형성의 빈도 및 체세포배 성숙의 빈도가 더 증가되어, 유전자이식 T0 식물의 재생이 전반적으로 증가된다. 아그로박테리움 균주 LBA4404를 사용하여, Pioneer의 대두 품종 PHY21의 미숙 자엽, 분할 종자, 단리된 배축, 성숙 자엽 마디, 하배축, 상배축, 또는 잎 조직을 포함한 다양한 외식편 유형을 형질전환시킨다. 아그로박테리움 감염이 시작되고 4일 후, 조직을 멸균 배양 배지로 세척하여 과잉의 박테리아를 제거한다. 9일 후 조직을 체세포배 성숙 배지로 옮기고, 이어 22일 후 유전자이식 체세포배를 건조 준비시킨다. 이 시점에, 잘 형성되고 성숙한 체세포배는 적절한 필터 세트를 구비한 형광 입체 현미경하에서 형광을 발한다. 발육된 체세포배는 기능적이고, 온실에서 발아되어 건강한 식물을 생성한다. 체세포배를 생성하고 발아시켜 식물을 형성하는 이 빠른 방법은 아그로박테리움 감염으로부터 유전자이식 T0 식물을 온실로 옮기는 데 소요되는 일반적인 기간을 4개월(종래의 대두 형질전환의 경우)에서 약 2~3개월로 단축시킨다.

[0284] 유사한 방식으로 시험된 다른 인핸서 요소들도 GM-HBSTART3 프로모터(서열 번호 1)을 단독으로 사용한 것에 비해 형질전환이 증가되는 것으로 나타난다. 이러한 인핸서는 내인성 식물 인핸서 요소뿐만 아니라 콜리플라워 모자이크 바이러스 35S와 미라빌리스 모자이크 바이러스 2xMMV와 같은 바이러스 인핸서를 포함한다.

[0285] **실시예 10. 애기장대 WUS 유전자의 동원체가 대두에서 체세포배 형성을 자극하는 기능을 함**

[0286] 하기 처리들을 비교하였다. 유전자총 형질전환 처리의 경우, 모두 GM-EF1A PRO::GM-EF1A INTRON1::ZS-YELLOW::NOS TERM + GM-SAMS PRO::GM-SAMS INTRON1::GM-ALS::GM-ALS TERM을 갖는 플라스미드 QC318(서열 번호 117)을 포함하였다. 처리는 1) 유전자가 첨가되지 않은 대조군, 2) 애기장대 WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 pVER9662(서열 번호 118), 3) *Glycine max* WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBIGMWUS(서열 번호 119), 4) *Medicago truncatula* WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBIMTWUS(서열 번호 120), 5) *Lotus japonica* WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBILJWUS(서열 번호 121), 6) *Phaseolus vulgaris* WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBIPVWUS(서열 번호 122), 및 7) 피튜니아 WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBIPYWUS(서열 번호 123)를 포함하였다.

[0287] 미숙 자엽을 종자로부터 단리하고, 2주 동안 사전 배양한 후, 유전자총으로 형질전환시켜, 두 플라스미드(첫 번째는 WUS 동원체 각각에 대한 cDNA 서열의 발현을 유도하는 AT-UBI PRO(pVER9662(서열 번호 118), UBIGMWUS(서열 번호 119), UBIMTWUS(서열 번호 120), UBILJWUS(서열 번호 121), UBIPVWUS(서열 번호 122), 및 UBIPYWUS(서열 번호 123))로 이루어진 발현 카세트, 및 ZS-YELLOW에 대한 발현 카세트(QC318(서열 번호 117))를 포함)의 혼합물을 공동 도입시켰다. 외식편을 2주 동안 배양하였다. 2주째, 형광 구형 체세포배의 수를 계수하고 각각의 처리에 대해 표로 만들었다.

[0288] 유전자총 형질전환 2주 후, 처리된 대조군 자엽에서 형광 구형 체세포배가 거의 관찰되지 않은 반면, 그 외 모든 처리(AT-UBI 프로모터에 의해 유도된 상이한 쌍떡잎식물 종의 WUS 유전자를 함유)의 경우, 처리된 자엽에서 많은 형광 체세포배가 관찰되었다.

[0289] **실시예 11. 애기장대 WUS 유전자의 동원체가 *BRASSICA*에서 체세포배 형성을 자극하는 기능을 함**

[0290] *Brassica*의 미숙 자엽, 분할 종자, 단리된 배축, 성숙 자엽 마디, 하배축, 상배축, 또는 잎 조직을 포함한 다양한 외식편 유형을 애기장대 WUS 유전자의 동원체로 형질전환시키기 위해 단리한다. 하기 처리들을 비교한다. 유전자총 형질전환 처리의 경우, 모두 GM-EF1A PRO::GM-EF1A INTRON1::ZS-YELLOW::NOS TERM + GM-SAMS PRO::GM-SAMS INTRON1::GM-ALS::GM-ALS TERM을 갖는 플라스미드 QC318(서열 번호 117)을 포함한다. 처리는 1) 유전자가 첨가되지 않은 대조군, 2) 애기장대 WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 pVER9662(서열 번호 118), 3) *Glycine max* WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBIGMWUS(서열 번호 119), 4) *Medicago truncatula* WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBIMTWUS(서열 번호 120), 5) *Lotus japonica* WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBILJWUS(서열 번호 121), 6) *Phaseolus vulgaris* WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBIPVWUS(서열 번호 122), 및 7) 피튜니아 WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBIPYWUS(서열 번호 123)를 포함한다.

[0291] *Brassica*의 미숙 자엽, 분할 종자, 단리된 배축, 성숙 자엽 마디, 하배축, 상배축, 또는 잎 조직을 포함한 다양한 외식편 유형을 유전자총으로 형질전환시키기 위해 단리하여, 두 플라스미드(첫 번째는 WUS 동원체 각각에 대한 cDNA 서열의 발현을 유도하는 AT-UBI PRO(pVER9662(서열 번호 118), UBIGMWUS(서열 번호 119), UBIMTWUS(서열 번호 120), UBILJWUS(서열 번호 121), UBIPVWUS(서열 번호 122), 및 UBIPYWUS(서열 번호 123))로 이루어진

발현 카세트, 및 ZS-YELLOW에 대한 발현 카세트(QC318(서열 번호 117))를 포함)의 혼합물을 공동 도입시킨다. 외식편을 2주 동안 배양한다. 2주째, 형광 구형 체세포배의 수를 계수하고 각각의 처리에 대해 표로 만든다.

[0292] 유전자총 형질전환 2주 후, 처리된 대조군 외식편 유형(*Brassica*의 미숙 자엽, 분할 종자, 단리된 배축, 성숙 자엽 마디, 하배축, 상배축, 또는 잎 조직을 포함)에서 형광 구형 체세포배가 거의 관찰되지 않는 반면, 그 외 모든 처리(AT-UBI 프로모터에 의해 유도된 상이한 쌍떡잎식물 종의 WUS 유전자를 함유)의 경우, 처리된 외식편 유형(*Brassica*의 미숙 자엽, 분할 종자, 단리된 배축, 성숙 자엽 마디, 하배축, 상배축, 또는 잎 조직을 포함)에서 많은 형광 체세포배가 관찰된다.

[0293] **실시예 12. 애기장대 WUS 유전자의 동원체가 해바라기에서 체세포배 형성을 자극하는 기능을 함**

[0294] 해바라기의 미숙 자엽, 분할 종자, 단리된 배축, 성숙 자엽 마디, 하배축, 상배축, 또는 잎 조직을 포함한 다양한 외식편 유형을 애기장대 WUS 유전자의 동원체로 형질전환시키기 위해 단리한다. 하기 처리들을 비교한다. 유전자총 형질전환 처리의 경우, 모두 GM-EF1A PRO::GM-EF1A INTRON1::ZS-YELLOW::NOS TERM + GM-SAMS PRO::GM-SAMS INTRON1::GM-ALS::GM-ALS TERM을 갖는 플라스미드 QC318(서열 번호 117)을 포함한다. 처리는 1) 유전자가 첨가되지 않은 대조군, 2) 애기장대 WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 pVER9662(서열 번호 118), 3) *Glycine max* WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBIGMWUS(서열 번호 119), 4) *Medicago truncatula* WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBIMTWUS(서열 번호 120), 5) *Lotus japonica* WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBILJWUS(서열 번호 121), 6) *Phaseolus vulgaris* WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBIPVWUS(서열 번호 122), 및 7) 피튜니아 WUS 유전자의 발현을 유도하는 AT-UBI PRO를 갖는 UBIPYWUS(서열 번호 123)를 포함한다.

[0295] 해바라기의 미숙 자엽, 분할 종자, 단리된 배축, 성숙 자엽 마디, 하배축, 상배축, 또는 잎 조직을 포함한 다양한 외식편 유형을 유전자총으로 형질전환시키기 위해 단리하여, 두 플라스미드(첫 번째는 WUS 동원체 각각에 대한 cDNA 서열의 발현을 유도하는 AT-UBI PRO(pVER9662(서열 번호 118), UBIGMWUS(서열 번호 119), UBIMTWUS(서열 번호 120), UBILJWUS(서열 번호 121), UBIPVWUS(서열 번호 122), 및 UBIPYWUS(서열 번호 123))로 이루어진 발현 카세트, 및 ZS-YELLOW에 대한 발현 카세트(QC318(서열 번호 117))를 포함)의 혼합물을 공동 도입시킨다. 외식편을 2주 동안 배양한다. 2주째, 형광 구형 체세포배의 수를 계수하고 각각의 처리에 대해 표로 만든다.

[0296] 유전자총 형질전환 2주 후, 처리된 대조군 외식편 유형(해바라기의 미숙 자엽, 분할 종자, 단리된 배축, 성숙 자엽 마디, 하배축, 상배축, 또는 잎 조직을 포함)에서 형광 구형 체세포배가 거의 관찰되지 않는 반면, 그 외 모든 처리(AT-UBI 프로모터에 의해 유도된 상이한 쌍떡잎식물 종의 WUS 유전자를 함유)의 경우, 처리된 외식편 유형(해바라기의 미숙 자엽, 분할 종자, 단리된 배축, 성숙 자엽 마디, 하배축, 상배축, 또는 잎 조직을 포함)에서 많은 형광 체세포배가 관찰된다.

[0297] **실시예 13. 애기장대 WUS 유전자의 동원체가 *BRASSICA*에서 형태형성 성장을 자극함.**

[0298] 12개의 상이한 쌍떡잎식물 종, 2개의 겉씨식물, 및 하나의 외떡잎식물 종의 WUS 유전자에 대해, 스펙티노마이신-함유 배지에서 선별 처리를 하면서 Brassica에서 유전자이식 녹색 싹 반응의 성장을 자극하는 능력을 평가함으로써 그 효능을 시험하였다. WUS 발현 카세트를 함유한 모든 처리에 대해, 구축물에 사용된 WUS 유전자를 제외하고 T-DNA의 구성은 동일하였다. 이 T-DNA 구성은 RB + CAMV35S PRO::***WUS***::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB였다(변수 WUS 유전자를 굵은 이탤릭체로 표시).

[0299] *Brassica napus*의 종자를 50% 클로록스 용액으로 표면 멸균 처리하고, MS 기본 염과 비타민을 함유하는 고체 배지에서 발아시켰다. 묘목을 28℃의 명조건에서 10 내지 14일 동안 성장시키고, 하배축을 자엽에서 분리하였다. 하배축 외식편을 200 mM의 아세토시린곤과 함께 10 ml의 20A 배지(표 3)를 함유한 100 x 25 mm 페트리 플레이트로 옮긴 후, 3~5 mm의 길이로 얇게 잘랐다. 전술한 발현 카세트를 함유한 아그로박테리움 용액(550 nM, 0.50 광학 밀도의 아그로박테리움 균주 LBA4404 THY-) 40 μl를 플레이트에 첨가하고, 하배축/아그로박테리움 혼합물을 함유한 페트리 플레이트를 쉐이커 플랫폼에 놓고 약하게 교반하였다. 10분 동안 약하게 교반한 후, 플레이트를 21℃의 약광 조건으로 옮겨 3일 동안 공동 배양하였다.

[0300] 공동 배양 후, 아그로박테리움 용액으로부터 하배축 외식편을 꺼내, 멸균 여과지에 가볍게 닦아낸 후 70A 선별 배지(표 3)(10 mg/l의 스펙티노마이신 함유)에 놓고, 광실(26℃ 및 밝은 빛)로 옮겼다. 외식편을 2차의 70A 선별로 옮기기 전에 70A 선별 배지에서 2주 동안 유지하였다(대안적으로, 2차 선별을 위해 20 mg/l의 스펙티노마이신을 함유한 70B 배지(표 3)로 외식편을 옮겼다). 2차 선별 후, 외식편을 2~3주 동안 70C 싹 신장 배지(표

3)로 옮기고 다시 광실에 놓았다. 이어서, 싹을 온실의 토양으로 옮기기 전에 90A 발근 배지(표 3)로 옮겼다.

표 3

카놀라 형질전환용 배지

감염 및 공동 배양 배지(20A); MS 염 및 비타민, 0.1 g/l 미오이노시톨, 0.5 g/l MES 완충액, 0.1 mg/l a-NAA, 1 mg/l BAP, 10 ug/l 지베렐린산, 50 mg/l 티미딘, pH 5.5.
제 1 선별 배지(70A); MS 염 및 비타민, 0.1 g/l 미오이노시톨, 0.5 g/l MES 완충액, 0.1 mg/l a-NAA, 1 mg/l BAP, 10 ug/l 지베렐린산, 40 mg/l 아데닌 헤미설페이트, 20 g/l 수크로스, 0.5 g/l PVP40, 2 mg/l 질산은, 0.5 g/l 카르베니실린, 5 mg/l 스펙티노마이신, 5 g/l TC 한천, pH 5.7.
제 2 선별 배지(70B); MS 염 및 비타민, 0.1 g/l 미오이노시톨, 0.5 g/l MES 완충액, 0.1 mg/l a-NAA, 1 mg/l BAP, 10 ug/l 지베렐린산, 40 mg/l 아데닌 헤미설페이트, 20 g/l 수크로스, 0.5 g/l PVP40, 2 mg/l 질산은, 0.5 g/l 카르베니실린, 10 mg/l 스펙티노마이신, 5 g/l TC 한천, pH 5.7.
재생 배지(70C); MS 염 및 비타민, 0.1 g/l 미오이노시톨, 0.5 g/l MES 완충액, 2.5 ug/l BAP, 40 mg/l 아데닌 헤미설페이트, 20 g/l 수크로스, 0.5 g/l PVP40, 0.5 g/l 카르베니실린, 10 mg/l 스펙티노마이신, 5 g/l TC 한천, pH 5.7.
발근 배지(90A); MS 염 및 비타민, 0.5 mg/l IBA, pH 5.7.

[0301]

[0302] 표 4에 나타낸 바와 같이, 상이한 종으로부터의 WUS 유전자는 카놀라에서 유전자이식 녹색 싹의 성장 반응을 자극하였다. 이러한 싹 발육의 자극 및 스펙티노마이신 저항성 싹을 재생하는 능력은 WUS 유전자의 공급원에 따라 가변적이었다. 오이의 경우, 이러한 유전자이식 싹의 자극은 음성 대조군 처리에서 볼 수 있는 수준보다 약간 높았지만, 다른 종으로부터의 WUS 유전자의 경우 *Gnetum gnemon*(겉씨식물)에 대해 최대 95%의 범위이었고, 포도, 거머리말(외떡잎식물), *Kalanchoe*, 피튜니아, 사과, 해바라기, 카사바, 및 *Gnetum*의 WUS 유전자의 경우 70%가 넘는 반응이 관찰되었다.

표 4

다양한 종에서 얻은 WUS 유전자의 효능*

플라스미드 번호	플라스미드 서열 번호	WUS 유전자 서열 번호	속 종	일반 명칭	총 외식편	새싹 수	WUS 반응%
RV021090	153	78	*Helianthus annuus*	해바라기	128	110	86%
RV026522	154	127	*Gnetum gnemon*	그네툼	41	39	95%
RV026526	155	82	*Vitis vinifera*	포도	81	58	72%
RV026534	156	90	*Petunia hybrida*	피튜니아	121	97	80%
RV026531	157	129	*Malus domestica*	사과	43	35	81%
RV026524	158	131	*Manihot esculenta*	카사바	38	34	89%
RV026523	159	133	*Kalanchoe fedtschenkoi*	라벤더 스칼럽스	40	31	78%
RV026530	160	135	*Gossypium hirsutum*	목화	41	22	54%
RV026527	161	137	*Zostera marina*	거머리말	40	30	75%
RV026529	162	139	*Amborella trichopoda*	암보렐라	44	20	45%
RV026528	163	141	*Aquilegia coerulea*	매발톱꽃	40	21	53%
RV026520	164	80	*Populus trichocarpa*	포플러	49	7	14%
RV026533	165	143	*Amaranthus hypochondriacus*	아마란스	41	6	15%
RV026521	166	145	*Cucumis sativus*	오이	69	4	6%
RV026525	167	147	*Pinus taeda*	소나무	43	5	12%
RV022814	168	해당 없음	없음	Wus 없음	38	2	5%
아그로박테리움 없음	해당 없음	해당 없음	없음	아그로박테리움 없음	124	2	2%

* 아그로박테리움 감염 후 28 일째 외식편(스펙티노마이신 선별)으로부터 녹색 형광의 싹을 생성한 출발 하배축 외식편의 백분율로 측정된 다양한 종으로부터의 WUS 유전자의 효능(출발 하배축 외식편은 전술한 바와 같이 WUS 유전자를 함유한 T-DNA 를 갖는 아그로박테리움에 의해 형질전환됨)

[0303]

[0304] **실시예 14. 해바라기 WUS 동원체의 발현을 제어하는 HD-ZIP IV 전사 인자 계열의 프로모터의 사용이 BRASSICA에서 형태형성 성장을 자극함**

[0305] 해바라기(*Helianthus annuus*)로부터 애기장대 WUS 유전자의 동원체의 발현을 유도하는 애기장대 프로모터에 대해, 스펙티노마이신-함유 배지에서 선별 처리를 하면서 Brassica에서 유전자이식 녹색 싹 반응의 성장을 자극하는 능력을 평가함으로써 그 효능을 시험하였다. 모든 처리에 대해, RB + ***HD-ZIP IV PRO***::HA-WUS::OS-T28 TERM + GM-UBQ PRO::GM-UBQ 5UTR::GM-UBQ INTRON1::ZS-YELLOW1 N1::NOS TERM + AT-UBIQ10 PRO::AT-UBIQ10 5UTR::AT-UBIQ10 INTRON1::CTP::SPCN::UBQ14 TERM + LB 발현 카세트를 함유하는 T-DNA를 사용하였다(변수 프로모터 HD-ZIP IV PRO를 굵은 이탤릭체로 표시).

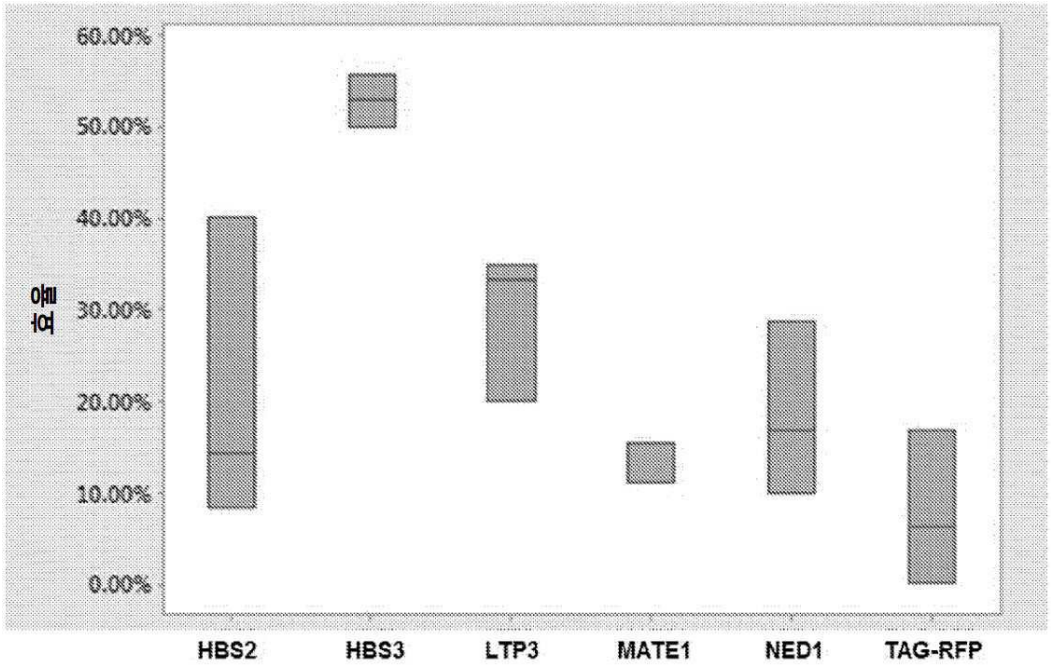
[0306] 시험한 프로모터는 애기장대에서 유래되었고, Protodermal Factor 1(PDF1, 서열 번호 149), Protodermal Factor2(PDF2, 서열 번호 150), Glabrous2(HDG2, 서열 번호 151), Meristem Layer1(ML1, 서열 번호 125), Eceriferum6(CER6, 서열 번호 126), 및 Anthocyanless(ANL1, 서열 번호 152)를 포함하였다. 이들 프로모터를 함유하는 플라스미드에 대한 T-DNA 구성은 표 1에 각각 RV027343, RV027344, RV027340, RV027342, RV027338, 및 RV027337로 표시되어 있고, 이들은 각각 서열 번호 175, 169, 173, 174, 172, 및 171에 해당한다. 이들 프로모터를 양성 대조군으로서의 CaMV35S PRO(T-DNA 정보는 RV021090, 서열 번호 153 참조) 및 음성 대조군으로서의 WUS 발현 카세트가 없는 T-DNA(T-DNA 정보는 RV026532, 서열 번호 170 참조)와 비교하였다.

[0307] *Brassica* 종자의 표면 멸균, 묘목을 생성하기 위한 발아, 하배축 외식편의 제조, 아그로박테리움 균주 LBA4404 THY-를 이용한 형질전환, 조직 배양 및 스펙티노마이신을 이용한 선별은 모두 실시예 13에 기재된 바와 같이 수행되었다.

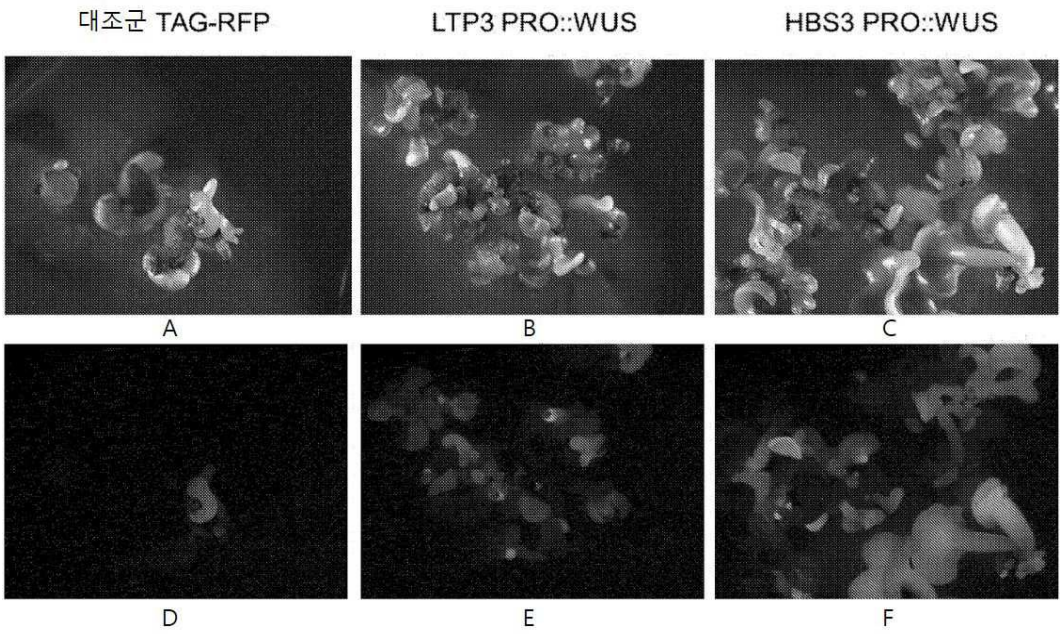
[0308] 결과는 0 내지 10의 스케일로 제시되며, 0은 음성 대조군의 반응을 초과하는 반응이 없는 것이고, 10은 CaMV35S PRO로 관찰된 강한 형태형성 반응 자극과 일치한다. 시험된 다양한 프로모터에 대해, PDF1 PRO로 관찰된 반응은 5였고, PDF2 PRO의 경우 반응은 2.3, HDG2 PRO의 경우 반응은 2.2, ML1의 경우 반응은2, CER6 PRO의 경우 반응은 2, ANL1 PRO의 경우 반응은 2였다. CaMV35S 프로모터는 WUS의 발현을 유도할 때 바람직하지 않을 수 있는 강한 항시성 프로모터이다(강한 초기 발현이 (싹 형성을 빠르게 자극하기 위해) T-DNA의 통합 직후에는 유익하지만, 전체 식물에서 강한 발현은 형태학적 이상 및 불임을 초래한다). 시험된 HD-ZIP IV 프로모터는 발육배 및 분열조직의 L1(표피) 층에서 발현되었고, 달리 전체 식물에서 발현되지 않았다. 따라서, 식물의 생장부 및 생식부가 발달함에 따라 WUS 발현이 하향 조절되는 T-DNA 전달 후 싹 형성의 양성 성장 자극이 바람직한 결과이며 달성되었다. 싹 형성의 이러한 빠른 성장 자극은 시험된 모든 HD-ZIP IV 프로모터에 대해 입증되었다.

도면

도면1



도면2



도면3a



도면3b



서 열 목 록

SEQUENCE LISTING

<110> PIONEER HI-BRED INTERNATIONAL, INC.

DA SILVA CONCEICAO, ALEXANDRE

FINCH, ALLISON M

GORDON-KAMM, WILLIAM J

KLEIN, THEODORE M

LOWE, KEITH S

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<211> 2817

<212> DNA

<213> Vitus vinifera

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<211> 2500

<212> DNA

<213> Vitus vinifera

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<211> 456

<212> DNA

<213> Zea mays

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<211> 2980

<212> DNA

<213> Setaria italica

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<212> DNA

<213> Sorghum bicolor

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<211> 2500

<212> DNA

<213> Setaria italica

<400> 57

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ccaaaccatg gccaaagtga ctgttgacgc aggcacgtaa atattcaatt gtcacagtgc 180

ggtttggaca ccacgtagac aaacgtttcc tggtcctgag cttttgattg gcccatttca 240

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<211> 2754

<212> DNA

<213> Oryza sativa

<400> 58

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caattttaaa taagcagatg atcacattta atgtgtagta tgaagggaac aggtagaata 180

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caagagagac gtgaaccgaa caggattgcc cttgcacacc tgcggcggat gtatggttgc 360

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<212> DNA
<213> Oryza sativa

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<211> 879

<212> DNA

<213> Arabidopsis thaliana

<400> 60

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<211> 292

<212> PRT

<213> Arabidopsis thaliana

<400> 61

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20 25 30

Arg Gln Thr Ser Thr Arg Trp Thr Pro Thr Thr Glu Gln Ile Lys Ile
35 40 45

Leu Lys Glu Leu Tyr Tyr Asn Asn Ala Ile Arg Ser Pro Thr Ala Asp
50 55 60

Gln Ile Gln Lys Ile Thr Ala Arg Leu Arg Gln Phe Gly Lys Ile Glu
65 70 75 80

Gly Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg
85 90 95

Gln Lys Lys Arg Phe Asn Gly Thr Asn Met Thr Thr Pro Ser Ser Ser
100 105 110

Pro Asn Ser Val Met Met Ala Ala Asn Asp His Tyr His Pro Leu Leu
115 120 125
His His His His Gly Val Pro Met Gln Arg Pro Ala Asn Ser Val Asn
130 135 140
Val Lys Leu Asn Gln Asp His His Leu Tyr His His Asn Lys Pro Tyr
145 150 155 160
Pro Ser Phe Asn Asn Gly Asn Leu Asn His Ala Ser Ser Gly Thr Glu

165 170 175
Cys Gly Val Val Asn Ala Ser Asn Gly Tyr Met Ser Ser His Val Tyr
180 185 190
Gly Ser Met Glu Gln Asp Cys Ser Met Asn Tyr Asn Asn Val Gly Gly
195 200 205
Gly Trp Ala Asn Met Asp His His Tyr Ser Ser Ala Pro Tyr Asn Phe
210 215 220
Phe Asp Arg Ala Lys Pro Leu Phe Gly Leu Glu Gly His Gln Glu Glu

225 230 235 240
Glu Glu Cys Gly Gly Asp Ala Tyr Leu Glu His Arg Arg Thr Leu Pro
245 250 255
Leu Phe Pro Met His Gly Glu Asp His Ile Asn Gly Gly Ser Gly Ala
260 265 270
Ile Trp Lys Tyr Gly Gln Ser Glu Val Arg Pro Cys Ala Ser Leu Glu
275 280 285
Leu Arg Leu Asn
290

<210> 62

<211> 876
<212> DNA
<213> Lotus japonicus
<400> 62

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<210> 63

<211> 292

<212> PRT

<213> Lotus japonicus

<400> 63

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Ser Ser Gly Gly Lys Gly Gly Phe Leu Ser Arg Gln Ser Ser Thr Arg

20 25 30

Trp Thr Pro Thr Thr Asp Gln Ile Arg Ile Leu Lys Glu Leu Tyr Tyr

35 40 45

Asn Asn Gly Ile Arg Ser Pro Ser Ala Glu Gln Ile Gln Arg Ile Ser

50 55 60

Ala Arg Leu Arg Gln Tyr Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr

65 70 75 80

Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys Lys Arg Phe Thr

85 90 95

Ser Glu Thr His Leu Leu Pro Asn Pro Ile Gln Gln Gln Gln Arg Asn

100 105 110

Gly Thr Asn Ala Ala Trp Lys Gln Pro Glu Glu Gln Pro Ile Ile Asn
115 120 125
His Thr Lys Tyr Ser Ser Asn Asn Ile Ser Ala Pro Ala Gly Ile Ile
130 135 140
Thr Ser Ala Pro Ser Ser Ser Ala Glu Ile Val Ser Val Gly Gln Ile
145 150 155 160
Gly Asn Phe Gly Tyr Gly Ser Leu Pro Met Glu Lys Ser Phe Arg Asp

165 170 175
Cys Ser Ile Ser Ala Gly Gly Asn Thr Gly Tyr Ala Gly Ser Ala Ile
180 185 190
Asn His Asn Leu Gly Trp Met Gly Val Asp Pro Tyr Ser Ser Ala Tyr
195 200 205
Thr Asn Phe Phe Asp Lys Ile Arg Pro Thr Glu Glu Glu Thr Met Glu
210 215 220
Glu Glu Gly Gln Glu Asn Gly Ser Pro Glu Ile Glu Thr Leu Pro Leu

225 230 235 240
Phe Pro Met His Gly Glu Asp Ile His Ser Gly Tyr Phe Asn Leu Lys
245 250 255
Ser Asn Ser Ser His Tyr Ala Gly Gly Trp Tyr Gln Thr Glu Glu Gly
260 265 270
Tyr Phe Asn Asn Gly Ser Arg Ala Ser Leu Glu Leu Ser Leu Lys Tyr
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Pro Asp Phe Ala
290

<210> 64

<211> 888
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<400> 64

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<211> 295

<212> PRT

<213> Glycine max

<400> 65

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20 25 30

Gln Ser Ser Thr Arg Trp Thr Pro Thr Asn Asp Gln Ile Arg Ile Leu
35 40 45

Lys Glu Leu Tyr Tyr Asn Asn Gly Ile Arg Ser Pro Ser Ala Glu Gln
50 55 60

Ile Gln Arg Ile Ser Ala Arg Leu Arg Gln Tyr Gly Lys Ile Glu Gly
65 70 75 80

Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln
85 90 95

Lys Lys Arg Phe Thr Ser Asp His Asn His Asn Asn Val Pro Met Gln
100 105 110

Arg Pro Pro Thr Asn Pro Ser Ala Ala Trp Lys Pro Asp Leu Ala Asp
115 120 125
Pro Ile His Thr Thr Lys Tyr Cys Asn Ile Ser Ser Thr Ala Gly Ile
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Ser Ser Ala Ser Ser Ser Val Glu Met Val Thr Val Gly Gln Met Gly
145 150 155 160
Asn Tyr Gly Tyr Gly Ser Val Pro Met Glu Lys Ser Phe Arg Asp Cys

165 170 175
Ser Ile Ser Ala Gly Gly Ser Ser Gly His Val Gly Leu Ile Asn His
180 185 190
Asn Leu Gly Trp Val Gly Val Asp Pro Tyr Asn Ser Ser Thr Tyr Ala
195 200 205
Asn Phe Phe Asp Lys Ile Arg Pro Ser Asp Gln Glu Thr Leu Glu Glu
210 215 220
Glu Ala Glu Asn Ile Gly Ala Thr Lys Ile Glu Thr Leu Pro Leu Phe

225 230 235 240
Pro Met His Gly Glu Asp Ile His Gly Tyr Cys Asn Leu Lys Ser Asn
245 250 255
Ser Tyr Asn Tyr Asp Gly Asn Gly Trp Tyr His Thr Glu Glu Gly Phe
260 265 270
Lys Asn Ala Ser Arg Ala Ser Leu Glu Leu Ser Leu Asn Ser Tyr Thr
275 280 285
Arg Arg Ser Pro Asp Tyr Ala
290 295

<210> 66
<211> 897
<212> DNA
<213> Camelina sativa
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gcagatcaga tccagaagat cactgctagg ctgcgacagt tcggaaagat cgagggcaag 240

aacgtctttt actggttcca gaaccacaag gctcgtgagc gtcagaagaa gagattcaac 300

ggcacaacca tgaccacacc gtcttcatct cccaactcgg ttatgatggc taatgatcat 360

tatcatcaac atctccctct acttcaccat catcatggcg ttcccatgca gagacctgcg 420

aactccgtca acgttaaact taaccaagag catcatctct atcatcagaa caagtcatat 480

cccagcttca ataacgggaa tttaaatcat gcaagctcag gtactgaatg tggtgctgtt 540

aatgcttcta atggctactc aagtagccat atctatggat ctatggaaca agactgttct 600

atgaattaca acaacgtagg tggaggatgg acggcaaaca tggatcataa tcatcattac 660

tcatcagcac cttacaactt cttcgataga ccaaaacctc cgtttggtct agacggtcat 720

caggaagaag aagaatgtgg tggcgatgct tatctggaac atcgacgtac gcttcctctc 780

ttccctatgc atggtgaaga tcacataaat ggtgccagct ggaagtatgg ccaactggac 840

ggtcatgatt gtcatggtag aggcccttgc gcttctcttg agcttcgttt gaactag 897

<210> 67

<211> 298

<212> PRT

<213> Camelina sativa

<400> 67

Met Glu Pro Pro Gln His Gln His His His His Gln Ala Asp Gln Glu
1 5 10 15

Ser Gly Asn Asn Asn Lys Ser Gly Ser Gly Gly Tyr Thr Cys Arg Gln
20 25 30

Thr Ser Thr Arg Trp Thr Pro Thr Thr Glu Gln Ile Lys Met Leu Lys
35 40 45

Glu Leu Tyr Tyr Asn Ser Gly Ile Arg Ser Pro Thr Ala Asp Gln Ile
50 55 60

Gln Lys Ile Thr Ala Arg Leu Arg Gln Phe Gly Lys Ile Glu Gly Lys
65 70 75 80

Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys
85 90 95

Lys Arg Phe Asn Gly Thr Thr Met Thr Thr Pro Ser Ser Ser Pro Asn
100 105 110

Ser Val Met Met Ala Asn Asp His Tyr His Gln His Leu Pro Leu Leu
115 120 125
His His His His Gly Val Pro Met Gln Arg Pro Ala Asn Ser Val Asn
130 135 140
Val Lys Leu Asn Gln Glu His His Leu Tyr His Gln Asn Lys Ser Tyr
145 150 155 160
Pro Ser Phe Asn Asn Gly Asn Leu Asn His Ala Ser Ser Gly Thr Glu

165 170 175
Cys Gly Ala Val Asn Ala Ser Asn Gly Tyr Ser Ser Ser His Ile Tyr
180 185 190
Gly Ser Met Glu Gln Asp Cys Ser Met Asn Tyr Asn Asn Val Gly Gly
195 200 205
Gly Trp Thr Ala Asn Met Asp His Asn His His Tyr Ser Ser Ala Pro
210 215 220
Tyr Asn Phe Phe Asp Arg Pro Lys Pro Pro Phe Gly Leu Asp Gly His

225 230 235 240
Gln Glu Glu Glu Glu Cys Gly Gly Asp Ala Tyr Leu Glu His Arg Arg
245 250 255
Thr Leu Pro Leu Phe Pro Met His Gly Glu Asp His Ile Asn Gly Ala
260 265 270
Ser Trp Lys Tyr Gly Gln Leu Asp Gly His Asp Cys His Gly Arg Gly
275 280 285
Pro Cys Ala Ser Leu Glu Leu Arg Leu Asn
290 295

<210> 68
<211> 897
<212> DNA
<213> Capsella rubella
<400> 68
atggagccac cacagcatca gcatcatcat catcaagccg accaagaaag cggcaacaac 60
aacaacaagt ccggctctgg tggctacacg tgtcgccaaa ccagcactag gtggacacca 120
acgacggagc agatcagaat cctcaaggat ctttactaca acagtgcgat ccggtcacca 180

acagccgatc agatccagaa gatcaccgcg aggctgagac agtacgggaa gatcgaaggc 240

aagaacgtct tttactggtt ccagaaccat aaggctcgtg agcgtcagaa gaagagattc 300

aacggcacaa ccatgaccac tccatcttca tctcccaact cggttatggt ggctaatgat 360

cactatcatc ctctacttca ccatcatcat ggtgttccca tgcaaaggcc tgctaactcc 420

gtcaacgtca aacttaacca agaccatcat ctctatcatc agaacaagtc atatcccacc 480

ttcaacaatg ggaatttaaa tcatggaagc tcaggtactg aatgtggtgt tgttaatgct 540

tctaatggct acatgagtag ccatgtctat ggatctatgg aacaagactg ttcaatgaac 600

tacaacaacg taggtactgg aggatacgga tcaaacatgg atcatagtca tcattactca 660

tctgcaccat acaacttctt tgatagatca aagcctctct ttggtctaga aggtcatcaa 720

gaagatgaag aagaatatgg tataggcgat gcttatctgg aacatcgacg tacgcttcct 780

ctcttcccta tgcatggtga agatcacatg aacggtagtg gtgctagctg gaagtttgat 840

ggccgtgatt gccatggtag aggcccttgc gcttctcttg agcttcgtct gaactag 897

<210> 69

<211> 298

<212> PRT

<213> Capsella rubella

<400> 69

Met Glu Pro Pro Gln His Gln His His His His Gln Ala Asp Gln Glu
1 5 10 15

Ser Gly Asn Asn Asn Asn Lys Ser Gly Ser Gly Gly Tyr Thr Cys Arg
20 25 30

Gln Thr Ser Thr Arg Trp Thr Pro Thr Thr Glu Gln Ile Arg Ile Leu
35 40 45

Lys Asp Leu Tyr Tyr Asn Ser Ala Ile Arg Ser Pro Thr Ala Asp Gln
50 55 60

Ile Gln Lys Ile Thr Ala Arg Leu Arg Gln Tyr Gly Lys Ile Glu Gly
65 70 75 80

Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln
85 90 95

Lys Lys Arg Phe Asn Gly Thr Thr Met Thr Thr Pro Ser Ser Ser Pro
100 105 110

Asn Ser Val Met Val Ala Asn Asp His Tyr His Pro Leu Leu His His
115 120 125
His His Gly Val Pro Met Gln Arg Pro Ala Asn Ser Val Asn Val Lys
130 135 140
Leu Asn Gln Asp His His Leu Tyr His Gln Asn Lys Ser Tyr Pro Thr
145 150 155 160
Phe Asn Asn Gly Asn Leu Asn His Gly Ser Ser Gly Thr Glu Cys Gly

165 170 175
Val Val Asn Ala Ser Asn Gly Tyr Met Ser Ser His Val Tyr Gly Ser
180 185 190
Met Glu Gln Asp Cys Ser Met Asn Tyr Asn Asn Val Gly Thr Gly Gly
195 200 205
Tyr Gly Ser Asn Met Asp His Ser His His Tyr Ser Ser Ala Pro Tyr
210 215 220
Asn Phe Phe Asp Arg Ser Lys Pro Leu Phe Gly Leu Glu Gly His Gln

225 230 235 240
Glu Asp Glu Glu Glu Tyr Gly Ile Gly Asp Ala Tyr Leu Glu His Arg
245 250 255
Arg Thr Leu Pro Leu Phe Pro Met His Gly Glu Asp His Met Asn Gly
260 265 270
Ser Gly Ala Ser Trp Lys Phe Asp Gly Arg Asp Cys His Gly Arg Gly
275 280 285
Pro Cys Ala Ser Leu Glu Leu Arg Leu Asn
290 295

<210> 70
<211> 588
<212> DNA
<213> Arabis alpina
<400> 70
atggctactc atgtagccat tagaagcatt aacagcacca cattcagaac ctgaacttgc 60
atgatttaaa ttcccgttat tgaagctggg atatgatttg ttctgatgaa gcagatggtg 120
ttcttggtca tgaagcttaa cgttgagaga agcaggtctc tgcatggaaa caccatgatg 180

atgttgaaga agaggatgat gatgatggtt atgatgataa cgatcactgg ccatcataac 240

tgagttgggc gatgaagacg ttggtgtggt ggtcatggtt gtgccattga atctcttctt 300

ctgacgctca cgagccttat ggttctgaaa ccagtaaaag acgtttttgc cctcgatctt 360

accatattgt ctcagccttg cagagatctt ctggatctca tcagcagttg gtgaccggat 420

tccactattg tagtaaagat ctttgaggat tctgatttgc tccgtggttg gagtccatct 480

cgtgctggtt tgacgacaag tgtaaccacc agaaccggac ttgttgttgt tgttgccgct 540

ttcttggtcg gcttgatgat gatgatgatg atgttgcggt ggctccat 588

<210> 71

<211> 304

<212> PRT

<213> Arabis alpina

<400> 71

Met Glu Pro Pro Gln His His His His His His Gln Ala Asp Gln Glu
1 5 10 15

Ser Gly Asn Asn Asn Asn Lys Ser Gly Ser Gly Gly Tyr Thr Cys Arg
20 25 30

Gln Thr Ser Thr Arg Trp Thr Pro Thr Thr Glu Gln Ile Arg Ile Leu
35 40 45

Lys Asp Leu Tyr Tyr Asn Ser Gly Ile Arg Ser Pro Thr Ala Asp Glu
50 55 60

Ile Gln Lys Ile Ser Ala Arg Leu Arg Gln Tyr Gly Lys Ile Glu Gly
65 70 75 80

Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln
85 90 95

Lys Lys Arg Phe Asn Gly Thr Thr Met Thr Thr Thr Pro Thr Ser Ser
100 105 110

Ser Pro Asn Ser Val Met Met Ala Ser Asp Arg Tyr His His Asn His
115 120 125

His His His Pro Leu Leu Gln His His His Gly Val Ser Met Gln Arg
130 135 140

Pro Ala Ser Leu Asn Val Lys Leu His Asp Gln Glu His His Leu Leu

145 150 155 160

His Gln Asn Lys Ser Tyr Pro Ser Phe Asn Asn Gly Asn Leu Asn His

165 170 175

Ala Ser Ser Gly Ser Glu Cys Gly Ala Val Asn Ala Ser Asn Gly Tyr

180 185 190

Met Ser Ser His Val Tyr Gly Ser Met Glu Gln Asp Tyr Ser Met Ser

195 200 205

Asn Asn Asn Ile Gly Gly Gly Gly Trp Ser Asn Met Asp His Asn His

210 215 220

His Tyr Ser Ser Pro Ala Tyr Asn Phe Phe Asp Arg Pro Lys Pro Leu

225 230 235 240

Phe Gly Leu Glu Gly His Gln Glu Glu Asp Gln Tyr Gly Gly Asp Ala

245 250 255

Tyr Leu Glu His Arg Arg Thr Leu Pro Leu Phe Pro Met His Gly Glu

260 265 270

Asp His Ile Asn Gly Gly Gly Gly Ala Ile Trp Lys Tyr Arg Gln Leu

275 280 285

Asp Gly Leu Glu Leu Arg Leu Asn Ser Tyr Ala Gly Val Ala Pro Glu

290 295 300

<210> 72

<211> 873

<212> DNA

<213> Raphanus sativus

<400> 72

atggagccac cgcaacatta ccatcaagcc gatcaagaaa gcggcaacaa caacaagtcc 60

gtctctggtg gttacacgtg tcgtcaaacg agcacaagat ggacaccaac aaccgatcaa 120

atcagaatac tcaaagatct ttactacaac aatggagtcc ggtcaccaac agccgaccag 180

atccagaaga tctctgcaac gctgagacag tacggaaaaa tcgagggaaa aaatgtcttt 240

tactggtttc aaaaccataa ggctcgtgag cgacagaaga agagattcaa cagcacaacc 300

atggccacac caacgtcttc atcgcccaac tcggttatga tgatggctag tgatcaccat 360

catcatcatg gtgttaccat ccagagacct gctttggtca acgttaagct cgaccaagaa 420

aatcatatgt ttcaccagaa cagatcatat cccagctcca ataacgggag tataaatcat 480

gcaagttcag gcacggaata tggtgttttc ggcgcttcta atggctacat tagtaaccat 540

atctatggat ctatggaaca agactgttca atgagctaca acagcgtagg tggaggatgg 600

acaaacatgg atcataatca tcattactca actccagctt acaacttctt tgatagacca 660

tcgcctctgt ctggactaga aggtcatcaa gaagaaggac aatatggtgg cgattcttat 720

ctggaacatc gacgtacact tcctctcttc cctttgcacg gtgaggatca catcaacggt 780

ggtggtggtt ccatcttgaa gtacggacaa tcagacggtt gtgatcgtta tggtagaggc 840

ccttgtgctt ctcttaagct atgtctgaac tga 873

<210> 73

<211> 290

<212> PRT

<213> Raphanus sativus

<400> 73

Met Glu Pro Pro Gln His Tyr His Gln Ala Asp Gln Glu Ser Gly Asn

1 5 10 15

Asn Asn Lys Ser Val Ser Gly Gly Tyr Thr Cys Arg Gln Thr Ser Thr

20 25 30

Arg Trp Thr Pro Thr Thr Asp Gln Ile Arg Ile Leu Lys Asp Leu Tyr

35 40 45

Tyr Asn Asn Gly Val Arg Ser Pro Thr Ala Asp Gln Ile Gln Lys Ile

50 55 60

Ser Ala Thr Leu Arg Gln Tyr Gly Lys Ile Glu Gly Lys Asn Val Phe

65 70 75 80

Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys Lys Arg Phe

85 90 95

Asn Ser Thr Thr Met Ala Thr Pro Thr Ser Ser Ser Pro Asn Ser Val

100 105 110

Met Met Met Ala Ser Asp His His His His His Gly Val Thr Ile Gln

115 120 125

Arg Pro Ala Leu Val Asn Val Lys Leu Asp Gln Glu Asn His Met Phe

130 135 140

His Gln Asn Arg Ser Tyr Pro Ser Ser Asn Asn Gly Ser Ile Asn His

145 150 155 160

Ala Ser Ser Gly Thr Glu Tyr Gly Val Phe Gly Ala Ser Asn Gly Tyr

165 170 175

Ile Ser Asn His Ile Tyr Gly Ser Met Glu Gln Asp Cys Ser Met Ser

180 185 190

Tyr Asn Ser Val Gly Gly Gly Trp Thr Asn Met Asp His Asn His His

195 200 205

Tyr Ser Thr Pro Ala Tyr Asn Phe Phe Asp Arg Pro Ser Pro Leu Ser

210 215 220

Gly Leu Glu Gly His Gln Glu Glu Gly Gln Tyr Gly Gly Asp Ser Tyr

225 230 235 240

Leu Glu His Arg Arg Thr Leu Pro Leu Phe Pro Leu His Gly Glu Asp

245 250 255

His Ile Asn Gly Gly Gly Gly Ser Ile Leu Lys Tyr Gly Gln Ser Asp

260 265 270

Gly Cys Asp Arg Tyr Gly Arg Gly Pro Cys Ala Ser Leu Lys Leu Cys

275 280 285

Leu Asn

290

<210> 74

<211> 885

<212> DNA

<213> Brassica napus

<400> 74

atggagccag cgcaacatca ccatcaagcc gaccaagaaa gcggcaacaa caacaagtcc 60

gtctctggtg gttacacgtg tcgtcaaacg agtacaagat ggacaccaac gaccgatcaa 120

atcagaatac tcaaagatct ttactacaac aacggagtcc ggtcaccaac agccgaccag 180

atccagaaga tctctgcaag gctgagacag tacggaaaga tcgagggaaa aaatgtcttt 240

tactggtttc aaaaccataa ggctcgtgag cgacagaaga agagattcaa cagcacaacc 300

atgaccatac caacgtcttc atcgcccaac tcggttatga tggctagtga tcactatcac 360

cataaccatc atcatcatgg cgttaccatc cagagacctg ctttggtcaa cgttaagctc 420

gaccaagaaa atcatatgtt tcatcagaac agatcatatc ccagcttcaa taacgggaat 480

acaaatcatg caagttcagg cacggaatat ggtgttttca gtgcttctaa tggctacatt 540

agtagccata tctatgaacc tatggaacaa gactgttcaa tgagctacaa caacgtaggt 600

ggaggatgga caaacataga tcataatcac cattactcaa ctccagccta caacttctta 660

gatagaccaa tgcctctgtc tggactagaa ggtcatcatc aagaagaagg agaatatggt 720

ggcgatgctt atctggaaca tagacgcaca cttcctctct tccctttgca cggtgaggat 780

cacatcaacg gcggtggtgg ttccatctgg aagtacagac aatcggacgg ttgtgatcgt 840

tatggtagag gcccttgtgc ttctcttaag ctgtgtctga actga 885

<210> 75

<211> 294

<212> PRT

<213> Brassica napus

<400> 75

Met Glu Pro Ala Gln His His His Gln Ala Asp Gln Glu Ser Gly Asn

1 5 10 15

Asn Asn Lys Ser Val Ser Gly Gly Tyr Thr Cys Arg Gln Thr Ser Thr

20 25 30

Arg Trp Thr Pro Thr Thr Asp Gln Ile Arg Ile Leu Lys Asp Leu Tyr

35 40 45

Tyr Asn Asn Gly Val Arg Ser Pro Thr Ala Asp Gln Ile Gln Lys Ile

50 55 60

Ser Ala Arg Leu Arg Gln Tyr Gly Lys Ile Glu Gly Lys Asn Val Phe

65 70 75 80

Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys Lys Arg Phe

85 90 95

Asn Ser Thr Thr Met Thr Ile Pro Thr Ser Ser Ser Pro Asn Ser Val

100 105 110

Met Met Ala Ser Asp His Tyr His His Asn His His His His Gly Val

115 120 125

Thr Ile Gln Arg Pro Ala Leu Val Asn Val Lys Leu Asp Gln Glu Asn

130 135 140

His Met Phe His Gln Asn Arg Ser Tyr Pro Ser Phe Asn Asn Gly Asn

145 150 155 160

Thr Asn His Ala Ser Ser Gly Thr Glu Tyr Gly Val Phe Ser Ala Ser

165 170 175

Asn Gly Tyr Ile Ser Ser His Ile Tyr Glu Pro Met Glu Gln Asp Cys

180 185 190

Ser Met Ser Tyr Asn Asn Val Gly Gly Gly Trp Thr Asn Ile Asp His

195 200 205

Asn His His Tyr Ser Thr Pro Ala Tyr Asn Phe Leu Asp Arg Pro Met

210 215 220

Pro Leu Ser Gly Leu Glu Gly His His Gln Glu Glu Gly Glu Tyr Gly

225 230 235 240

Gly Asp Ala Tyr Leu Glu His Arg Arg Thr Leu Pro Leu Phe Pro Leu

245 250 255

His Gly Glu Asp His Ile Asn Gly Gly Gly Gly Ser Ile Trp Lys Tyr

260 265 270

Arg Gln Ser Asp Gly Cys Asp Arg Tyr Gly Arg Gly Pro Cys Ala Ser

275 280 285

Leu Lys Leu Cys Leu Asn

290

<210> 76

<211> 885

<212> DNA

<213> Brassica oleracea

<400> 76

atggagccag cgcaacatca ccatcaagcc gaccaagaaa gcggcaacaa caacaagtcc 60

gtctctggtg gttacacgtg tcgtcaaacg agtacaagat ggacaccaac gaccgatcaa 120

atcagaatac tcaaagatct ttactacaac aacggagtcc ggtcaccaac agccgaccag 180

atccagaaga tctctgcaag gctgagacag tacggaaaga tcgagggaaa aaatgtcttt 240

tactggtttc aaaaccataa ggctcgtgag cgacagaaga agagattcaa cagcacaacc 300

atgaccatac caacgtcttc atcgcccaac tcggttatga tggctagtga tcactatcac 360

cataaccatc atcatcatgg cgttaccatc cagagacctg ctttggtcaa cgttaagctc 420

gaccaagaaa atcatatgtt tcatcagaac agatcatatc ccagcttcaa taacgggaat 480

ataaatcatg caagttcagg cacggaatat ggtgttttca gtgcttctaa tggctacatt 540

agtagccata tctatggacc tatggaacaa gactgttcaa tgagctacaa caacgtaggt 600

ggaggatgga caaatataga tcataatcac cattactcaa ctccagccta caacttctta 660

gatagaccaa tgcctctgtc tggactagaa ggtcatcatc aagaagaagg agaatatggt 720

ggcgatgctt atctggaaca tagacgcaca cttcctctct tccctttgca cggtgaggat 780

cacatcaacg gcggtggtgg ttccatctgg aagtacagac aatcggacgg ttgtgatcgt 840

tatggtagag gcccttgtgc ttctcttaag ctgtgtctga actga 885

<210> 77

<211> 294

<212> PRT

<213> Brassica oleracea

<400> 77

Met Glu Pro Ala Gln His His His Gln Ala Asp Gln Glu Ser Gly Asn

1 5 10 15

Asn Asn Lys Ser Val Ser Gly Gly Tyr Thr Cys Arg Gln Thr Ser Thr

20 25 30

Arg Trp Thr Pro Thr Thr Asp Gln Ile Arg Ile Leu Lys Asp Leu Tyr

35 40 45

Tyr Asn Asn Gly Val Arg Ser Pro Thr Ala Asp Gln Ile Gln Lys Ile

50 55 60

Ser Ala Arg Leu Arg Gln Tyr Gly Lys Ile Glu Gly Lys Asn Val Phe

65 70 75 80

Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys Lys Arg Phe

85 90 95

Asn Ser Thr Thr Met Thr Ile Pro Thr Ser Ser Ser Pro Asn Ser Val

100 105 110

Met Met Ala Ser Asp His Tyr His His Asn His His His His Gly Val

115 120 125

Thr Ile Gln Arg Pro Ala Leu Val Asn Val Lys Leu Asp Gln Glu Asn

130 135 140

His Met Phe His Gln Asn Arg Ser Tyr Pro Ser Phe Asn Asn Gly Asn

145 150 155 160

Ile Asn His Ala Ser Ser Gly Thr Glu Tyr Gly Val Phe Ser Ala Ser

165 170 175

Asn Gly Tyr Ile Ser Ser His Ile Tyr Gly Pro Met Glu Gln Asp Cys

180 185 190

Ser Met Ser Tyr Asn Asn Val Gly Gly Gly Trp Thr Asn Ile Asp His

195 200 205

Asn His His Tyr Ser Thr Pro Ala Tyr Asn Phe Leu Asp Arg Pro Met

210 215 220

Pro Leu Ser Gly Leu Glu Gly His His Gln Glu Glu Gly Glu Tyr Gly

225 230 235 240

Gly Asp Ala Tyr Leu Glu His Arg Arg Thr Leu Pro Leu Phe Pro Leu

245 250 255

His Gly Glu Asp His Ile Asn Gly Gly Gly Gly Ser Ile Trp Lys Tyr

260 265 270

Arg Gln Ser Asp Gly Cys Asp Arg Tyr Gly Arg Gly Pro Cys Ala Ser

275 280 285

Leu Lys Leu Cys Leu Asn

290

<210> 78

<211> 882

<212> DNA

<213> Helianthus annuus

<400> 78

atggaaactc aacaacaaca agcagatatt caagattttg ggaacaaaaa cagtaacacc 60

tatgcatgca ggcaaagcag tactaggtgg acacccacaa gtgatcagat cagaatactg 120

aaagagctct actacaacaa tggaatcaga tcaccaactg ctgatcagat tcagagaatc 180

gctgctcaac tcagacagta cggcaagatt gaaggcaaga acgtgtttta ttggtttcag 240

aaccataaag ctcgtgaacg ccagaagaaa cggttcactc ctactgctcc tccaccaccg 300

tctcacttct ccgatcatat taaccaccat cttcccaacg ctgcccccat gcagattccg 360

tctcaccacc accactacca ccatcaagaa ccaccacatg tctatgctca ccctcacaaa 420

ctctacacca ctcatcacat tggggttggt tcttcttcac aaggagtgat gggtgtaggg 480

tgtggctatg gatctgttgc tatggagaag agtttcagga agtgttcaat atcaccaccg 540

ggagagagta aggccacagg aggaattggc cggaatatcg gttcaagatc acggataagt 600

gtcgattcgt gttccttctt cgacacaatt aagccaaaga catatgagat gtttgagaat 660

catgatcaag atgaagaaca aggagagcct tcaactgaga tcgaaacact tccattattc 720

ccgattcacg acggcaacca tcatgatttc ttcggcatga ggacggcaaa tctgcaattg 780

gagcaaggca ctggaggtta ctacactgga ggaagctggt atcggtctga tgagagggcg 840

tctcttgagc tcagtctcaa ctcatatggt tattataact aa 882

<210> 79

<211> 293

<212> PRT

<213> Helianthus annuus

<400> 79

Met Glu Thr Gln Gln Gln Gln Ala Asp Ile Gln Asp Phe Gly Asn Lys

1 5 10 15

Asn Ser Asn Thr Tyr Ala Cys Arg Gln Ser Ser Thr Arg Trp Thr Pro

20 25 30

Thr Ser Asp Gln Ile Arg Ile Leu Lys Glu Leu Tyr Tyr Asn Asn Gly

35 40 45

Ile Arg Ser Pro Thr Ala Asp Gln Ile Gln Arg Ile Ala Ala Gln Leu

50 55 60

Arg Gln Tyr Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr Trp Phe Gln

65 70 75 80

Asn His Lys Ala Arg Glu Arg Gln Lys Lys Arg Phe Thr Pro Thr Ala

85 90 95

Pro Pro Pro Pro Ser His Phe Ser Asp His Ile Asn His His Leu Pro

100 105 110

Asn Ala Ala Pro Met Gln Ile Pro Ser His His His His Tyr His His

115 120 125

Gln Glu Pro Pro His Val Tyr Ala His Pro His Lys Leu Tyr Thr Thr

130 135 140

His His Ile Gly Val Gly Ser Ser Ser Gln Gly Val Met Gly Val Gly

145 150 155 160

Cys Gly Tyr Gly Ser Val Ala Met Glu Lys Ser Phe Arg Lys Cys Ser

165 170 175

Ile Ser Pro Pro Gly Glu Ser Lys Ala Thr Gly Gly Ile Gly Arg Asn

180 185 190

Ile Gly Ser Arg Ser Arg Ile Ser Val Asp Ser Cys Ser Phe Phe Asp

195 200 205

Thr Ile Lys Pro Lys Thr Tyr Glu Met Phe Glu Asn His Asp Gln Asp

210 215 220

Glu Glu Gln Gly Glu Pro Ser Thr Glu Ile Glu Thr Leu Pro Leu Phe

225 230 235 240

Pro Ile His Asp Gly Asn His His Asp Phe Phe Gly Met Arg Thr Ala

245 250 255

Asn Leu Gln Leu Glu Gln Gly Thr Gly Gly Tyr Tyr Thr Gly Gly Ser

260 265 270

Trp Tyr Arg Ser Asp Glu Arg Ala Ser Leu Glu Leu Ser Leu Asn Ser

275 280 285

Tyr Gly Tyr Tyr Asn

290

<210> 80

<211> 795

<212> DNA

<213> Populus trichocarpa

<400> 80

atggaacctc accaacaaca accaaatgag gataataacg gtggcgccaa agggaacttt 60

ctttgcaggc aaaccagtac aaggtggaat cccactactg accagataag aatattgaag 120

gaactgtact acatcaaagg tgttaggtcc ccaaatggag ctgagattca gcagatctct 180

gctaggctta gaaagtacgg caagattgaa ggcaagaatg tattttattg gtttcagaac 240

cataaagctc gtgagaggca aaagaaaaga ctcaccaatg aagtccccat gcaacaaaga 300

actgcctgga aacctgaaga ttattactct tacaagtact ccaacagtaa taataatcct 360

gggttttctt cggcttcttc atctgcaaat accggtgtgg ttactgttgg gcagacagat 420

agccatggat atggatcggt aaccatgcag gagaagaatt cttgggactg ttcagcacca 480

gctggtggta gcaatggtgc tggtagtgga tccatgagca atattaacta tggatcaggg 540

gttgacatta actctcacag ttcatcctat gctgtatttg gtcaagaaca agaagcagct 600

gcaaagattg agactcttcc tctatttcca atgcttggtg aggacatcag tagctctttc 660

aacatcaaca atatcaaccc agacttttat tacagtagcg gctgtggcta tggtgattat 720

ggcaatgaca cttcttcccg tacttccctg gaccttagtc tctactccta caatggccaa 780

ccacaagatt attaa 795

<210> 81

<211> 264

<212> PRT

<213> Populus trichocarpa

<400> 81

Met Glu Pro His Gln Gln Gln Pro Asn Glu Asp Asn Asn Gly Gly Ala
1 5 10 15

Lys Gly Asn Phe Leu Cys Arg Gln Thr Ser Thr Arg Trp Asn Pro Thr
20 25 30

Thr Asp Gln Ile Arg Ile Leu Lys Glu Leu Tyr Tyr Ile Lys Gly Val
35 40 45

Arg Ser Pro Asn Gly Ala Glu Ile Gln Gln Ile Ser Ala Arg Leu Arg
50 55 60

Lys Tyr Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr Trp Phe Gln Asn
65 70 75 80

His Lys Ala Arg Glu Arg Gln Lys Lys Arg Leu Thr Asn Glu Val Pro
85 90 95

Met Gln Gln Arg Thr Ala Trp Lys Pro Glu Asp Tyr Tyr Ser Tyr Lys
100 105 110

Tyr Ser Asn Ser Asn Asn Asn Pro Gly Phe Ser Ser Ala Ser Ser Ser
115 120 125

Ala Asn Thr Gly Val Val Thr Val Gly Gln Thr Asp Ser His Gly Tyr
130 135 140

Gly Ser Val Thr Met Gln Glu Lys Asn Ser Trp Asp Cys Ser Ala Pro
145 150 155 160

Ala Gly Gly Ser Asn Gly Ala Gly Ser Gly Ser Met Ser Asn Ile Asn
165 170 175
Tyr Gly Ser Gly Val Asp Ile Asn Ser His Ser Ser Ser Tyr Ala Val
180 185 190
Phe Gly Gln Glu Gln Glu Ala Ala Ala Lys Ile Glu Thr Leu Pro Leu
195 200 205

Phe Pro Met Leu Gly Glu Asp Ile Ser Ser Ser Phe Asn Ile Asn Asn
210 215 220
Ile Asn Pro Asp Phe Tyr Tyr Ser Ser Gly Cys Gly Tyr Gly Asp Tyr
225 230 235 240
Gly Asn Asp Thr Ser Ser Arg Thr Ser Leu Asp Leu Ser Leu Tyr Ser
245 250 255
Tyr Asn Gly Gln Pro Gln Asp Tyr
260

<210> 82
<211> 843
<212> DNA
<213> Vitus vinifera

<400> 82
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gatagcggca gcagcaaagg cagttttctg tgcaggcaaa gcagtacacg ctggactcct 120
acaactgacc agataagaat attgaaggac ctttactaca acaatggagt taggtcccca 180
agtgctgaac agattcagag gatctcagct aggctgaggc agtacggcaa gatcgaaggc 240
aagaacgtct tttattggtt tcagaaccat aaagctcgtg aaaggcagaa gaagcgattc 300
actactgata tgcccatgca aagatcccct ggaaatgccg gttggagacc tgatgatccc 360
attcacaaca aatttcatac cattcccact cctgggatat cttccccatc ttcttcttct 420

tcacccagtg tgcttgctgt tgggcagatg ggaagctttg gatatggatc tgtggagagg 480
agttttacag actgttcaat atcagcaggt ggtggtcgtg gtggggttgg tggatctata 540
aaccagagct tcgaatgggt tggcatggac ccatattctt catcttatgc actctttgac 600
aagagaaaaa caatgggtga aagcttcgaa gaagagcaag aagaagaagc aactccggag 660
attgaaacgc tcccactctt tcccatgcac gctgaggata tcactggctt ttgcaacatt 720
aagcccgaat ccgacgccta ctactccggc tggtacaggc ccgccgacgc caagaccagt 780

tcccgcactt ctcttgagct tagcctcaac tcctacgccg gcaggtcccc ggattccccc 840

tga 843

<210> 83

<211> 280

<212> PRT

<213> Vitus vinifera

<400> 83

Met Glu Pro Gln Gln Gln Leu Gln Gln Gln Gln Gln Asn Gln Gln
1 5 10 15

Gln Pro Asn Glu Asp Ser Gly Ser Ser Lys Gly Ser Phe Leu Cys Arg
20 25 30

Gln Ser Ser Thr Arg Trp Thr Pro Thr Thr Asp Gln Ile Arg Ile Leu
35 40 45

Lys Asp Leu Tyr Tyr Asn Asn Gly Val Arg Ser Pro Ser Ala Glu Gln
50 55 60

Ile Gln Arg Ile Ser Ala Arg Leu Arg Gln Tyr Gly Lys Ile Glu Gly
65 70 75 80

Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln
85 90 95

Lys Lys Arg Phe Thr Thr Asp Met Pro Met Gln Arg Ser Leu Gly Asn
100 105 110

Ala Gly Trp Arg Pro Asp Asp Pro Ile His Asn Lys Phe His Thr Ile
115 120 125

Pro Thr Pro Gly Ile Ser Ser Pro Ser Ser Ser Ser Ser Pro Ser Val
130 135 140

Leu Ala Val Gly Gln Met Gly Ser Phe Gly Tyr Gly Ser Val Glu Arg
145 150 155 160

Ser Phe Thr Asp Cys Ser Ile Ser Ala Gly Gly Gly Arg Gly Gly Val
165 170 175

Gly Gly Ser Ile Asn Gln Ser Phe Glu Trp Val Gly Met Asp Pro Tyr
180 185 190

Ser Ser Ser Tyr Ala Leu Phe Asp Lys Arg Lys Thr Met Gly Glu Ser
195 200 205

Phe Glu Glu Glu Gln Glu Glu Glu Ala Thr Pro Glu Ile Glu Thr Leu
210 215 220

Pro Leu Phe Pro Met His Ala Glu Asp Ile Thr Gly Phe Cys Asn Ile
225 230 235 240

Lys Pro Glu Ser Asp Ala Tyr Tyr Ser Gly Trp Tyr Arg Pro Ala Asp
245 250 255

Ala Lys Thr Ser Ser Arg Thr Ser Leu Glu Leu Ser Leu Asn Ser Tyr
260 265 270

Ala Gly Arg Ser Pro Asp Ser Pro
275 280

<210> 84

<211> 879

<212> DNA

<213> Arabidopsis thaliana

<400> 84

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cctaccactg agcagatcaa gatcctgaag gagttgtact acaacaatgc catccgctcc 180

cctactgctg atcagattca gaagatcacc gctcgcctgc gtcaattcgg taagatcgag 240

ggtaagaacg tcttctactg gttccagaac cacaaggctc gtgagaggca gaagaagcgt 300

ttcaacggga ctaacatgac cactccttcc tcatcaccca attctgttat gatggccgcc 360

aacgaccact accatccttt gcttcatcac caccacggag ttcctatgca aagacccgca 420

aactccgtca acgtgaagtt gaaccaggac catcacctct accaccacaa caagccctac 480

ccctctttca acaacgggaa tctcaaccat gcctcctctg gaaccgaatg tggagttgtc 540

aacgcctcca acggctacat gtcttcccac gtgtacggct ctatggagca agattgctcc 600

atgaactaca acaacgttgg aggcggatgg gcaaacatgg atcaccacta ctcctccgct 660

ccctacaact tcttcgatag ggctaaacct ctcttcggct tggagggaca ccaagaagaa 720

gaggaatgcg gaggtgatgc ttacttggaa cacagaagga ctctccctct ctttccaatg 780

cacggtgagg accacatcaa cggaggatct ggcgctatct ggaagtatgg acagtccgag 840

gtcagaccat gcgcttccct ggaattgaga ctgaactag 879

<210> 85

<211> 292

<212> PRT

<213> Arabidopsis thaliana

<400> 85

Met Glu Pro Pro Gln His Gln His His His His Gln Ala Asp Gln Glu
1 5 10 15

Ser Gly Asn Asn Asn Asn Asn Lys Ser Gly Ser Gly Gly Tyr Thr Cys
20 25 30

Arg Gln Thr Ser Thr Arg Trp Thr Pro Thr Thr Glu Gln Ile Lys Ile
35 40 45

Leu Lys Glu Leu Tyr Tyr Asn Asn Ala Ile Arg Ser Pro Thr Ala Asp
50 55 60

Gln Ile Gln Lys Ile Thr Ala Arg Leu Arg Gln Phe Gly Lys Ile Glu
65 70 75 80

Gly Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg
85 90 95

Gln Lys Lys Arg Phe Asn Gly Thr Asn Met Thr Thr Pro Ser Ser Ser
100 105 110

Pro Asn Ser Val Met Met Ala Ala Asn Asp His Tyr His Pro Leu Leu
115 120 125

His His His His Gly Val Pro Met Gln Arg Pro Ala Asn Ser Val Asn
130 135 140

Val Lys Leu Asn Gln Asp His His Leu Tyr His His Asn Lys Pro Tyr
145 150 155 160

Pro Ser Phe Asn Asn Gly Asn Leu Asn His Ala Ser Ser Gly Thr Glu
165 170 175

Cys Gly Val Val Asn Ala Ser Asn Gly Tyr Met Ser Ser His Val Tyr
180 185 190

Gly Ser Met Glu Gln Asp Cys Ser Met Asn Tyr Asn Asn Val Gly Gly
195 200 205

Gly Trp Ala Asn Met Asp His His Tyr Ser Ser Ala Pro Tyr Asn Phe
210 215 220

Phe Asp Arg Ala Lys Pro Leu Phe Gly Leu Glu Gly His Gln Glu Glu
225 230 235 240

Glu Glu Cys Gly Gly Asp Ala Tyr Leu Glu His Arg Arg Thr Leu Pro
245 250 255

Leu Phe Pro Met His Gly Glu Asp His Ile Asn Gly Gly Ser Gly Ala
260 265 270

Ile Trp Lys Tyr Gly Gln Ser Glu Val Arg Pro Cys Ala Ser Leu Glu
275 280 285

Leu Arg Leu Asn
290

<210> 86
<211> 879
<212> DNA
<213> Lotus japonicus
<400> 86

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aaaggagggt tcctcagtag gcagtccagt actaggtgga cacccaccac agaccagatt 120

cgcatcctca aggagctcta ctacaacaac ggaatacgct caccctccgc agaacagatc 180

cagaggatct ccgcaagact cagacagtac ggcaagatcg agggcaagaa cgtcttctac 240

tggttccaaa accacaaagc ccgcgaaagg cagaagaagc gcttcaccag tgagacacac 300

ttgctgccca acccaattca gcagcaacag cgcaacggaa caaacgcagc ttggaagcag 360

ccagaggagc aacccatcat caaccacacc aagtactcct ccaacaacat atccgcaccc 420

gcagggatta tcacaagtgc cccatcttct tccgcagaaa tagtctccgt ggggcagatc 480

ggaaacttcg gatacggatc tctgccaatg gagaagtcct tcagggactg cagtatctca 540

gccggaggta acacaggata cgccgggtct gctattaacc acaacctcgg atggatggga 600

gtcgacccat actcctctgc ttacaccaac ttcttcgaca aaatacgccc aacagaggag 660

gagactatgg aggaagaagg acaggagaac ggctcaccag aaatcgagac cctgcctttg 720

ttcccaatgc acggagagga catccactcc ggctacttca acctcaagag taactcatcc 780

cactacgctg gcggatggta ccaaactgag gaaggctact tcaacaacgg atcacgcgcc 840

agtctggagc tgtccctgaa gtaccctgac ttcgcttag 879

<210> 87

<211> 292

<212> PRT

<213> Lotus japonicus

<400> 87

Met Glu Gln Pro Pro Gln Gln Gln Gln Asn Glu Asp Gly Gly Gly
1 5 10 15
Ser Ser Gly Gly Lys Gly Gly Phe Leu Ser Arg Gln Ser Ser Thr Arg
20 25 30
Trp Thr Pro Thr Thr Asp Gln Ile Arg Ile Leu Lys Glu Leu Tyr Tyr
35 40 45
Asn Asn Gly Ile Arg Ser Pro Ser Ala Glu Gln Ile Gln Arg Ile Ser
50 55 60

Ala Arg Leu Arg Gln Tyr Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr
65 70 75 80
Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys Lys Arg Phe Thr
85 90 95
Ser Glu Thr His Leu Leu Pro Asn Pro Ile Gln Gln Gln Gln Arg Asn
100 105 110
Gly Thr Asn Ala Ala Trp Lys Gln Pro Glu Glu Gln Pro Ile Ile Asn
115 120 125

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

Asn His Asn Leu Gly Trp Met Gly Val Asp Pro Tyr Ser Ser Ala Tyr
195 200 205

Thr Asn Phe Phe Asp Lys Ile Arg Pro Thr Glu Glu Glu Thr Met Glu
210 215 220

Glu Glu Gly Gln Glu Asn Gly Ser Pro Glu Ile Glu Thr Leu Pro Leu
225 230 235 240

Phe Pro Met His Gly Glu Asp Ile His Ser Gly Tyr Phe Asn Leu Lys
245 250 255

Ser Asn Ser Ser His Tyr Ala Gly Gly Trp Tyr Gln Thr Glu Glu Gly
260 265 270

Tyr Phe Asn Asn Gly Ser Arg Ala Ser Leu Glu Leu Ser Leu Lys Tyr
275 280 285

Pro Asp Phe Ala
290

<210> 88
<211> 909
<212> DNA
<213> Medicago truncatula
<400> 88

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atcatggggt ccagacagag ttccaccaga tggaccccaa caaccgacca gatccgcatt 120

ctcaaagacc tctactacaa caacggaata aggtctccaa gtgccgagca aatccagagg 180

atctccgcaa ggctcagaca gtacgggaag atcgagggca agaacgtctt ctactggttc 240

caaaaccaca aagccagaga gcgccagaaa aagcgcttca cctccgacgt caacgtcgtc 300

cctatcatcc agcgcgcccc caacaacaat actatcataa gtgccgccaa ctggaagcct 360

gaccaccacg aacaacagca gaacattaat gtccacacaa accactccac atacaacatc 420

tcctccgctg gcctcagttc tgcttcctgc tcctcagcag aaatggtcac tgtggggcaa 480

ataggcaact acggctacgg gtctgtccca atggagaagt ctttccgcga atgcaccatc 540

tcagctggat gctcatcatc ccaggttggc tcaaccataa acccccacat cggctggatc 600

ggacaccacg tcgacccata ttcaagtgcc tacgctaacc tcttcgagaa aatacgcccc 660

aacgaggaga tcatggaaga gtacgaccag ggacaggaaa acggttcccc agagatcgag 720

acactcccac tcttccctat gcacggagaa gacatccacg gagggtattg caacctcaaa 780

tcaaattcct ccaactacgg aggatggtac caggcagaag acgcagggtt catgtacgga 840

agtcgtacca cttctctcga gctcagtctg aactcctacg gatgcaggtc ccccgactac 900

gccaattaa 909

<210> 89

<211> 302

<212> PRT

<213> Medicago truncatula

<400> 89

Met Glu Gln Pro Gln Gln Gln Gln Gln Pro Gln Thr Gln Gln His Ser
1 5 10 15

Pro Asn Asn Gly Ile Met Gly Ser Arg Gln Ser Ser Thr Arg Trp Thr
20 25 30

Pro Thr Thr Asp Gln Ile Arg Ile Leu Lys Asp Leu Tyr Tyr Asn Asn
35 40 45

Gly Ile Arg Ser Pro Ser Ala Glu Gln Ile Gln Arg Ile Ser Ala Arg

50 55 60

Leu Arg Gln Tyr Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr Trp Phe
65 70 75 80

Gln Asn His Lys Ala Arg Glu Arg Gln Lys Lys Arg Phe Thr Ser Asp
85 90 95

Val Asn Val Val Pro Ile Ile Gln Arg Ala Pro Asn Asn Asn Thr Ile
100 105 110

Ile Ser Ala Ala Asn Trp Lys Pro Asp His His Glu Gln Gln Gln Asn

115 120 125

Ile Asn Val His Thr Asn His Ser Thr Tyr Asn Ile Ser Ser Ala Gly
130 135 140

Leu Ser Ser Ala Ser Cys Ser Ser Ala Glu Met Val Thr Val Gly Gln
145 150 155 160

Ile Gly Asn Tyr Gly Tyr Gly Ser Val Pro Met Glu Lys Ser Phe Arg
165 170 175

Glu Cys Thr Ile Ser Ala Gly Cys Ser Ser Ser Gln Val Gly Ser Thr

180 185 190

Ile Asn Pro His Ile Gly Trp Ile Gly His His Val Asp Pro Tyr Ser

195 200 205

Ser Ala Tyr Ala Asn Leu Phe Glu Lys Ile Arg Pro Asn Glu Glu Ile

210 215 220

Met Glu Glu Tyr Asp Gln Gly Gln Glu Asn Gly Ser Pro Glu Ile Glu

225 230 235 240

Thr Leu Pro Leu Phe Pro Met His Gly Glu Asp Ile His Gly Gly Tyr

245 250 255

Cys Asn Leu Lys Ser Asn Ser Ser Asn Tyr Gly Gly Trp Tyr Gln Ala

260 265 270

Glu Asp Ala Gly Phe Met Tyr Gly Ser Arg Thr Thr Ser Leu Glu Leu

275 280 285

Ser Leu Asn Ser Tyr Gly Cys Arg Ser Pro Asp Tyr Ala Asn

290 295 300

<210> 90

<211> 924

<212> DNA

<213> Petunia x hybrida

<400> 90

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atcggacaag gaacaaacat cgaggacggc agtaacaaga acaactcctc caacttcatg 120

tgcaggcaaa atagtacccg ctggacacca accactgacc agatcaggat attaaaggac 180

ctctactaca acaacggagt cagatcacct accgctgaac agatccagag aatctccgcc 240

aagctcaggc agtacggcaa gatcgagggc aagaacgtct tctactggtt ccagaaccat 300

aaagcccgcg agagacaaaa gaagcgcctc atcgccgcag ccaccaccga taacactaac 360

ctcccaatgc agatgcagtt ccagagagga gtctggagat cctctgcaga cgaccccatc 420

caccacaagt atactaatcc aggagtgcac tgcccttcag cttcttccca cggagttctc 480

gcagtcggac agaacggcaa tcacggatac ggagccttgg caatggaaaa atccttccgc 540

gactgctcca tcagtccagg cagttccatg tcacaccacc accaccaaaa cttcgcttgg 600

gcaggagtcg atccttattc ctcaaccaca acctaccctt tcctcgagaa gaccaagcac 660

ttcgagaatg aaaccctcga agctgatgaa gagcagcaag aggaggacca ggagaactac 720

tactaccaaa ggaccacctc tgccatcgaa accctgcctc tcttccctat gcacgaggag 780

aacatctcct ccttctgcaa cctcaagcac caggagtcat ccggaggatt ctacaccgag 840

tggtacagag cagacgacaa cctcgcagca gccagagctt ccctcgagct ctccttgaac 900

tccttcatcg gcaactcctc ctag 924

<210> 91

<211> 307

<212> PRT

<213> Petunia x hybrida

<400> 91

Met Glu Thr Ala Gln His Gln Gln Asn Asn Gln Gln His Tyr Leu His
1 5 10 15

Gln His Leu Ser Ile Gly Gln Gly Thr Asn Ile Glu Asp Gly Ser Asn
20 25 30

Lys Asn Asn Ser Ser Asn Phe Met Cys Arg Gln Asn Ser Thr Arg Trp
35 40 45

Thr Pro Thr Thr Asp Gln Ile Arg Ile Leu Lys Asp Leu Tyr Tyr Asn
50 55 60

Asn Gly Val Arg Ser Pro Thr Ala Glu Gln Ile Gln Arg Ile Ser Ala
65 70 75 80

Lys Leu Arg Gln Tyr Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr Trp
85 90 95

Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys Lys Arg Leu Ile Ala
100 105 110

Ala Ala Thr Thr Asp Asn Thr Asn Leu Pro Met Gln Met Gln Phe Gln
115 120 125

Arg Gly Val Trp Arg Ser Ser Ala Asp Asp Pro Ile His His Lys Tyr
130 135 140

Thr Asn Pro Gly Val His Cys Pro Ser Ala Ser Ser His Gly Val Leu
145 150 155 160

Ala Val Gly Gln Asn Gly Asn His Gly Tyr Gly Ala Leu Ala Met Glu
165 170 175

Lys Ser Phe Arg Asp Cys Ser Ile Ser Pro Gly Ser Ser Met Ser His

180 185 190

His His His Gln Asn Phe Ala Trp Ala Gly Val Asp Pro Tyr Ser Ser

195 200 205

Thr Thr Thr Tyr Pro Phe Leu Glu Lys Thr Lys His Phe Glu Asn Glu

210 215 220

Thr Leu Glu Ala Asp Glu Glu Gln Gln Glu Glu Asp Gln Glu Asn Tyr

225 230 235 240

Tyr Tyr Gln Arg Thr Thr Ser Ala Ile Glu Thr Leu Pro Leu Phe Pro

245 250 255

Met His Glu Glu Asn Ile Ser Ser Phe Cys Asn Leu Lys His Gln Glu

260 265 270

Ser Ser Gly Gly Phe Tyr Thr Glu Trp Tyr Arg Ala Asp Asp Asn Leu

275 280 285

Ala Ala Ala Arg Ala Ser Leu Glu Leu Ser Leu Asn Ser Phe Ile Gly

290 295 300

Asn Ser Ser

305

<210> 92

<211> 864

<212> DNA

<213> Phaseolus vulgaris

<400> 92

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ggatttctct ccagacagtc ctccactagg tggactccaa ccactgacca gatcagaata 120

ctcaaagacc tctactacaa caacggaatc agatcccctt ccgcagaaca gatccaaagg 180

atctccgcta ggctcagaca gtacgggaag atcgagggca agaacgtctt ctactggttc 240

caaaaccaca aggccaggga gagacagaag aagaggttca cctccgacaa cgtccccatg 300

caaagacctg caccaacaaa tgctgcacct ccttggaaac ccgaccaaga ccccattcac 360

accaagtact ccaacatctc ctccacagga atctccagtg catcctcctc ctcagtcgag 420

atgatcaccg tcggacaaat ggggaactac gggtacggaa gtgtgccaat ggaaaagtcc 480

ttcagagact gctccatatc cgccggagga tcttccggtc atgttggaat aaaccacaac 540

ctcggctggg tgggagtcga tccttactct tctgcatacg ccaacttctt cgataaaata 600

agaccaaacg aagagaccct cgaggaagaa gaagaggagg aggaggagga agaagaagag 660

gacggaggag cagaaattga gaccctcccc ctgttcccaa tgcacggaga ggacattcac 720

ggctactgca acctcaagtc caacagttac aactacgacg gcaacggctg gtatcacagt 780

gaggatggct tcaaaaacgg cagtagggcc tccctcgagc tctccctcaa ctcttacacc 840

agaagatccc ccgacttcgc atag 864

<210> 93

<211> 287

<212> PRT

<213> Phaseolus vulgaris

<400> 93

Met Glu Pro Gln Gln Pro Gln Gly Ser Gln Pro Asn Glu Asp Gly Gly
1 5 10 15

Ser Gly Lys Gly Gly Phe Leu Ser Arg Gln Ser Ser Thr Arg Trp Thr
20 25 30

Pro Thr Thr Asp Gln Ile Arg Ile Leu Lys Asp Leu Tyr Tyr Asn Asn
35 40 45

Gly Ile Arg Ser Pro Ser Ala Glu Gln Ile Gln Arg Ile Ser Ala Arg
50 55 60

Leu Arg Gln Tyr Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr Trp Phe
65 70 75 80

Gln Asn His Lys Ala Arg Glu Arg Gln Lys Lys Arg Phe Thr Ser Asp
85 90 95

Asn Val Pro Met Gln Arg Pro Ala Pro Thr Asn Ala Ala Pro Pro Trp
100 105 110

Lys Pro Asp Gln Asp Pro Ile His Thr Lys Tyr Ser Asn Ile Ser Ser
115 120 125

Thr Gly Ile Ser Ser Ala Ser Ser Ser Ser Val Glu Met Ile Thr Val
130 135 140

Gly Gln Met Gly Asn Tyr Gly Tyr Gly Ser Val Pro Met Glu Lys Ser
145 150 155 160

Phe Arg Asp Cys Ser Ile Ser Ala Gly Gly Ser Ser Gly His Val Gly

165 170 175

Ile Asn His Asn Leu Gly Trp Val Gly Val Asp Pro Tyr Ser Ser Ala

180 185 190

Tyr Ala Asn Phe Phe Asp Lys Ile Arg Pro Asn Glu Glu Thr Leu Glu

195 200 205

Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Asp Gly Gly Ala

210 215 220

Glu Ile Glu Thr Leu Pro Leu Phe Pro Met His Gly Glu Asp Ile His

225 230 235 240

Gly Tyr Cys Asn Leu Lys Ser Asn Ser Tyr Asn Tyr Asp Gly Asn Gly

245 250 255

Trp Tyr His Ser Glu Asp Gly Phe Lys Asn Gly Ser Arg Ala Ser Leu

260 265 270

Glu Leu Ser Leu Asn Ser Tyr Thr Arg Arg Ser Pro Asp Phe Ala

275 280 285

<210> 94

<211> 963

<212> DNA

<213> Zea mays

<400> 94

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cggtcgccca actcggagca gatccagcgc atcaccgcca tgctgcggca gcacggcaag 240

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cgccgcctca ccaacctcga cgtcaacgtg cccgtcgccg ccgacgacag cgcccaccgc 360

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atgggcggcg cgtcaccgtg ggcatgctcc tcctcgtcgt cggaggaccc gatggcggcg 600

ctggcgctgg cgccgaaggt gacccgggcg cccgagacgc tccctctctt cccgaccggc 660

ggcggcggag acgataggca gcccccgcgg ccgcggcagt ctgtcccagc aggcgaggcc 720

atccgcggcg gcagcagcag cagcagctac cttccgttct ggggtgccgc gcccacccca 780

actggcagtg ccacttccgt tgcgatccag cagcaacacc agctgatgca gatgcaagag 840
cagtacagct tttacagcaa cgcccagctg ctgcccggca ccggcagcca ggatgcagca 900
gcaacatccc tggagctgag cctcagctcc tggtgctccc cttaccctgc agggaccatg 960
tga 963

<210> 95
<211> 320
<212> PRT
<213> Zea mays
<400> 95

Met Ala Ala Asn Val Gly Ala Gly Arg Ser Ala Gly Gly Gly Gly Ala
1 5 10 15
Gly Thr Gly Thr Gly Thr Ala Ala Gly Ser Gly Gly Val Ser Thr Ala

20 25 30
Val Cys Arg Pro Ser Gly Ser Arg Trp Thr Pro Thr Pro Glu Gln Ile
35 40 45
Arg Ile Leu Lys Glu Leu Tyr Tyr Gly Cys Gly Ile Arg Ser Pro Asn
50 55 60
Ser Glu Gln Ile Gln Arg Ile Thr Ala Met Leu Arg Gln His Gly Lys
65 70 75 80
Ile Glu Gly Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg

85 90 95
Glu Arg Gln Lys Arg Arg Leu Thr Asn Leu Asp Val Asn Val Pro Val
100 105 110
Ala Ala Asp Asp Ser Ala His Arg Leu Gly Val Leu Ser Leu Ser Pro
115 120 125
Ser Ser Gly Cys Ser Gly Ala Ala Pro Pro Ser Pro Thr Leu Gly Phe
130 135 140
Tyr Ala Gly Gly Asn Gly Ser Ala Val Met Leu Asp Thr Ser Ser Asp

145 150 155 160
Trp Gly Ser Ala Ala Ala Met Ala Thr Glu Ala Cys Phe Met Gln Asp

165 170 175

Tyr Met Gly Val Met Gly Gly Ala Ser Pro Trp Ala Cys Ser Ser Ser

180 185 190

Ser Ser Glu Asp Pro Met Ala Ala Leu Ala Leu Ala Pro Lys Val Thr

195 200 205

Arg Ala Pro Glu Thr Leu Pro Leu Phe Pro Thr Gly Gly Gly Gly Asp

210 215 220

Asp Arg Gln Pro Pro Arg Pro Arg Gln Ser Val Pro Ala Gly Glu Ala

225 230 235 240

Ile Arg Gly Gly Ser Ser Ser Ser Ser Tyr Leu Pro Phe Trp Gly Ala

245 250 255

Ala Pro Thr Pro Thr Gly Ser Ala Thr Ser Val Ala Ile Gln Gln Gln

260 265 270

His Gln Leu Met Gln Met Gln Glu Gln Tyr Ser Phe Tyr Ser Asn Ala

275 280 285

Gln Leu Leu Pro Gly Thr Gly Ser Gln Asp Ala Ala Ala Thr Ser Leu

290 295 300

Glu Leu Ser Leu Ser Ser Trp Cys Ser Pro Tyr Pro Ala Gly Thr Met

305 310 315 320

<210> 96

<211> 978

<212> DNA

<213> Zea mays

<400> 96

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gcgccggcgg tgtgccgccc cagcggctcg cggtggacgc cgacgccgga gcagatcagg 120

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cgcatcaccg ccatgctgcg gcagcacggc aagatcgagg gcaagaacgt cttctactgg 240

ttccagaacc acaaggcccg cgagcgccag aagcgccgcc tcaccagcct cgacgtcaac 300

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ggcggcggat cggctgggct gctggacacg agttccgact ggggcagcag cggcgctgcc 480

atggccaccg agacatgctt cctgcaggac tacatgggcg tgacggacac gggcagctcg 540

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cgggtggcga cgacgcgggc gcccgagaca ctccctctct tcccgacctg cggcgacgac 660
gacgacgacg acagccagcc cccgccgcgg ccgcggcacg cagtcccagt cccggcaggc 720
gagaccatcc gcggcggcgg cggcagcagc agcagctact tgccgttctg gggtgccggt 780
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caggagcagt acagctttta cagcaacagc acccagctgg ccggcaccgg cagccaagac 900
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gctgcaggga gcatgtga 978

<210> 97
<211> 325
<212> PRT
<213> Zea mays
<400> 97

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Gly Ser Val Ala Ala Pro Ala Val Cys Arg Pro Ser Gly Ser Arg Trp
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Thr Pro Thr Pro Glu Gln Ile Arg Met Leu Lys Glu Leu Tyr Tyr Gly
35 40 45

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

Gln Leu Gly Val Leu Ser Leu Ser Ser Pro Pro Ser Gly Ala Ala Pro
115 120 125
Pro Ser Pro Thr Leu Gly Phe Tyr Ala Ala Gly Asn Gly Gly Gly Ser

130 135 140

Ala Gly Leu Leu Asp Thr Ser Ser Asp Trp Gly Ser Ser Gly Ala Ala
145 150 155 160

Met Ala Thr Glu Thr Cys Phe Leu Gln Asp Tyr Met Gly Val Thr Asp
165 170 175

Thr Gly Ser Ser Ser Gln Trp Pro Cys Phe Ser Ser Ser Asp Thr Ile
180 185 190

Met Ala Ala Ala Ala Ala Ala Ala Arg Val Ala Thr Thr Arg Ala Pro
195 200 205

Glu Thr Leu Pro Leu Phe Pro Thr Cys Gly Asp Asp Asp Asp Asp Asp
210 215 220

Ser Gln Pro Pro Pro Arg Pro Arg His Ala Val Pro Val Pro Ala Gly
225 230 235 240

Glu Thr Ile Arg Gly Gly Gly Gly Ser Ser Ser Ser Tyr Leu Pro Phe
245 250 255

Trp Gly Ala Gly Ala Ala Ser Thr Thr Ala Gly Ala Thr Ser Ser Val
260 265 270

Ala Ile Gln Gln Gln His Gln Leu Gln Glu Gln Tyr Ser Phe Tyr Ser
275 280 285

Asn Ser Thr Gln Leu Ala Gly Thr Gly Ser Gln Asp Val Ser Ala Ser
290 295 300

Ala Ala Ala Leu Glu Leu Ser Leu Ser Ser Trp Cys Ser Pro Tyr Pro
305 310 315 320

Ala Ala Gly Ser Met
325

<210> 98

<211> 666

<212> DNA

<213> Zea mays

<400> 98

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atccagcgca tctccaccca cctcagcgcc ttcggcaagg tggagagcaa gaacgtcttc 180

tactggttcc agaaccacaa ggcccgcgag cgccaccacc acaagaagcg acgccgcggc 240

gcgtcgtcgt cctcccccga cagcggcagc ggcaggggaa gcaacaacga ggaagacggc 300

cgtggtgccg cctcgcagtc gcacgacgcc gacgccgacg ccgacctcgt gctgcaaccg 360

ccagagagca agcgggaggc cagaagctat ggccaccatc accggctcgt gacatgctac 420

gtcagggacg tggtggagca gcaggaggcg tcgccgtcgt gggagcggcc gacgagggag 480

gtggagacgc tagagctctt ccccctcaag tcgtacggcg acctcgaggc ggcggagaag 540

gtccggtcgt acgtcagagg aagcggcgcc accagcgagc agtgcaggga gttgtccttc 600

ttcgacgtcg tctccgccgg ccgggatccg ccgctcgagc tcaggctctg cagcttcggt 660

ccctag 666

<210> 99

<211> 221

<212> PRT

<213> Zea mays

<400> 99

Met Glu Ala Leu Ser Gly Arg Val Gly Val Lys Cys Gly Arg Trp Asn
1 5 10 15

Pro Thr Ala Glu Gln Val Lys Val Leu Thr Glu Leu Phe Arg Ala Gly
20 25 30

Leu Arg Thr Pro Ser Thr Glu Gln Ile Gln Arg Ile Ser Thr His Leu
35 40 45

Ser Ala Phe Gly Lys Val Glu Ser Lys Asn Val Phe Tyr Trp Phe Gln
50 55 60

Asn His Lys Ala Arg Glu Arg His His His Lys Lys Arg Arg Arg Gly
65 70 75 80

Ala Ser Ser Ser Ser Pro Asp Ser Gly Ser Gly Arg Gly Ser Asn Asn
85 90 95

Glu Glu Asp Gly Arg Gly Ala Ala Ser Gln Ser His Asp Ala Asp Ala
100 105 110

Asp Ala Asp Leu Val Leu Gln Pro Pro Glu Ser Lys Arg Glu Ala Arg
115 120 125

Ser Tyr Gly His His His Arg Leu Val Thr Cys Tyr Val Arg Asp Val
130 135 140

Val Glu Gln Gln Glu Ala Ser Pro Ser Trp Glu Arg Pro Thr Arg Glu
145 150 155 160

Val Glu Thr Leu Glu Leu Phe Pro Leu Lys Ser Tyr Gly Asp Leu Glu
165 170 175

Ala Ala Glu Lys Val Arg Ser Tyr Val Arg Gly Ser Gly Ala Thr Ser
180 185 190

Glu Gln Cys Arg Glu Leu Ser Phe Phe Asp Val Val Ser Ala Gly Arg
195 200 205

Asp Pro Pro Leu Glu Leu Arg Leu Cys Ser Phe Gly Pro
210 215 220

<210> 100

<211> 975

<212> DNA

<213> Zea mays

<400> 100

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cggtggaacc cgaccaagga gcaggtggcc gtgctggagg ggctgtacga gcacggcctg 180

cgcaccccca gcgcggagca gatacagcag atcacgggca ggctgcggga gcacggcgcc 240

atcgagggca agaacgtctt ctactggttc cagaaccaca aggcccgcca gcgccagagg 300

cagaagcagg acagcttcgc ctacttcagc aggctcctcc gccggccccc gccgctgccc 360

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tactactacc cacagccaca gcagcagcag cagcagcagg tgacagtcat gtaccagtac 600

ccgagaatgg aggtagccgg ccaggacaag atgatgacca gggccgcggc gcaccagcag 660

cagcagcaca acggcgccgg gcaacaaccg ggacgcgccg gccaccccag ccgcgagacg 720

ctccagctgt tcccgctcca gcccaccttc gtgctgcggc acgacaaggg gcgcgccgcc 780

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<210> 101

<211> 324

<212> PRT

<213> Zea mays

<400> 101

Met Glu Thr Pro Gln Gln Gln Ser Ala Ala Ala Ala Ala Ala Ala Ala
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His Gly Gln Asp Asp Gly Gly Ser Pro Pro Met Ser Pro Ala Ser Ala
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Ala Ala Ala Ala Leu Ala Asn Ala Arg Trp Asn Pro Thr Lys Glu Gln
35 40 45

Val Ala Val Leu Glu Gly Leu Tyr Glu His Gly Leu Arg Thr Pro Ser
50 55 60

Ala Glu Gln Ile Gln Gln Ile Thr Gly Arg Leu Arg Glu His Gly Ala
65 70 75 80

Ile Glu Gly Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg
85 90 95

Gln Arg Gln Arg Gln Lys Gln Asp Ser Phe Ala Tyr Phe Ser Arg Leu
100 105 110

Leu Arg Arg Pro Pro Pro Leu Pro Val Leu Ser Met Pro Pro Ala Pro
115 120 125

Pro Tyr His His Ala Arg Val Pro Ala Pro Pro Ala Ile Pro Met Pro
130 135 140

Met Ala Pro Pro Pro Pro Ala Ala Cys Asn Asp Asn Gly Gly Ala Arg
145 150 155 160

Val Ile Tyr Arg Asn Pro Phe Tyr Val Ala Ala Pro Gln Ala Pro Pro
165 170 175

Ala Asn Ala Ala Tyr Tyr Tyr Pro Gln Pro Gln Gln Gln Gln Gln Gln

180 185 190

Gln Val Thr Val Met Tyr Gln Tyr Pro Arg Met Glu Val Ala Gly Gln

195 200 205

Asp Lys Met Met Thr Arg Ala Ala Ala His Gln Gln Gln Gln His Asn

210 215 220

Gly Ala Gly Gln Gln Pro Gly Arg Ala Gly His Pro Ser Arg Glu Thr

225 230 235 240

Leu Gln Leu Phe Pro Leu Gln Pro Thr Phe Val Leu Arg His Asp Lys

245 250 255

Gly Arg Ala Ala Asn Gly Ser Asn Asn Asp Ser Leu Thr Ser Thr Ser

260 265 270

Thr Ala Thr Ala Thr Ala Thr Ala Thr Ala Thr Ala Ser Ala Ser Ile

275 280 285

Ser Glu Asp Ser Asp Gly Leu Glu Ser Gly Ser Ser Gly Lys Gly Val

290 295 300

Glu Glu Ala Pro Ala Leu Pro Phe Tyr Asp Phe Phe Gly Leu Gln Ser

305 310 315 320

Ser Gly Gly Arg

<210> 102

<211> 543

<212> DNA

<213> Zea mays

<400> 102

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agggtgctgg agatgctgta ccgcgggggg atgcgcacgc ccaactcgtt ccagatcgag 180

cagatcacgg aggagctcgg caagtacggc cggatcgagg gcaagaacgt cttctactgg 240

ttccagaacc acaaggcccg cgagcgccag aagcagaagc gagctgccct cctcaccctc 300

agcaccacca ccactgcttc cacgctgcta ccaccagctg ctgaaaccaa ggagggagtg 360

gagacgaaaa aagaagaagc gtgtgaagat gcatcgagcc gcaagcggag gtgcagggcc 420

tgggaagatg tcgtcgtcca tggtggcggc gacgatgccg gtacggaggt agctgacgac 480

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taa 543

<210> 103

<211> 180

<212> PRT

<213> Zea mays

<400> 103

Met His Gly Val Arg Thr Tyr Leu Leu Ile Phe Ile Val Ser Lys Leu
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Gln Val Gln Val Pro Pro Thr Thr Thr Thr Val Gly Gly Thr Thr Arg
20 25 30

Trp Asn Pro Ser Pro Asp Gln Ile Arg Val Leu Glu Met Leu Tyr Arg
35 40 45

Gly Gly Met Arg Thr Pro Asn Ser Phe Gln Ile Glu Gln Ile Thr Glu

50 55 60

Glu Leu Gly Lys Tyr Gly Arg Ile Glu Gly Lys Asn Val Phe Tyr Trp
65 70 75 80

Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys Gln Lys Arg Ala Ala
85 90 95

Leu Leu Thr Leu Ser Thr Thr Thr Thr Ala Ser Thr Leu Leu Pro Pro
100 105 110

Ala Ala Glu Thr Lys Glu Gly Val Glu Thr Lys Lys Glu Glu Ala Cys

115 120 125

Glu Asp Ala Ser Ser Arg Lys Arg Arg Cys Arg Ala Trp Glu Asp Val

130 135 140

Val Val His Gly Gly Gly Asp Asp Ala Gly Thr Glu Val Ala Asp Asp
145 150 155 160

Tyr Tyr Thr Asp Asp Asp Val Thr Leu Glu Leu Phe Pro Leu Arg Pro
165 170 175

Asp Gln Gly Lys
180

<210> 104

<211> 708
<212> DNA
<213> Zea mays
<400> 104

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gaccagatcc agcgcatctc cgcccacctc ggcgccttcg gcaaggtgga gagcaagaac 180

gtcttctact ggttccagaa ccacaaggcc cgcgagcgcc accaccacaa gaagaagaag 240

cgccgccgac gcggcgcggc aatggcatcg tcgtcctccc ccgacagcgg cagcggcagc 300

gggagcagcg aggaagacgg ccgtgttgcc gccgccgccg acgccgacct cgtgctgcaa 360

ccatcgccgg gaagaagcaa aagcaaacgg gaggccagaa gaagctacgg cggcggccac 420

catcgccggc tcgttacatg taagcagcta gcgcacacac atcgctacgt cagggacgtt 480

gtggagcagc aggaggagga cacgtgggag cggccgacga gggaggcggt ggagactctg 540

gagctcttcc ccctcaagtc gtacgtcgac ctcgaggcgg agaaggtccg gcggtacgtc 600

gtcagggccg gcgccagcga gcagtgcagg gacttctcct tcttcgacgt gtccggcggt 660

cgggatccgc cgcttcttga gcttaggctc tgcagcttcg gtccctag 708

<210> 105
<211> 235
<212> PRT
<213> Zea mays

<400> 105

Met Asp Thr Glu Ala Pro Ser Gly Arg Val Gly Val Lys Cys Gly Arg
1 5 10 15

Trp Asn Pro Thr Pro Glu Gln Val Lys Val Leu Thr Glu Leu Phe Arg
20 25 30

Ala Gly Leu Arg Thr Pro Ser Thr Asp Gln Ile Gln Arg Ile Ser Ala
35 40 45

His Leu Gly Ala Phe Gly Lys Val Glu Ser Lys Asn Val Phe Tyr Trp
50 55 60

Phe Gln Asn His Lys Ala Arg Glu Arg His His His Lys Lys Lys Lys

65 70 75 80

Arg Arg Arg Arg Gly Ala Ala Met Ala Ser Ser Ser Ser Pro Asp Ser

85 90 95

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

100 105 110

Ala Asp Ala Asp Leu Val Leu Gln Pro Ser Pro Gly Arg Ser Lys Ser

115 120 125

Lys Arg Glu Ala Arg Arg Ser Tyr Gly Gly Gly His His Arg Arg Leu

130 135 140

Val Thr Cys Lys Gln Leu Ala His Thr His Arg Tyr Val Arg Asp Val

145 150 155 160

Val Glu Gln Gln Glu Glu Asp Thr Trp Glu Arg Pro Thr Arg Glu Ala

165 170 175

Val Glu Thr Leu Glu Leu Phe Pro Leu Lys Ser Tyr Val Asp Leu Glu

180 185 190

Ala Glu Lys Val Arg Arg Tyr Val Val Arg Ala Gly Ala Ser Glu Gln

195 200 205

Cys Arg Asp Phe Ser Phe Phe Asp Val Ser Gly Gly Arg Asp Pro Pro

210 215 220

Leu Leu Glu Leu Arg Leu Cys Ser Phe Gly Pro

225 230 235

<210> 106

<211> 1545

<212> DNA

<213> Zea mays

<400> 106

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atcccccgca tccgcatgcg cctgcaggag tacggccagg tcggcgacgc caacgtcttc 360

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actggtcaca ggacatgcgc ctggggcccg gcggggctcg ggcagttctg gcccgtgggc 960

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<210> 107

<211> 514

<212> PRT

<213> Zea mays

<400> 107

Met Ala Ser Ser Phe Asn Asn Lys Thr Ser His Trp Pro Ser Met Phe
1 5 10 15

Arg Ser Lys His Ala Ala Glu Pro Trp Gln Ala Gln Pro Asp Ile Ser
20 25 30

Ser Ser Pro Pro Ser Leu Leu Ser Gly Gly Gly Ser Ser Ser Thr Thr
35 40 45

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

Pro Arg Asp Glu Ile Pro Arg Ile Arg Met Arg Leu Gln Glu Tyr Gly
100 105 110
Gln Val Gly Asp Ala Asn Val Phe Tyr Trp Phe Gln Asn Arg Lys Ser
115 120 125
Arg Ser Lys Asn Lys Gln Arg Thr Gly Gln Leu Gly Leu Gly Leu Ala
130 135 140
Arg Ala Pro Gly Cys Gly Ala Ala Ala Pro Pro Val Thr Pro Gln Pro
145 150 155 160

Leu Ile Gln Asn Gln Phe Gln Val Leu Ala Ser Pro Ala Gln Ala Pro
165 170 175
Ala Ser Ser Ser Ser Ser Ser Ser Asp Arg Ser Ser Gly Ser Ser Lys
180 185 190
Pro Ala Pro Gln Pro Met Ser Ala Thr Ala Ala Ala Met Asn Phe Pro
195 200 205
Gly Pro Leu Gly Ala Ala Cys Ala Gln Met Tyr Tyr Gln Ala His Pro
210 215 220

Val Ala Pro Val Ser Ala Leu Pro Ala His Lys Val Gln Asp Pro Val
225 230 235 240
Ala Ser Asp Glu Pro Val Phe Gln Pro Trp Pro Gln Gly Tyr Phe Leu
245 250 255
Ser Ala Ala Glu Val Ala Ser Ile Leu Gly Gly Gln Tyr Arg His Asp
260 265 270
Val Pro Val Gln Gln Gln Pro Pro Ala Thr Leu Pro Ala Gly Ala Phe
275 280 285

Leu Gly Leu Tyr Asn Glu Val Thr Glu Pro Thr Val Thr Gly His Arg

290 295 300

Thr Cys Ala Trp Gly Pro Ala Gly Leu Gly Gln Phe Trp Pro Val Gly
305 310 315 320
Gly Ala Asp His His Gln His His Lys His Asn Thr Thr Ala Ala Thr
325 330 335
Asn Thr Val Ala Arg Asp Ala Ala His Glu His Ala Thr Thr Leu Gly
340 345 350

Leu Leu Gln Tyr Gly Phe Glu Ala Ser Ala Ala Met Glu Thr Ala Ser
355 360 365
Ala Ala Val Pro Leu Ala Ala Ser Pro Gly Thr Ala Ala Ser Val Ala
370 375 380
Thr Ala Gly Leu Thr Ser Leu Pro Ala Ser Thr Asn Ala Val Val Val
385 390 395 400
Asn Tyr Asp Leu Leu Gln Gly Leu Ala Val Pro Gly Ser Gly Ser Gly
405 410 415

Ala Val Gly Val Ser Thr Gly Gly Ala Pro Pro Val Ala Val Ala Ala
420 425 430
Ala Pro Thr Ala Ala Gln Glu Gly Val Val Val Ala Leu Cys Ile Thr
435 440 445
Asp Ser Val Thr Gly Lys Ser Val Ala His Asn Val Ala Ala Ala Arg
450 455 460
Leu Asp Val Arg Ala Gln Phe Gly Glu Ala Ala Val Leu Leu Arg Ala
465 470 475 480

Val Gly Asp Arg Gly Gly Leu Asp Leu Val Pro Val Pro Val Asp Ala
485 490 495
Leu Gly Cys Thr Val Glu Pro Leu Gln His Gly Ala Phe Tyr Tyr Val
500 505 510
Leu Val

<210> 108
<211> 2000
<212> DNA

<213> Glycine max

<400> 108

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cttggatctc acgggtttca gtgtcaaacc tcatttaata agtcacccca tctcccaccc 180

ttaatttcca ccatccaatt ctccaatctc cttcttcata aattaacggc tgtgatctat 240

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ctcccaaatt aaaaaattgc caattaccaa acatgccatc tctcaaaaag tacaaagtgt 360

tactattatt tctatttaat tagtatagta gtactctttc taataaattt aatattaaaa 420

tcacataatt gtacgtacta cttagtaatt gagattcttc aaaataaaga gaataactct 480

caggtaattt agtgaattct gaaagacaat aaattgtgtt tttgacaaat gttaatacaa 540

caattttata cagtagtaat atttttttata tcacaaaaac taaatataat ttcatctgaa 600

taatctattt atttttttaat gtaaatattt atcacttaga ttatcaagat tacgattttt 660

aaagaaaaga ttaacaagat tacgaattgg gttaagccaa tttgtataag aaaattggat 720

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ctactaacgt tggcattgaa gttgctttta gaagtaccta tcaattttcc acgatatttc 900

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tgaatgagaa taatgttttg atagtttaaa gctttttatt tgtttggttt ctgatatggg 1860

aaagttttgt tgtggggtgt tagagacagg tttggctcaa ctgaagtaag gtttggttat 1920

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<210> 109

<211> 2000

<212> DNA

<213> Glycine max

<400> 109

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gaaaattaaa ctcatgaaca atataaatat actttttat ctgaaataaa cgagggaaaa 180

tatctcttat ttgagaaaaa aaatttaaaa atatttttgt tactactatg tcataagtta 240

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tggcaagaaa actcatactt gtgggtatcc gtctgaattc gtttcgactt tgacgggtaa 420

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cacttaaaat ctttaaattt ttttgcataa tttaatcaaa aggcataaaa tttttattac 600

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tcgcatgggt actaaaatta tttgtcctta ctttatataa acaaattcca acaaaatcaa 480

gtttttgcta aaactagttt atttgcgggt tatttaatta cctatcatat tacttgtaat 540

atcattcgta tgttaacggg taaaccaaac caaaccggat attgaactat taaaaatctt 600

gtaaatttga cacaaactaa tgaatatcta aattatgtta ctgctatgat aacgaccatt 660

tttgtttttg agaaccataa tataaattac aggtacgtga caagtactaa gtatttatat 720

ccacctttag tcacagtacc aatattgcgc ctaccgggca acgtgaacgt gatcatcaaa 780

tcaaagtagt taccaaacgc tttgatctcg ataaaactaa aagctgacac gtcttgctgt 840

ttcttaattt atttctctta caacgacaat tttgagaaat atgaaatttt tatatcgaaa 900

gggaacagtc cttatcattt gctcccatca cttgcttttg tctagttaca actggaaatc 960

gaagagaagt attacaaaaa cattttttctc gtcatttata aaaaaatgac aaaaaattaa 1020

atagagagca aagcaagagc gttgggtgac gttggtctct tcattaactc ctctcatcta 1080

ccccttcctc tgttcgcctt tatatccttc accttccctc tctcatcttc 1130

<210> 127

<211> 663

<212> DNA

<213> Gnetum gnemon

<400> 127

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ccggagcagc tgagcattct gaaagagctg tatcatggcc gcggaatcag gtcgcccagc 120

gccgaacaga tccaccatat ctcatggaaa ctcagctcct acggcaaaat cgagggcaag 180

aacgtcttct actggttcca gaaccataag gcccgccagc gtcaaaaaga gcgtctcggt 240

tccgtcatgc ctgcttctgc ttcttcttct tcttttgcat ctccttctca gggagctgtc 300

ggcattcgaa ggggaagtac ggtgctatgt cttaaggaaa cttcagcttc atcttcatct 360

tattcttctg ttccgcgttc ggtatcagct gtgcgccagt gtgctgcatc tcgtgactgt 420

ggcttgcacc atgatctgac aactctggag ctgtttcccc tgcaccgtga aagtagaata 480

gacgctgggg aggaagacga ggagcaacag gaggtggtgg tgctagataa taataataac 540

acctggacaa ggcaccaaaa tgtaaatgtg aatcatgcaa ataatgacga tgagccacag 600

catggtcatg ggcagattga tattgatgta gggcttcgtc tttgcctcgg tggatttcgc 660

tga 663

<210> 128

<211> 220

<212> PRT

<213> Gnetum gnemon

<400> 128

Met Glu Glu Asn Ser Asn Ser Ser Arg Ser Ser Asn Ser Gly Ala Arg

1 5 10 15

Trp Asn Pro Thr Pro Glu Gln Leu Ser Ile Leu Lys Glu Leu Tyr His

20 25 30

Gly Arg Gly Ile Arg Ser Pro Ser Ala Glu Gln Ile His His Ile Ser

35 40 45

Trp Lys Leu Ser Ser Tyr Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr

50 55 60

Trp Phe Gln Asn His Lys Ala Arg Gln Arg Gln Lys Glu Arg Leu Gly

65 70 75 80

Ser Val Met Pro Ala Ser Ala Ser Ser Ser Ser Phe Ala Ser Pro Ser

85 90 95

Gln Gly Ala Val Gly Ile Arg Arg Gly Ser Thr Val Leu Cys Leu Lys

100 105 110

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

115 120 125

Ser Ala Val Arg Gln Cys Ala Ala Ser Arg Asp Cys Gly Leu His His

130 135 140

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

145 150 155 160

Asp Ala Gly Glu Glu Asp Glu Glu Gln Gln Glu Val Val Val Leu Asp

165 170 175

Asn Asn Asn Asn Thr Trp Thr Arg His Gln Asn Val Asn Val Asn His

180 185 190

Ala Asn Asn Asp Asp Glu Pro Gln His Gly His Gly Gln Ile Asp Ile

195 200 205

Asp Val Gly Leu Arg Leu Cys Leu Gly Gly Phe Arg

210 215 220

<210> 129

<211> 987

<212> DNA

<213> Malus domestica

<400> 129

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aaggaccttt actacaacaa tggcattagg tcacccagcg ctgagcagat tcagaggatc 180

tctgctaagc tgcgacagtt cggcaagatc gaaggcaaga acgttttcta ttggtttcag 240
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catcatcatg cagcaccacc agtgccagct gtaccaccag aatccaataa tatcaggcaa 360
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cccattaatc accaccgcaa gtattccaac atttctgctc caccagccgg gttttcttct 480
gcatcttctt cttcagttgg tgtgaatgtt tctgttgggg cacagatggg aaactatggg 540
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actagcactg gtcatgtggg tggatctaat tataatactt ttaatcacaa ctatggatca 660
tgggttggtg ttgatccata ttcctcgccc tacactatct ttgacaagaa atcctcatca 720
aaaccagtgt ttggtgacca ggaaaatatg acggaagaag aatactacaa tctgcaagct 780
tcccaagaga ttgagactct cccactcttc cccatgcacg gtgaagacat tcatggcttt 840
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ggcaacgatg gtggctctcg cacttccctt gagcttagcc tcaattccta cggtcacatg 960
acccatgatt acttcagatc atattga 987

<210> 130
<211> 328
<212> PRT
<213> Malus domestica
<400> 130

Met Asp Pro Gln Gln Asn Pro Asn Glu Val Gln Glu Gly Gly Asn Arg
1 5 10 15
Gln Gly Gly Gly Ile Met Cys Arg Gln Ser Ser Thr Arg Trp Thr Pro
20 25 30
Thr Thr Asp Gln Ile Lys Ile Leu Lys Asp Leu Tyr Tyr Asn Asn Gly
35 40 45
Ile Arg Ser Pro Ser Ala Glu Gln Ile Gln Arg Ile Ser Ala Lys Leu
50 55 60
Arg Gln Phe Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr Trp Phe Gln
65 70 75 80
Asn His Lys Ala Arg Glu Arg Gln Lys Lys Arg Phe Thr Ser Ser Ser

85 90 95

Ala Pro Asp His His His His Ala Ala Pro Pro Val Pro Ala Val Pro

100 105 110

Pro Glu Ser Asn Asn Ile Arg Gln Arg Ser Ser Gly Val Gly Ile Asp

115 120 125

Ile Asn Ala Thr Ala Ala Ala Ala Tyr Glu Gln Ala Pro Ile Asn His

130 135 140

His Arg Lys Tyr Ser Asn Ile Ser Ala Pro Pro Ala Gly Phe Ser Ser

145 150 155 160

Ala Ser Ser Ser Ser Val Gly Val Asn Val Ser Val Gly Ala Gln Met

165 170 175

Gly Asn Tyr Gly Tyr Gly Ser Ile Ala Met Glu Lys Ser Phe Arg Glu

180 185 190

Cys Ser Ile Ser Ser Gly Gly Ser Thr Ser Thr Gly His Val Gly Gly

195 200 205

Ser Asn Tyr Asn Thr Phe Asn His Asn Tyr Gly Ser Trp Val Gly Val

210 215 220

Asp Pro Tyr Ser Ser Pro Tyr Thr Ile Phe Asp Lys Lys Ser Ser Ser

225 230 235 240

Lys Pro Val Phe Gly Asp Gln Glu Asn Met Thr Glu Glu Glu Tyr Tyr

245 250 255

Asn Leu Gln Ala Ser Gln Glu Ile Glu Thr Leu Pro Leu Phe Pro Met

260 265 270

His Gly Glu Asp Ile His Gly Phe Gly Asn Ile Lys Ser Ser Ser Met

275 280 285

Asp Gly Tyr Tyr Ser Gly Trp Tyr Arg Ser Asp Gly Gly Asn Asp Gly

290 295 300

Gly Ser Arg Thr Ser Leu Glu Leu Ser Leu Asn Ser Tyr Gly His Met

305 310 315 320

Thr His Asp Tyr Phe Arg Ser Tyr

325

<210> 131

<211> 867

<212> DNA

<213> Manihot esculenta

<400> 131

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actgaccaaa taagaatatt gaaggacctt tactacaaca gtggagttag gtccccaagt 180

gcagagcaga ttcagaggat atctgctagg cttagacagt acggtaagat tgaaggcaag 240

aatgtctttt attggtttca gaaccataaa gctcgtgaga ggcagaagaa aaggttcacc 300

actgatgtcc ccatgcaaca aagaactgtt tcgaatgctt ctagttggaa gcctgaagac 360

tattccttcc acaacaaata ccccaacatt gctcctgggt tttcttctgc atctccatcc 420

tcagctgctg ggcttcctgt tggacagatg ggaaactatg ggtatggatc tgtaactatg 480

gagaagagtt ttagggactg ctcaatatca gctactgcca acggtggtgt tggtggatcc 540

atgagcccca actgtgggtg ggttggcatt gatccctact gttcatccta ctctctcttt 600

gacaagcaaa aatcaactaa tgaaacccta gaagatgaag aacaagatca agatcaagaa 660

gaggaagcaa ctccagggat tgaaaccctc cctctcttcc ctacccagag agaagacatc 720

aatggcttct gcaacatgaa gcaccacccc atctgctact ccgaaaacta ctgctatggc 780

tctgacgatg gaaacaacgc ttcgcgtact tccctcgaac ttagcctcaa ctcttacagc 840

aatgggcagg cacgggattc catctaa 867

<210> 132

<211> 288

<212> PRT

<213> Manihot esculenta

<400> 132

Met Glu Pro Gln Gln Gln Gln Gln Gln Gln Asn Gln Gln Gln Pro
1 5 10 15

Asn Asp Asp Asn Ser Ser Gly Ala Lys Gly Asn Phe Leu Cys Arg Gln
20 25 30

Ser Ser Thr Arg Trp Thr Pro Thr Thr Asp Gln Ile Arg Ile Leu Lys
35 40 45

Asp Leu Tyr Tyr Asn Ser Gly Val Arg Ser Pro Ser Ala Glu Gln Ile

50 55 60

Gln Arg Ile Ser Ala Arg Leu Arg Gln Tyr Gly Lys Ile Glu Gly Lys
65 70 75 80

Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys
85 90 95

Lys Arg Phe Thr Thr Asp Val Pro Met Gln Gln Arg Thr Val Ser Asn
100 105 110

Ala Ser Ser Trp Lys Pro Glu Asp Tyr Ser Phe His Asn Lys Tyr Pro
115 120 125

Asn Ile Ala Pro Gly Phe Ser Ser Ala Ser Pro Ser Ser Ala Ala Gly
130 135 140

Leu Pro Val Gly Gln Met Gly Asn Tyr Gly Tyr Gly Ser Val Thr Met
145 150 155 160

Glu Lys Ser Phe Arg Asp Cys Ser Ile Ser Ala Thr Ala Asn Gly Gly
165 170 175

Val Gly Gly Ser Met Ser Pro Asn Cys Gly Trp Val Gly Ile Asp Pro
180 185 190

Tyr Cys Ser Ser Tyr Ser Leu Phe Asp Lys Gln Lys Ser Thr Asn Glu
195 200 205

Thr Leu Glu Asp Glu Glu Gln Asp Gln Asp Gln Glu Glu Glu Ala Thr
210 215 220

Pro Gly Ile Glu Thr Leu Pro Leu Phe Pro Thr Gln Arg Glu Asp Ile
225 230 235 240

Asn Gly Phe Cys Asn Met Lys His His Pro Ile Cys Tyr Ser Glu Asn
245 250 255

Tyr Cys Tyr Gly Ser Asp Asp Gly Asn Asn Ala Ser Arg Thr Ser Leu
260 265 270

Glu Leu Ser Leu Asn Ser Tyr Ser Asn Gly Gln Ala Arg Asp Ser Ile
275 280 285

<210> 133

<211> 960

<212> DNA

<213> Kalanchoe fedtschenkoi

<400> 133

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aataacaacg gcggcagaag aagcagcacg aggtggacac caacaacaga ccagataaga 180

atactcaaag atctttacta caacaatgga gtgaggtctc ccacggtgga gcagattcag 240

aggatctctg ctaggttgag acagtacggt aagatcgagg gcaaaaatgt cttctactgg 300

tttcagaacc aaaaggctcg tgaaagacag aagaggaggt tcaacacaac cagcaccacc 360

aacaacaaca ctgctcccgc tcctctccct ccatcttcta ctgtttgtaa accttcttcg 420

ctagactcat ccactccaca cattcacact gcagcagcag ctgctgctgc tgcctccaaa 480

tactcagaca tcaacaacaa ccaaaacctt atctacccag ctgggcacaa tcagatgtca 540

ggtttcggat atgctgcctc cgtaacaatg gagaagagct tcagagattg ttctatatcg 600

acgggcgggt catctttcgg ttgggctaaa ggaggaatga cagatcacca tctgtctcgt 660

catccctacc cgcacacttc accctatgca atctacaacc agcggaaatc aataaacagt 720

gatgagtatc acgagctact ggcatcatca acttcggatc atcatgaaca tggggataat 780

cggggtccgg agattgaaac cctgccacta tttcccatgc acggcgagga catcaactac 840

ggcttctgcc accttaattc agccgagact gatggctggt tcaatcgcga tgtgggtagt 900

ggctctcgtg cgtccctcga gctcagtctc aactcttacg caggaatgga ccacacctag 960

<210> 134

<211> 319

<212> PRT

<213> Kalanchoe fedtschenkoi

<400> 134

Met Glu Ala Gln Gln Gln Gln His Asn Thr Met Met Gly Asp Asp Leu

1 5 10 15

Gly Gly Gly Lys Leu Ala Ala Thr Ser Phe Thr Arg Ala Gln Thr Asn

20 25 30

Ser Ser Asn Asn His Ser Leu Val Asn Asn Asn Gly Gly Arg Arg Ser

35 40 45

Ser Thr Arg Trp Thr Pro Thr Thr Asp Gln Ile Arg Ile Leu Lys Asp

50 55 60

Leu Tyr Tyr Asn Asn Gly Val Arg Ser Pro Thr Val Glu Gln Ile Gln

65 70 75 80

Arg Ile Ser Ala Arg Leu Arg Gln Tyr Gly Lys Ile Glu Gly Lys Asn

85 90 95

Val Phe Tyr Trp Phe Gln Asn Gln Lys Ala Arg Glu Arg Gln Lys Arg

100 105 110

Arg Phe Asn Thr Thr Ser Thr Thr Asn Asn Asn Thr Ala Pro Ala Pro

115 120 125

Leu Pro Pro Ser Ser Thr Val Cys Lys Pro Ser Ser Leu Asp Ser Ser

130 135 140

Thr Pro His Ile His Thr Ala Ala Ala Ala Ala Ala Ala Ala Ser Lys

145 150 155 160

Tyr Ser Asp Ile Asn Asn Asn Gln Asn Leu Ile Tyr Pro Ala Gly His

165 170 175

Asn Gln Met Ser Gly Phe Gly Tyr Ala Ala Ser Val Thr Met Glu Lys

180 185 190

Ser Phe Arg Asp Cys Ser Ile Ser Thr Gly Gly Ser Ser Phe Gly Trp

195 200 205

Ala Lys Gly Gly Met Thr Asp His His Leu Ser Arg His Pro Tyr Pro

210 215 220

His Thr Ser Pro Tyr Ala Ile Tyr Asn Gln Arg Lys Ser Ile Asn Ser

225 230 235 240

Asp Glu Tyr His Glu Leu Leu Ala Ser Ser Thr Ser Asp His His Glu

245 250 255

His Gly Asp Asn Arg Gly Pro Glu Ile Glu Thr Leu Pro Leu Phe Pro

260 265 270

Met His Gly Glu Asp Ile Asn Tyr Gly Phe Cys His Leu Asn Ser Ala

275 280 285

Glu Thr Asp Gly Trp Phe Asn Arg Asp Val Gly Ser Gly Ser Arg Ala

290 295 300

Ser Leu Glu Leu Ser Leu Asn Ser Tyr Ala Gly Met Asp His Thr

305 310 315

<210> 135

<211> 840

<212> DNA

<213> Gossypium hirsutum

<400> 135

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ggcagtggca aaggaagctt tctttgcagg caaagtagta cacggtggac tcctaccact 120

gaccagatta gaatattgaa ggacctttac tacaacaatg gagttaggtc cccgagtgct 180

gatcagattc aaaggatctc tgctagactt agacagtacg gaaaaatcga aggcaagaat 240

gtcttttatt ggtttcagaa ccacaaggct cgtgagaggc agaagaaaag gttcaccacc 300

acgactaatc atcatgtccc catgcaaagc cctaccactg ctgcaaataa caagtatccc 360

aacataactc ctggttattc tcctacatct ctttcttcaa ctggtgctct tactgttggg 420

cagataggaa actatggcta tgggtctata actatggaaa agagttttag ggattgctca 480

atatcagcat gtggtagtag tacaagtgta ggcgaatccc tgagtcacaa ctttggatgg 540

gttgggttcg atcctactta ttcttcgtcc tacacattct ttgagcacaa aaaattcatg 600

gaagagagac aaaaagatga agatgatgca gaagaagaag ctgctgctcc acaaattgag 660

accctccctc tctttcccat gcacagtgaa gacgctaatg gtttttgcag taatattagg 720

cccagactag acagctgcta ctctgggtgg tacaggtctg aagatggcta cactggttct 780

cgtgcctcac ttgagctcag cctcaaccca tatgcaggca gaccgcaaga ttctatctga 840

<210> 136

<211> 279

<212> PRT

<213> Gossypium hirsutum

<400> 136

Met Glu Pro Gln Gln Gln Gln Gln Asn Gln Gln Ala Pro Asn Glu

1 5 10 15

Asp Cys Gly Gly Gly Ser Gly Lys Gly Ser Phe Leu Cys Arg Gln Ser

20 25 30

Ser Thr Arg Trp Thr Pro Thr Thr Asp Gln Ile Arg Ile Leu Lys Asp

35 40 45

Leu Tyr Tyr Asn Asn Gly Val Arg Ser Pro Ser Ala Asp Gln Ile Gln

50 55 60

Arg Ile Ser Ala Arg Leu Arg Gln Tyr Gly Lys Ile Glu Gly Lys Asn

65 70 75 80

Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys Lys

85 90 95

Arg Phe Thr Thr Thr Thr Asn His His Val Pro Met Gln Ser Pro Thr

100 105 110

Thr Ala Ala Asn Asn Lys Tyr Pro Asn Ile Thr Pro Gly Tyr Ser Pro

115 120 125

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

130 135 140

Tyr Gly Tyr Gly Ser Ile Thr Met Glu Lys Ser Phe Arg Asp Cys Ser

145 150 155 160

Ile Ser Ala Cys Gly Ser Ser Thr Ser Val Gly Glu Ser Leu Ser His

165 170 175

Asn Phe Gly Trp Val Gly Phe Asp Pro Thr Tyr Ser Ser Ser Tyr Thr

180 185 190

Phe Phe Glu His Lys Lys Phe Met Glu Glu Arg Gln Lys Asp Glu Asp

195 200 205

Asp Ala Glu Glu Glu Ala Ala Ala Pro Gln Ile Glu Thr Leu Pro Leu

210 215 220

Phe Pro Met His Ser Glu Asp Ala Asn Gly Phe Cys Ser Asn Ile Arg

225 230 235 240

Pro Arg Leu Asp Ser Cys Tyr Ser Gly Trp Tyr Arg Ser Glu Asp Gly

245 250 255

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

260 265 270

Gly Arg Pro Gln Asp Ser Ile

275

<210> 137

<211> 969

<212> DNA

<213> Zostera marina

<400> 137

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gaagagatga acaacaatgg gagcgggggt gggagtagta agagcagttt tctttgcaga 120

cagtcgagca caaggtggat acccactact gatcagataa ggatactgaa ggatctatac 180

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ttctccaaca acactaatat gtcttcttct tcttctccct cagggatttc tcctgcttct 420

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gagagaactt cttacatgga gtgttccttg ccaggtggga tgccgatgcc cgtacctgaa 540

cccaatgtgg gagtcggagt gggatgggtg agtgttgaaa caggagctac tactatgcaa 600

caattctact accatcaaca gagaccaacc acacccttтc ctgctcctca tcatcaccac 660

cattactacg acgacggtta ctattccaat aataacaaac ttcaagccca tcacctacat 720

atacgcgagg aaaatcaaca tccagaagat gacgacgaag aagaagaaga agaagaagaa 780

gatgaagaga tagaaacact tccattgttc ccaatccact cacaggagac acagtcttac 840

ttcctggacc aacaacatgc aatccacaat caagaaagca ataacatgga aacctctttg 900

gagctcaccc taaactccta ctatgctgct tccagtagca gtactccaca tggatcagag 960

catgattaa 969

<210> 138

<211> 322

<212> PRT

<213> Zostera marina

<400> 138

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1 5 10 15

Met Ser Glu Asn Glu Glu Met Asn Asn Asn Gly Ser Gly Gly Gly Ser
20 25 30

Ser Lys Ser Ser Phe Leu Cys Arg Gln Ser Ser Thr Arg Trp Ile Pro
35 40 45

Thr Thr Asp Gln Ile Arg Ile Leu Lys Asp Leu Tyr Tyr Asn Ser Gly

50 55 60

Leu Arg Ser Pro Asn Ala Asp Gln Ile Gln Arg Ile Ser Ala Arg Leu

65 70 75 80

Arg Gln Tyr Gly Lys Ile Glu Gly Lys Asn Val Phe Tyr Trp Phe Gln

85 90 95

Asn His Lys Ala Arg Glu Arg Gln Lys Lys Arg Leu Thr Val Asp Ile

100 105 110

Ala Ala Ala Ala Thr Thr Asn Lys Phe Ser Asn Asn Thr Asn Met Ser

115 120 125

Ser Ser Ser Ser Pro Ser Gly Ile Ser Pro Ala Ser His Ala Val Tyr

130 135 140

Ala Gly Leu Gly Gln Met Gly Ser Asn Gly Gly Gly Ser Val Val Met

145 150 155 160

Glu Arg Thr Ser Tyr Met Glu Cys Ser Leu Pro Gly Gly Met Pro Met

165 170 175

Pro Val Pro Glu Pro Asn Val Gly Val Gly Val Gly Trp Val Ser Val

180 185 190

Glu Thr Gly Ala Thr Thr Met Gln Gln Phe Tyr Tyr His Gln Gln Arg

195 200 205

Pro Thr Thr Pro Phe Pro Ala Pro His His His His His Tyr Tyr Asp

210 215 220

Asp Gly Tyr Tyr Ser Asn Asn Asn Lys Leu Gln Ala His His Leu His

225 230 235 240

Ile Arg Glu Glu Asn Gln His Pro Glu Asp Asp Asp Glu Glu Glu Glu

245 250 255

Glu Glu Glu Glu Asp Glu Glu Ile Glu Thr Leu Pro Leu Phe Pro Ile

260 265 270

His Ser Gln Glu Thr Gln Ser Tyr Phe Leu Asp Gln Gln His Ala Ile

275 280 285

His Asn Gln Glu Ser Asn Asn Met Glu Thr Ser Leu Glu Leu Thr Leu

290 295 300

Asn Ser Tyr Tyr Ala Ala Ser Ser Ser Ser Thr Pro His Gly Ser Glu

305 310 315 320

His Asp

<210> 139

<211> 693

<212> DNA

<213> Amborella trichopoda

<400> 139

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caaatcagaa tccttagaga actttactac tctaacggtg ttagatctcc aaccgctgaa 180

caaatccaaa gaatctctgc tagacttaga caatacggta agatcgaagg taagaacgtt 240

ttttactggt ttcaaaacca taaggctaga gaaagacaaa agaagagact taccgttgat 300

cttgctgttc aaagagctgc tgctacctct ggtggtcttc caccaggtgt tcttaacgtt 360

tctcatatcg ctggttctac ctgcggttct gttcttatgg aatcttctct tatggattgc 420

tgctctacct ctgctcttga aaaccataac ggtagaaagg aaagagctaa cggtgatatg 480

atcgttgaaa gagaaagatc tgctccaggt aaggaaaccc ttcaactttt tccaatgcat 540

cattctccac aaaacgatga taccatcgct gatgattact tttttaacga tgaagaagaa 600

aagccagttt gcatggcttc tcttgaactt acccttaact cttttgatag agtttaccat 660

ccagattctg attttagatc tcattcttct taa 693

<210> 140

<211> 230

<212> PRT

<213> Amborella trichopoda

<400> 140

Met Asp His Ser Asp Asn Cys Pro Gly Asn Asn Asn Asn Ser Gly Ser

1 5 10 15

Gly Ser Gly Ser Gly Thr Lys Ser Gly Tyr Met Cys Arg Gln Ser Asn

20 25 30

Ser Arg Trp Ile Pro Thr Ala Glu Gln Ile Arg Ile Leu Arg Glu Leu

35 40 45

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

50 55 60

Ile Ser Ala Arg Leu Arg Gln Tyr Gly Lys Ile Glu Gly Lys Asn Val

65 70 75 80

Phe Tyr Trp Phe Gln Asn His Lys Ala Arg Glu Arg Gln Lys Lys Arg

85 90 95

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

100 105 110

Leu Pro Pro Gly Val Leu Asn Val Ser His Ile Ala Gly Ser Thr Cys

115 120 125

Gly Ser Val Leu Met Glu Ser Ser Leu Met Asp Cys Cys Ser Thr Ser

130 135 140

Ala Leu Glu Asn His Asn Gly Arg Lys Glu Arg Ala Asn Gly Asp Met

145 150 155 160

Ile Val Glu Arg Glu Arg Ser Ala Pro Gly Lys Glu Thr Leu Gln Leu

165 170 175

Phe Pro Met His His Ser Pro Gln Asn Asp Asp Thr Ile Ala Asp Asp

180 185 190

Tyr Phe Phe Asn Asp Glu Glu Glu Lys Pro Val Cys Met Ala Ser Leu

195 200 205

Glu Leu Thr Leu Asn Ser Phe Asp Arg Val Tyr His Pro Asp Ser Asp

210 215 220

Phe Arg Ser His Ser Ser

225 230

<210> 141

<211> 834

<212> DNA

<213> Aquilegia coerulea

<400> 141

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tggacaccca caagtgatca gataagaatc ttgaaagacc tttactacaa caatggagtt 180

aggtctccaa gtgcagagca aatacagaga atctcagcaa ggttaaggca gtacggtaag 240

attgaaggca agaatgtttt ctattggttt cagaaccaca aagctcgtga gagacaaaag 300

aagagattca caaatgatac aacaaccatg tccatgcaaa gagctactac tgcaactggt 360

catgccaaat acattactac ttctcctggg atttcatctc catcttctgc tggtgtactt 420

ggtgttggac agatgggaag caacatggga tatggatctg ttgttatgga gaagagtttt 480

agagactgtt caatatctcc tgctgctggt ggtggtggtg gtggtggcag taatatgggt 540

ggatatggat ccatgaacaa caattttggt tggattggtg cgggtgtcga gccatatcca 600

tttttcaaca agacaaagca tattcaaaaa tatgaagaag aagatttctc ccaaactgag 660

attgaaacac taccactatt tcccatgcat ggtgaagaca gtacaaggtt tggttcatct 720

ggcaacccaa acccgaattg gaactggttt cgaacaggag atgaaatgat tggatctaga 780

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<210> 142

<211> 277

<212> PRT

<213> Aquilegia coerulea

<400> 142

Met Glu Pro Gln Gln Asp Pro Asn Asn Gln Gln Gln Gln Leu Gln Gln
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Gln Gln His Glu Glu Asn Ser Ser Gly Ser Ser Ser Lys Ser Ser Phe
20 25 30

Leu Cys Arg Gln Thr Ser Thr Arg Trp Thr Pro Thr Ser Asp Gln Ile
35 40 45

Arg Ile Leu Lys Asp Leu Tyr Tyr Asn Asn Gly Val Arg Ser Pro Ser
50 55 60

Ala Glu Gln Ile Gln Arg Ile Ser Ala Arg Leu Arg Gln Tyr Gly Lys
65 70 75 80

Ile Glu Gly Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg
85 90 95

Glu Arg Gln Lys Lys Arg Phe Thr Asn Asp Thr Thr Thr Met Ser Met
100 105 110

Gln Arg Ala Thr Thr Ala Thr Gly His Ala Lys Tyr Ile Thr Thr Ser
115 120 125

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

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

Ile Gly Ser Arg Thr Ser Leu Glu Leu Ser Leu Asn Ser Tyr Ser Gly
260 265 270
Gly Ser Ile Asn Asn
275

<210> 143
<211> 969
<212> DNA
<213> Amaranthus hypochondriacus
<400> 143
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tggactccaa caacagaaca aataaagctt ttaaaagatc tttattattt acaaggaatt 180
agatctccta ctgctgatca aattcaaagc atttgtgcta ggttacgtcg ttatggaaaa 240

attgaaggca aaaatgtttt ttattggttt caaaatcata aggctaggga aagacaaaaa 300

aagaagctta ctcctacttc tcctaataat aataataata ataataataa taatttcata 360

agaaataata ataataataa taataatgga aatattaaca cttttcatgc taaatatcct 420

tgttccttca ataataatcc cggggtttct tgttcaagtt caccagcagg tgtgatttct 480

ggaggtgggc aattgatggg gagttttggg tatggagctg tgactatgga gactaaattt 540

agggtaagag gaggtattcc tattccaaat ggagcaaatc aatggagtaa ctatttcaac 600

ccaaacaatc caacatctta ctcaaatttc tttattgaca aggtatcatc aaatgttgat 660

catcattatc catacattaa ccatcatgat catgatcatg atcatgatca tcatgatcat 720

gatcattatg gcccacaatc acctcatgag attcagactc ttcctctctt tcccatgcac 780

aatgatcatc atgatctcga tctcgatctc gatctcgatc acgatcaagg gttctacacc 840

tataagcctc aatcggacgg ctgtaattat accggaggat atcgatggtc atctaatgtt 900

aatggtggac atacttctct tgagcttagt cttaactctt atggatttgg atgctattca 960

cctaattaa 969

<210> 144

<211> 322

<212> PRT

<213> Amaranthus hypochondriacus

<400> 144

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Lys Met Ser Asn Asn Thr Ser Asn Asn Asn Asn Asn Asn Asn Asn Tyr
20 25 30

Leu Cys Arg Gln Ser Ser Thr Arg Trp Thr Pro Thr Thr Glu Gln Ile
35 40 45

Lys Leu Leu Lys Asp Leu Tyr Tyr Leu Gln Gly Ile Arg Ser Pro Thr
50 55 60

Ala Asp Gln Ile Gln Ser Ile Cys Ala Arg Leu Arg Arg Tyr Gly Lys
65 70 75 80

Ile Glu Gly Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg
85 90 95

Glu Arg Gln Lys Lys Lys Leu Thr Pro Thr Ser Pro Asn Asn Asn Asn
100 105 110

Asn Asn Asn Asn Asn Asn Phe Ile Arg Asn Asn Asn Asn Asn Asn Asn
115 120 125

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

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

Phe Pro Met His Asn Asp His His Asp Leu Asp Leu Asp Leu Asp Leu
260 265 270
Asp His Asp Gln Gly Phe Tyr Thr Tyr Lys Pro Gln Ser Asp Gly Cys
275 280 285
Asn Tyr Thr Gly Gly Tyr Arg Trp Ser Ser Asn Val Asn Gly Gly His
290 295 300
Thr Ser Leu Glu Leu Ser Leu Asn Ser Tyr Gly Phe Gly Cys Tyr Ser
305 310 315 320

Pro Asn

<210> 145
<211> 924
<212> DNA

<213> Cucumis sativus

<400> 145

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actccaacaa ctgaccaaat acgaatattg aaagagcttt attataacaa tggtgttcgc 180

tccccaagcg ctgatcaaat tcagaggatc tctgctaggc ttagacagta cggtaaaatc 240

gaaggcaaaa acgtttttta ttggtttcaa aaccataagg ctcgtgaaag acagaagaaa 300

agattcactt caaattctac ttctcctatt actacctcca atactactac aactactact 360

gattttaaaa tttcttcaac taataataat attaactgga agtctgaaga tcattcctcc 420

tcttctcata acaaatttcc tccaggaata acaattgctt taattgattc ttgtttttgc 480

agctccttct tcttcggtta tggtcgccgt tggccatatg ggaaattacg gatatgggag 540

tgttcaatat cgtcgggagg aaatagcagt gtcgtcggtt accggagcca caacatggga 600

tcatggatcg gtatcgatcc gtattcttct ccggcagctg ccgcggcggg gggatcagcc 660

aatgtgtttg aaaaaacaaa atatgttgaa gaaagcatgg aagatcacga ggagcaagag 720

atcgaaactc tccccctttt ccccattcat ggtgaccgga ataatctcgg tggtttctgc 780

agcatgaagc cggaatattc agagagctac tacaccacca cttggtacgg gcggtctgac 840

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atctcacctg atgatggaat gtga 924

<210> 146

<211> 307

<212> PRT

<213> Cucumis sativus

<400> 146

Met Glu His Pro Ala Lys Gln Gln Gln Gln Glu Gln Gly Ile Asn Asp
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Gln Gly Gly Glu Gly Gly Gly Gly Ser Asn Gly Lys Gly Gly Phe Leu
20 25 30

Cys Arg Gln Ser Ser Ser Arg Trp Thr Pro Thr Thr Asp Gln Ile Arg
35 40 45

Ile Leu Lys Glu Leu Tyr Tyr Asn Asn Gly Val Arg Ser Pro Ser Ala
50 55 60

Asp Gln Ile Gln Arg Ile Ser Ala Arg Leu Arg Gln Tyr Gly Lys Ile

65 70 75 80

Glu Gly Lys Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg Glu
85 90 95

Arg Gln Lys Lys Arg Phe Thr Ser Asn Ser Thr Ser Pro Ile Thr Thr
100 105 110

Ser Asn Thr Thr Thr Thr Thr Thr Asp Phe Lys Ile Ser Ser Thr Asn
115 120 125

Asn Asn Ile Asn Trp Lys Ser Glu Asp His Ser Ser Ser Ser His Asn
130 135 140

Lys Phe Pro Pro Gly Ile Thr Ile Ala Leu Ile Asp Ser Cys Phe Cys
145 150 155 160

Ser Ser Phe Phe Phe Gly Tyr Gly Arg Arg Trp Pro Tyr Gly Lys Leu
165 170 175

Arg Ile Trp Glu Cys Ser Ile Ser Ser Gly Gly Asn Ser Ser Val Val
180 185 190

Gly Tyr Arg Ser His Asn Met Gly Ser Trp Ile Gly Ile Asp Pro Tyr
195 200 205

Ser Ser Pro Ala Ala Ala Ala Ala Gly Gly Ser Ala Asn Val Phe Glu
210 215 220

Lys Thr Lys Tyr Val Glu Glu Ser Met Glu Asp His Glu Glu Gln Glu
225 230 235 240

Ile Glu Thr Leu Pro Leu Phe Pro Ile His Gly Asp Arg Asn Asn Leu
245 250 255

Gly Gly Phe Cys Ser Met Lys Pro Glu Tyr Ser Glu Ser Tyr Tyr Thr
260 265 270

Thr Thr Trp Tyr Gly Arg Ser Asp Asp Asn Gly Ala Ala Gly Ser Arg
275 280 285

Ala Ser Leu Glu Leu Ser Leu Asn Ser Tyr Ala Thr Ile Ser Pro Asp
290 295 300

Asp Gly Met
305

<210> 147

<211> 840

<212> DNA

<213> Pinus taeda

<400> 147

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agatctccaa ccgttgatga aatccataga atctctatga agctttctag atacggtaag 180

atcgaaggta agaacgtttt ttactggttt caaaaccata aggctagaca tagacaaaag 240

accagacttt ctgctatgaa cgttggtaag ccagctgctt ctccagctct ttctcaaacc 300

accagaaagc ataagcaatg ctacatcacc accaacatcg ttcttaccct taaccaacat 360

tgctacacca agaccaacat ggttcttcaa atcatctggt ctcttggtct tggtaacaag 420

tctcaaacca ccaagatggt ttgggaaatc aagatcaccg cttttccaac catccatacc 480

gaatcttttt acgaaaacag aatggctggt gaaaactctt cttgcaagga agctgcttgc 540

tgctctcttg aacaaatgta cgctgttaag catcatgatg ttatgaaggc tatggaagaa 600

aaccaacttc aagaaggtaa gatcaacaga catgttgaaa cccttgaact ttttccaacc 660

cattgcgaag atagatctcc agaaccatct gaaaagaagt cttgctgctg gggttcttgc 720

gaaaccaagg aaaaccataa caacaaccca ccaggtggtt acaagggtaa gcaaagagat 780

tctgttcttg atctttgcct ttctcttggt aacaagtctt gcggtcttca tgataactaa 840

<210> 148

<211> 279

<212> PRT

<213> Pinus taeda

<400> 148

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1 5 10 15

Ser Ser Thr Arg Trp Asn Pro Thr Ser Glu Gln Leu Ser Ile Leu Arg
20 25 30

Glu Leu Tyr Tyr Thr Asn Gly Ile Arg Ser Pro Thr Val Asp Glu Ile
35 40 45

His Arg Ile Ser Met Lys Leu Ser Arg Tyr Gly Lys Ile Glu Gly Lys
50 55 60

Asn Val Phe Tyr Trp Phe Gln Asn His Lys Ala Arg His Arg Gln Lys
65 70 75 80
Thr Arg Leu Ser Ala Met Asn Val Gly Lys Pro Ala Ala Ser Pro Ala
85 90 95

Leu Ser Gln Thr Thr Arg Lys His Lys Gln Cys Tyr Ile Thr Thr Asn
100 105 110
Ile Val Leu Thr Leu Asn Gln His Cys Tyr Thr Lys Thr Asn Met Val
115 120 125
Leu Gln Ile Ile Trp Ser Leu Gly Leu Gly Asn Lys Ser Gln Thr Thr
130 135 140
Lys Met Val Trp Glu Ile Lys Ile Thr Ala Phe Pro Thr Ile His Thr
145 150 155 160

Glu Ser Phe Tyr Glu Asn Arg Met Ala Gly Glu Asn Ser Ser Cys Lys
165 170 175
Glu Ala Ala Cys Cys Ser Leu Glu Gln Met Tyr Ala Val Lys His His
180 185 190
Asp Val Met Lys Ala Met Glu Glu Asn Gln Leu Gln Glu Gly Lys Ile
195 200 205
Asn Arg His Val Glu Thr Leu Glu Leu Phe Pro Thr His Cys Glu Asp
210 215 220

Arg Ser Pro Glu Pro Ser Glu Lys Lys Ser Cys Cys Trp Gly Ser Cys
225 230 235 240
Glu Thr Lys Glu Asn His Asn Asn Asn Pro Pro Gly Gly Tyr Lys Gly
245 250 255
Lys Gln Arg Asp Ser Val Leu Asp Leu Cys Leu Ser Leu Gly Asn Lys
260 265 270
Ser Cys Gly Leu His Asp Asn
275

<210> 149
<211> 1500
<212> DNA

<213> Arabidopsis thaliana

<400> 149

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aaaatagagg gaaattcaaa tgaaaaaaaa agtttcatct acagaaaaaa aaacacgtca 180

aaattccaaa aaacttgaac gtcttgaaca acggtataac cgtataagaa agggaactta 240

aaacttaaac gtctacatga ttgaaagggt aaaaatattc atattacaat ggtataagaa 300

aatggagaag ccagaggagc aactccaaag aagaagatgc agcaagagag tgtacattat 360

tataattata agttggtata tatgtagttg ctaaaaaaaa aaaagagtgt acaatatttt 420

ctgaagaagc atcgagctgg ttaatattta agacccacca aaacctacca acaatgttat 480

tctcatctct aagcaaaagt ttgatctcat ctttttttt aatttttttt ttgatgggtc 540

ctcttctacc tctgcatctc cacgtgctat acaagtggcc tcgctgtatg tattatttac 600

cactctctta ttgttactaa aaacttgaat gtcacttgaa tgtcacttga atgcaaacta 660

tatatttagg tgaataccag atcaaatagc aataaataaa ccaacaattt atgctaatta 720

aaaacttgaa aaaacacttt ccattaccta tgatttttta atccaatctt ggggtttgat 780

gcccataaaa cgaagtcgaa aagagaaaag cgaccttaat tgtaataaat gcagctattg 840

acttctctat caacttcaag tcttcaagta actacaaatt taataatctc cttagcttat 900

cttagagaag cacaaaacaa aagggtctac gcttagtctt cctagattct tgatttgagt 960

cttgatggcc acttgctttt tatgtctcaa tcctaagagt tgatacttac ttagtattaa 1020

tcaggtttca cattatctca gacctagctt gcatttttga tacaaacatg tcatgcaata 1080

cacataagca acacgtaccg agtcattgat tgaaaaacat gacctaaata gtaatacgga 1140

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tttgcatgaa agattaaata attcaactct ttcacttatc ttattttaat ttactttgca 1260

gaattccaag ttttattgca atcaccaggg gtattaaatg ggatagtgta aatgcacctg 1320

caacacatca actaaccaca cactcctcca cctgtttgta tttacttcaa aaacttaatt 1380

caaaaatccc tttgcttctt tccttccctc tatttataaa ccctagtatc cggtttttat 1440

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<210> 150

<211> 1500

<212> DNA

<213> Arabidopsis thaliana

<400> 150

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cttgtgaatc tggttctctc tctctcttct cttactagct tttgagtcga caaaagagag 180

agaaaagatc attaaactcg aatccaaaac ttcaaccaac aaaagggaaa aaaagaaaaa 240

ataactacaa agagatctat ctttagggtt tcaaaacttt cttgagtttc agatcgaatt 300

gagtaagtat ttatattatt taagtgttct tcttactgta tcatttctga ccatacacca 360

aactagggtt tcttaaagat cataactctc tctctctcgc ttcttcctac actagatctg 420

tccgatagat ctgatgcttt gagaagtctt gtgactttca agattgattt ttctttttgt 480

ttgtttgttt atatgtacac atacttggag atagatatac tctcatatat gttactgtgc 540

atatggatgg atccatctta agtgtgaata aaaactaccc aaatctcatt tttgtttgtt 600

tcttatctca tttgttgttt ttgtaaggcc gtcattataa atctaaaaca atttatccgt 660

tttcgtaaag aaaatcgctt tccaaaaaaa aaaactttga agtaaaaata tcattttggg 720

tctatattca aatctctcct catatttcca tctcattatt tgtaaaatta atatattagg 780

tcattatcat attgattata gagtttatat gttatgttat aaagtaaaag ttatatatgt 840

aaatattacg tttgtaatat atatatttat acatatgtac gtatacataa tttgtatgta 900

tatgtgcctc atttttaaca aatattatcg ttaatcttta gtttcattat aatttttag 960

ggatgtatac attatataaa tattttatgt tatttatatc tgaaatttct atactataac 1020

atcttagata gttttcatac gtaattaaga gttttaatta atagttacat aattatgatt 1080

agatgaattt atctataaat tgtatgcaaa aatatataca tatccaaccg aaaaaatgga 1140

gagtaaacaa aaacctaaca attaatggtt tagtacttgt gaaaatgata aaattattaa 1200

tttaatatat agtttcataa cagctagaaa taactttccc ttaatataat tattttcttc 1260

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aatcttttac taattcgatt gtgattttga ttttgggcag tggaagatat acttggagaa 1380

gaaaatcaaa tcacgaaaat atattgatca gtgccttgaa ggaaaccaaa gttgacaact 1440

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<210> 151

<211> 1500

<212> DNA

<213> Arabidopsis thaliana

<400> 151

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tatatcctgt atgttgaatt ttcagttagt catctatatt ttgtcttaaa ttttgccttc 180

tttagcatta attaattgat ttcttgcctc taatatcttt cattcctctc ctaatttgct 240

ttctgacttt ctgatttatt gtattcttct ttaaataatc ttatcaattt ctgcatagtc 300

agagatgaaa ccttcaagaa aacaataaag aggaaagatt aaaggttagt attcccttgc 360

ctaccaaaaa aaacagaaag gtttgtattc tcttcgtata tccttatttt cattatacgt 420

ttggttttat atataaacac gtgtatatac tttatagtag tagtatacat ttacatatta 480

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tccgtcgcac aggtgcacag atataatact atttttttca tttcaatttt tttttctttc 660

ttaatcgcca aatagtagtt tacacttttt cctgaagagt ttttattttt tgaaaatcta 720

ctaatgatta tatttaatta aattttgttt tgttctcacg aaaatattgt ttttttaata 780

aacaggatag agttgggaat ttatttgtac ctacttttc ccattttatt tgctttgaac 840

aaatgagtcc aacatttctc ttttcgtctt ctctttccta tcccatctaa atcaaaactg 900

atcaaaatac ctcgaaattt taaattaaat caccatccat caaagctatt atagtgtaga 960

aattttaaag ttttttcttt ggacaacaat tgatttccat ctacactaca tatgatatga 1020

ctcttgaatc aatcatagtt tttatttcag tctgctgatt agtttttttc ttttctttta 1080

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ggttggttat tcaataacaa attaatttgt gattgtgatg tctttttttat gcaaggctct 1200

tttttcttgc tttcaattag tgggtccttt gtttcctaca atcatctcca attcttctgt 1260

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taagttattg ttgttcatac aaaaagaagg ttgtaaagtt gttgacgtgg acaaaagtat 1380

tgccatattt ttccagcctc gaagaaattt cccttcttct tcaaacccta aattttgaca 1440

aaactctcta ctttttttttg cttctagggt ttgctttcag tttccggagt ataagaaaag 1500

<210> 152

<211> 1905

<212> DNA

<213> Arabidopsis thaliana

<400> 152

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ccaaaaagaa aaaagcacaa aaacaaaata ctactactat actataatgg tgaaacacac 180

attacattag cattaaccgc tattccttaa aagatatatg agcagactac aaggaagtac 240

atttaaatca gtgtgctaag ttttctcaat tatcaatcta ataattgtac ttacataaat 300

ttaccaaatc atctcaccat ttagggcata caatttttgc tcaataatgg agcttgaaaa 360

gactaattat agaatgtctt tttggttgac acaagctcgc ttcttcatgt cgtttacttt 420

aaaaattgta gattctttca caacttataa gattctatag cctatttgat tatcctttag 480

gatgatgagt ataatatctc aaactataaa ataaaaatgt tcacatcaat aattgaaaaa 540

aaatgcatgt atgaattcac gaaaatatgg ttacggttgt gtgttgctgc tagacaaaag 600

tctttcatat attcagttat atttcttaaa actaatcaaa attgttttat gcaattttgg 660

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agtcaaccga gtagttctgg taagagagtc tcgtgcattc gtaaggtttc caagagtcca 780

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tgattccgtt gataaaattt gttcgttata tagaaataat cagaatataa ttagggttaa 900

ttttggagta aagaccattc aatcaatggt ttggcttgat tcttgtttga aattctatgt 960

ttaaaaaggg taatacttac aaacttgact ttattagtat tttaaaaaat gacaaaactt 1020

aaaatggatt tgaaatctag catatttaac tttaatacag ttttggttta agtttcactc 1080

tcttttctat caaaaatata attttaaaat ttgaaagatg ttaaaataga caaatgtcat 1140

tagtttgttc agatttagtt atgtttttaa tatacagaca attatattaa aataataata 1200

aaaaagtcag atcagttata aaagaaagtc agttcagttt taagacttaa gtttgagtct 1260

tttggatttg aatttaatag attcactttt attaaaaatt gttgaaacac tttttaaaaa 1320

ctaaaacatt tgtcaaaaaa tgcatttaac caaaaaaatg aaaatgaaag gtagtaagta 1380

ataaaagaag aaaacaaaat ctaaaaaagt gtctttaaaa tctcaaaatt aaaatataca 1440

aaaaagtaca agaaaataaa agaaaagggg gaaaaagaga gtcaatatca acatgcaagt 1500

gtgaatatta gactcttctt ctctcatcag ttaccccaaa actagggttt acccatttc 1560

atcatcttct tcttccccac atcactctct gaaagacaat aagagagaga gacacacaca 1620

ccaaaaaaaa aaccccattg atttttaaga gagagagaga gactgagaac gaagagcaaa 1680

tgagagagaa gttgaatcaa atttccgctt tcgttctctt tctctctcct ccatttcagt 1740

aataaacact ccttcctctt taatttttct gggtttcttc ttcttccctc ttttgttgtt 1800

tctgtgtgat tcagttctcg atccatcttt tcgtgaaaag tatcaatctt ttaattctct 1860

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<210> 153

<211> 11210

<212> DNA

<213> Artificial Sequence

<220><223> RV021090 T-DNA

<400> 153

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