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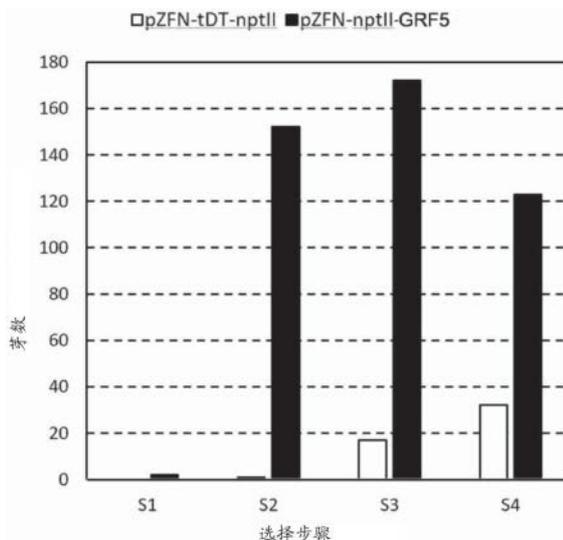
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(54) 发明名称
 遗传修饰植物的再生

(57) 摘要

本发明涉及植物育种领域,特别是涉及从细胞和其它组织产生植物。更特别地,本发明提供了用于改善植物再生的方法和工具,特别是从转化的或遗传修饰的植物细胞中再生。



1. 一种改善从植物愈伤组织或分生组织的植物细胞再生芽的方法,包括以下步骤
(a1) 平行或顺序地将以下引入植物细胞
 - i. 至少一种目的核苷酸序列;以及
 - ii. 包含编码GRF5多肽的多核苷酸的表达盒、编码GRF5多肽的mRNA或GRF5多肽;或(a2) 将至少一种目的核苷酸序列引入植物细胞;以及
在所述植物细胞中平行或顺序诱导编码GRF5多肽的内源基因的增强的表达水平;以及
(b) 任选地,在一定条件下培养(a1)或(a2)的植物细胞或衍生自(a1)或(a2)的植物细胞的植物细胞,在所述条件中在所述植物细胞中,GRF5多肽由表达盒表达,GRF5多肽由引入的mRNA翻译,GRF5多肽由内源基因增强表达,或存在GRF5多肽,
其中所述GRF5多肽由SEQ ID NO:2,106,108或209所示的序列组成。
2. 改善从基因组工程化植物的植物愈伤组织或分生组织的细胞再生芽的方法,包括步骤
(a1) 将如下引入植物细胞:包含编码GRF5多肽的多核苷酸的表达盒、编码GRF5多肽的mRNA或GRF5多肽;或
(a2) 在植物细胞中诱导编码GRF5多肽的内源基因的增强的表达水平;以及
(b) 在一定条件下培养(a1)或(a2)的植物细胞或衍生自(a1)或(a2)的植物细胞的植物细胞,在所述条件中在所述植物细胞中,GRF5多肽由表达盒表达,GRF5多肽由引入的mRNA翻译,GRF5多肽由内源基因增强表达,或存在GRF5多肽;
(c) 通过双链DNA断裂(DSB)诱导酶和任选地通过修复核酸分子修饰(b)的植物细胞的基因组,所述诱导酶识别所述细胞的基因组中的预定位点。
其中在所述预定位点的所述基因组的修饰选自
 - i. 至少一个核苷酸的置换;
 - ii. 至少一个核苷酸的缺失;
 - iii. 至少一个核苷酸的插入;或
 - iv. i-iii的任何组合;以及其中步骤(c)与步骤(a1)/(a2)和/或(b)同时进行,在步骤(a1)/(a2)之前进行,在步骤(a1)/(a2)和(b)之间进行或在步骤(b)之后进行,
其中所述GRF5多肽由SEQ ID NO:2,106,108或209所示的序列组成。
3. 一种产生具有改善的从植物愈伤组织或分生组织的植物细胞再生芽能力的转基因植物的方法,包括步骤
(a) 根据权利要求1的方法转化植物细胞,和
(b) 从(a)的植物细胞或从(a)的植物细胞衍生的植物细胞再生包含至少一个细胞的植物,所述细胞包含作为转基因的至少一个目的核苷酸序列。
4. 一种产生具有改善的从植物愈伤组织或分生组织的植物细胞再生芽能力的遗传修饰植物的方法,包括步骤
(a) 根据权利要求2的方法修饰植物细胞的基因组,和
(b) 从(a)的植物细胞或从衍生自(a)的植物细胞再生在至少一个细胞中包含基因组修饰的植物。
5. 产生具有改善的从植物愈伤组织或分生组织再生芽能力的单倍体植物胚的方法,包

括步骤

(a1) 将如下引入未成熟雄配子体或小孢子中: 包含编码GRF5多肽的多核苷酸的表达盒、编码GRF5多肽的mRNA或GRF5多肽; 或

(a2) 在未成熟雄配子体或小孢子中诱导编码GRF5多肽的内源基因的增强的表达水平; 以及

(b) 在一定条件下培养(a)的未成熟雄配子体或小孢子, 其中在未成熟雄配子体或小孢子中, GRF5多肽从表达盒表达, GRF5多肽从引入的mRNA翻译, GRF5多肽从内源基因增强表达, 或存在GRF5多肽; 以及

(c) 选择来源于步骤(b)的未成熟雄性配子体或小孢子的单倍体植物胚, 其中所述GRF5多肽由SEQ ID NO: 2, 106, 108或209所示的序列组成。

6. 如权利要求1-5中任一项所述的方法, 其中所述编码GRF5多肽的多核苷酸为

(i) SEQ ID NO: 1, 105, 107或208所示的核苷酸序列;

(ii) 在遗传密码简并性范围内编码由(i)编码的多肽的核苷酸序列; 或

(iii) 与(i)或(ii)的核苷酸序列互补的核苷酸序列。

7. 如权利要求1至5中任一项所述的方法, 其中将包含编码GRF5多肽的多核苷酸的表达盒引入植物细胞导致其稳定整合到所述植物细胞的基因组中, 或其中将包含编码GRF5多肽的多核苷酸的表达盒、编码GRF5多肽的mRNA或GRF5多肽引入植物细胞中, 或在植物细胞中诱导编码GRF5多肽的内源基因的表达水平增强导致GRF5多肽在所述植物细胞或其后代细胞中瞬时出现。

8. 权利要求1至5任一项的方法, 其中编码GRF5多肽的多核苷酸与适合于在植物细胞中表达GRF5多肽的至少一种调节序列可操作连接。

9. 权利要求1至4的方法, 其中步骤(a1)或(a2)的植物细胞是体细胞组织的细胞。

10. 权利要求1至4的方法, 其中步骤(a1)或(a2)的植物细胞是愈伤组织、分生组织或胚组织的细胞, 或原生质体。

遗传修饰植物的再生

[0001] 本发明涉及植物育种和生物技术领域,并且特别涉及从细胞和其他组织产生植物。更特别地,本发明提供了用于改善植物再生,特别是从转化的或遗传修饰的植物细胞中再生的方法和工具。

[0002] 在植物育种中,为了产生用于特定目的所需基因型和表型,从人类文明的开始临近就已经实践了植物物种的操作过程。随着基因工程的发展,这个农业领域在过去的几十年中已经有了显著的变化。已经开发了多种植物遗传工程方法。转化方法的选择取决于许多变量,主要是待转化的植物物种、实验目的和必要设备的可用性。绝大多数植物转化技术需要使用具有高再生能力的外植体作为起始材料。此外,基因编辑构成了一种新的分子生物学方法,通过该方法可以将特定的修饰如插入、缺失或点突变或其组合引入植物基因组中。为此,需要特异性分子仪器,其首先具有核酸酶活性,但最重要的是可以以足够的特异性引导至待修饰的靶序列,以编程和进行特异性和定点诱变。在过去的几年中,在植物生物技术中,特异性基因组编辑已经发展为常规育种和转基因策略的替代。然而,由于植物的经编辑起始材料的有限再生能力,目前可用的工具,例如大范围核酸酶、锌指核酸酶(ZFNs)、“转录激活剂样效应物核酸酶”(TALEN)或CRISPR系统仅在有限程度上用于植物生物技术中。

[0003] 多种细胞具有发育为胚胎的潜力,包括单倍体配子体细胞,例如花粉和胚囊的细胞(参见Forster,B.P.,等人(2007) Trends Plant Sci.12:368-375 and Seguí-Simarro, J.M. (2010) Bot.Rev.76:377-404),以及衍生自植物的所有三个基本组织层的体细胞(Gaj, M.D. (2004) Plant Growth Regul.43:27-47或Rose,R.,等人(2010) “Developmental biology of somatic embryogenesis” in: Plant Developmental Biology- Biotechnological Perspectives, Pua E-C和Davey MR, 编著(Berlin Heidelberg: Springer), pp.3-26)。

[0004] 再生成植物的能力通常限于某些植物物种中的特定基因型,并且在转化和遗传修饰的植物细胞和其它前体组织中减弱。即使转化和遗传修饰植物细胞的步骤是成功的,这也不一定意味着实际上可以从修饰的细胞获得期望的植物。据推测,植物细胞和其它前体组织为了实现遗传修饰而经受的处理影响植物发育和再生。因此,本发明的目的是提高先前已知的用于产生转基因和遗传修饰植物的方法的功效,并支持从修饰的植物细胞和其它植物前体再生植物。

[0005] 在本发明中,令人惊奇地发现拟南芥属基因GRF5 (GROWTH-REGULATING FACTOR 5, 生长调节因子5) 在促进植物再生中具有作用,这并未针对这种基因或其GRF基因家族的对应物而被报道过。

[0006] 在van der Knaap等人(2000; “A novel gibberellin-induced gene from rice and its potential regulatory role in stem growth”, Plant physiology, 122 (3), 695-704.)中,作者鉴定并表征了GRF基因家族在稻中的第一个成员(OsGRF1) 这是居间分生组织中赤霉素诱导的基因。拟南芥属中的过表达导致茎生长受损、雌性不育和雄性育性降低。赤霉素的应用不能恢复转化植物的茎伸长缺陷,表明OsGRF1能参与GA诱导的茎伸长。2003 Kim等人 (“The AtGRF family of putative transcription factors is involved

in leaf and cotyledon growth in Arabidopsis”, *The Plant Journal*, 36(1), 94-104.) 中已经表征了拟南芥属GRF家族,并确定了这些基因主要在活跃生长的组织中表达。对过表达AtGRF1和AtGRF2的无效突变体和转基因植物的分析表明GRF家族的一些成员参与了叶和子叶生长过程中细胞扩增的调节。此外,过表达的植物显示延迟的抽苔时间,揭示了开花中的推定作用。在确定协调发育叶中细胞增殖的分子机制的研究中,Rodriguez等发现miR396拮抗其靶标GRF转录因子在拟南芥属中的表达模式((2010),“Control of cell proliferation in Arabidopsis thaliana by microRNA miR396”, *Development*, 137(1), 103-112.)。因此,miR396和GRF之间的平衡控制叶中细胞的最终数目。此外,作者显示靶向miR396的GRF可调节茎顶端分生组织的大小。

[0007] 2005年,Horiguchi et al. (“The transcription factor AtGRF5 and the transcription coactivator AN3 regulate cell proliferation in leaf primordia of Arabidopsis thaliana”, *The Plant Journal*, 43(1), 68-78.) 首次表征了AtGRF5及其相互作用配偶体ANGUSTIFOLIA3 (AN3)。敲除突变体atgrf5和an3由于细胞数目减少而产生狭窄的叶,而在AtGRF5和AN3过表达株系中叶原基中的细胞增殖增强。Kuijt等人((2014),的细胞增殖增强。) “The transcription factor AtGRF5 and the transcription coactivator AN3 regulate cell proliferation in leaf primordia of Arabidopsis thaliana”, 显示GRF家族在网络中作用为参与者控制KNOTTED1-LIKE HOMEBOX (KNOX) 基因的表达,其参与枝条芽顶端分生组织的细胞分化限制。AtGRF4、AtGRF5和AtGRF6能够结合KNOX基因的启动子,抑制其表达。过表达AtGRF4、AtGRF5或AtGRF6的拟南芥幼苗在芽顶端分生组织中表现出发育异常。在Vercruyssen等人((2015), n. 过表达组织的细gulating factor 5 stimulates Arabidopsis chloroplast division, photosynthesis, and leaf longevity”, *Plant physiology*, pp-114.) 的最近研究中,过量表达GRF5的拟南芥属叶显示了每个细胞更高的叶绿体数目、增加的叶绿素含量和延迟的叶衰老。

[0008] 总之,众所周知拟南芥属基因AtGRF5和其它GRF基因在叶片形态发生和茎发育中起作用。此外,据报道GRF基因在开花、种子和根发育中起作用,以控制胁迫条件下的植物生长和调节植物寿命。然而,在他们的研究过程中,本发明的发明人惊奇地发现了该基因家族的另一和新的功能。GRF5能够提供加强植物再生的积极作用,因此允许更有效地回收转基因植物。本发明允许改善来自不同组织或细胞(例如小孢子)的再生,可以克服对植物再生的不顺应性,特别是基因型依赖性,通过例如目的基因和GRF5的共表达改善转基因植物的恢复,通过例如基因组编辑组分和GRF5的瞬时共表达改善基因组工程化植物的恢复,以及缩短产生转基因株系和恢复的时间。因此,本发明的第一方面是GRF5多肽用于改善植物再生能力的用途。

[0009] 下文任何对本发明方法中有用的多肽或蛋白质的提及均指本文定义的GRF5多肽或GRF5蛋白。下文任何对本发明方法中有用的核酸或多核苷酸(除了被转化的目的核苷酸序列或任选用作修饰植物基因组的修复模板的核酸分子)的提及,被认为是指能够编码此类GRF5多肽或GRF5蛋白的核酸或多核苷酸。在一个实施方案中,任何提及可用于本发明方法的多肽/蛋白质或核酸/多核苷酸应理解为指可用于本发明方法、构建体、表达盒、植物细胞、植物、种子、可收获部分和产物的蛋白质或核酸。待引入植物细胞或植物(并因此可用于实施本发明方法)的包括mRNA的核酸/多核苷酸是编码现在将描述的多肽/蛋白质类型的任

何核酸/多核苷酸,下文也称为“GRF5核酸”、“GRF5多核苷酸”、“GRF5基因”或“GRF5mRNA”等。

[0010] 本文定义的“GRF5多肽”或“GRF5蛋白”指任何转录因子,当用Interproscan软件(www.ebi.ac.uk/Interpro)分析时,优选14-3-3样蛋白GF14v,更优选其包含PFAM结构域PF08880(也称为QLQ结构域)和PFAM结构域PF08879(也称为WRC结构域),甚至更优选包含发现在GRF5多肽N末端或其附近至少90%、至少91%、至少92%、至少93%、至少94%、至少95%、至少96%、至少97%、至少98%、至少99%或100%覆盖度匹配的PFAM结构域PF08880,以及发现与位于GRF5多肽的PFAM结构域PF08880的C末端至少90%、至少91%、至少92%、至少93%、至少94%、至少95%、至少96%、至少97%、至少98%、至少99%或100%覆盖度匹配的PFAM结构域PF08879,即匹配PF08879的氨基酸区段按翻译方向位于匹配PF08880的氨基酸区段后。优选地,至少一个匹配具有至少95%、至少96%、至少97%、至少98%、至少99%或100%的覆盖度。

[0011] 在一个实施方案中,两个匹配氨基酸区段均位于GRF5多肽的N-末端一半,优选地,匹配PFAM结构域PF08880的氨基酸区段位于GRF5多肽的N-末端四分之一。优选地,PFAM结构域PF08880与GRF5多肽的氨基酸残基匹配,所述匹配始于GRF5多肽的残基5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或25,优选地始于残基9、10、11、12、13、14、15、16、17、18、19、20或21,且PFAM结构域PF08879与GRF5多肽的氨基酸残基匹配,所述匹配始于GRF5多肽的残基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或99,优选地始于残基72、73、74、75、76、77、78、79、80、81、82、83、84、85、86、87、88、89、90、91、92、93、94或95。优选地,匹配PFAM结构域PF08880的氨基酸区段的起始氨基酸残基与匹配PFAM结构域PF08879的氨基酸区段的起始氨基酸残基之间的距离是在GRF5多肽中的60至82个氨基酸,更优选地60至75个氨基酸,更优选61至75个氨基酸,甚至更优选在GRF5多肽中62-73个氨基酸。

[0012] 在又一个实施方案中,本文使用的GRF5多肽包含指示物基序:[D]-[PL]-[E]-[P]-[G]-[R]-[C]-[R]-[R]-[T]-[D]-[G]-[K]-[K]-[W]-[R]-[C]-[SA]-[RK]-[ED]-[A]-[YH]-[P]-[D]-[S]-[K]-[Y]-[C]-[E]-[KR]-[H]-[M]-[H]-[R]-[G]-[RK]-[N]-[R],其中GRF5多肽与指示物基序相比显示最多三个错配,即在各GRF5多肽和指示物基序之间的序列比对中最多可出现三个错配,优选与指示物基序相比至多两个错配,即在各GRF5多肽和指示物基序之间的序列比对中最多可出现二个错配,更优选地与指示物基序相比至多一个错配,即在各GRF5多肽和指示物基序之间的序列比对中最多可出现仅一个错配,并且更优选地与指示物基序相比没有错配。在特别优选的实施方案中,错配或单一错配之一位于指示物基序的位置2,更优选地,错配是[A]而不是[P]。错配是指根据指示物基序的某一位置上的氨基酸被不同的氨基酸取代,或者通过插入至少一个额外的氨基酸而缺失或移位。方括号内的指示物基序的字母表示氨基酸残基(单字母代码),基序代表本发明任何GRF5多肽中存在的从N-末端到C-末端方向的氨基酸残基顺序。如果在一个方括号内有两个字母,则它们表示可选。该基序已经来自16个不同植物物种(包括单子叶和双子叶植物)的GRF5多肽序列的全面比较中推导出来,并允许将GRF5多肽与GRF蛋白质家族的其它成员如GRF1区分开(参见图5A)。图5B显示了通过序列比对/比较进行的示例性基序分析,用于确定错配的数目。优选地,基序位于GRF5多肽的N-末端一半,更优选地基序位于GRF5多肽的N-末端一半,并含有与PFAM结构域PF08879匹配的氨基酸区段亚区,即基序与PFAM结构域PF08879匹配的氨基酸区

段具有连续重叠。优选地,指示物基序由SEQ ID NO:41至SEQ ID NO:104或SEQ ID NO:113至SEQ ID NO:176的任何氨基酸序列组成。相应地,GRF5多肽优选包含由氨基酸序列SEQ ID NO:41至SEQ ID NO:104或SEQ ID NO:113至SEQ ID NO:176中的任何组成的一个连续基序。

[0013] 用于本发明方法的来自各种植物物种的GRF5多肽的实例在下文进一步描述。在表1中,显示了PFAM结构域PF08880和PFAM结构域PF08879的位置以及在任何所显示的GRF5序列中的单个覆盖。

[0014] 表1:来自16个不同植物物种的GRF5多肽中的结构域分析

[0015]

[SEQ ID NO]	Pfam 结构域 (HMM)	Pfam 结构域描述	Pfam 结构域长度	(i)E ₋ 值	分数	Pfam 结构域自	Pfam 结构域至	序列自	序列至	转化的 Pfam 结构域的长度 %
2	PF08879	WRC	43	1,60E-20	72,5	1	41	82	124	95
2	PF08880	QLQ	35	7,80E-16	57,4	1	35	16	50	100
4	PF08879	WRC	43	1,10E-20	73	1	43	88	130	100
4	PF08880	QLQ	35	3,00E-16	58,8	1	34	20	54	97
6	PF08879	WRC	43	1,90E-20	72,3	1	43	91	133	100
6	PF08880	QLQ	35	7,00E-14	51,2	1	33	19	53	94
8	PF08879	WRC	43	2,00E-20	72,2	1	43	94	136	100
8	PF08880	QLQ	35	2,20E-15	56	1	34	21	55	97
10	PF08879	WRC	43	1,20E-20	72,9	1	41	82	124	95
10	PF08880	QLQ	35	3,30E-16	58,6	1	35	16	50	100
12	PF08879	WRC	43	1,80E-20	72,3	1	41	82	124	95
12	PF08880	QLQ	35	3,30E-16	58,6	1	35	16	50	100
14	PF08879	WRC	43	1,20E-20	72,9	1	41	82	124	95
14	PF08880	QLQ	35	3,30E-16	58,6	1	35	16	50	100
16	PF08879	WRC	43	1,20E-20	72,9	1	41	82	124	95
16	PF08880	QLQ	35	4,90E-16	58,1	1	35	16	50	100
18	PF08879	WRC	43	1,80E-20	72,3	1	43	90	132	100
18	PF08880	QLQ	35	6,90E-14	51,2	1	33	18	52	94
20	PF08879	WRC	43	2,50E-21	75,1	1	43	88	130	100
20	PF08880	QLQ	35	2,30E-15	55,9	1	34	18	52	97
22	PF08879	WRC	43	2,30E-20	72	1	43	72	114	100
22	PF08880	QLQ	35	8,50E-16	57,3	1	35	10	44	100
24	PF08879	WRC	43	1,10E-20	73	1	43	94	136	100
24	PF08880	QLQ	35	2,20E-15	56	1	34	21	55	97
26	PF08879	WRC	43	1,90E-20	72,2	1	43	94	136	100
26	PF08880	QLQ	35	2,20E-15	56	1	34	21	55	97
28	PF08879	WRC	43	2,80E-21	74,9	1	43	79	121	100
28	PF08880	QLQ	35	8,50E-13	47,7	1	34	13	47	97
30	PF08879	WRC	43	2,30E-21	75,2	1	43	75	117	100
30	PF08880	QLQ	35	2,50E-15	55,8	2	34	9	43	94
32	PF08879	WRC	43	1,90E-20	72,3	1	43	91	133	100
32	PF08880	QLQ	35	3,50E-15	55,4	1	34	18	52	97

[0016]	106	PF08879	WRC	43	2,80E-21	74,9	1	43	79	121	100
	106	PF08880	QLQ	35	8,50E-13	47,7	1	34	13	47	97
	108	PF08879	WRC	43	1,20E-20	72,9	1	41	82	124	95
	108	PF08880	QLQ	35	3,30E-16	58,6	1	35	16	50	100
	110	PF08879	WRC	-	-	-	2	43	95	137	-
	110	PF08880	QLQ	-	-	-	1	37	13	49	-
	112	PF08879	WRC	-	-	-	1	43	90	131	-
	112	PF08880	QLQ	-	-	-	1	33	15	49	-
	209	PF08879	WRC	-	-	-	1	43	90	131	-
	209	PF08880	QLQ	-	-	-	1	33	15	49	-

[0017] 根据一个方面,本发明提供了一种转化植物细胞的方法,包括以下步骤

[0018] (a1) 将以下引入植物细胞

[0019] (i) 至少一种目的核苷酸序列;以及

[0020] (ii) 包含编码GRF5多肽的多核苷酸的表达盒、编码GRF5多肽的mRNA或GRF5多肽,其中(i)和(ii)可平行或以任何顺序相继引入,或

[0021] (a2) 将至少一种目的核苷酸序列引入植物细胞;和在所述植物细胞中平行或顺序诱导编码GRF5多肽的内源基因的增强的表达水平;以及

[0022] (b) 任选地在一定条件下培养(a1)或(a2)的植物细胞或源自(a1)或(a2)的植物细胞的植物细胞,在所述条件中在所述植物细胞中,GRF5多肽由所述表达盒表达,GRF5多肽由所引入的mRNA翻译,GRF5多肽由所述内源基因增强/增加表达,或GRF5多肽存在,优选与野生型植物细胞或其中包含编码GRF5多肽的多核苷酸的表达盒、编码GRF5多肽的mRNA或GRF5多肽未根据步骤(a1)引入或其中编码GRF5多肽的内源基因的增强表达水平未根据步骤(a2)诱导的植物细胞中的量相比,所述植物细胞中的量增加。

[0023] 根据本发明的方法产生了修饰或转化的植物细胞,由于GRF5的存在或GRF5的存在的量增加,所述植物细胞具有改善的再生能力。然而,优选在修饰的植物细胞中,GRF5的存在或GRF5以提高的量存在是瞬时的。

[0024] 植物细胞的转化是指将核酸分子以引起稳定整合到植物细胞基因组中或在植物细胞中瞬时出现的方式引入植物细胞中,导致核酸序列的表达,其例如与特定组织或某些发育阶段等组成性、暂时性或特异性相关。单子叶和双子叶植物细胞的转化和再生现在是常规的,并且最合适的转化技术的选择将由实践者确定。方法的选择将随待转化的植物类型或基因型而变化;本领域技术人员将认识到特定方法对于给定植物类型或基因型的适用性。合适的方法可以包括但不限于:植物原生质体的电穿孔;脂质体介导的转化;聚乙二醇(PEG)介导的转化;使用病毒的转化;微量注射植物细胞;植物细胞的微弹轰击;真空浸渗;和农杆菌介导的转化。

[0025] 引入至少一个目的核苷酸序列的步骤(a1)(i)或(a2)可使用本领域公知的任何合适的方法进行。有许多方法可用于将目的核酸转移到植物细胞中。示例性的载体介导的方法是农杆菌介导的转化,例如Lindsay&Gallois,1990,Journal of Experimental Botany, and Kischenko等人,2005,Cell Biology International(用于甜菜),by Ishida等人,2007,("Agrobacterium-mediated transformation of maize."Nature protocols,2(7),1614-1621)(用于玉米),或通过来自Japan Tobacco公司的PureWheat Technology(用于小

麦)。其它合适的技术包括粒子轰击和电穿孔。

[0026] 本发明的目的核苷酸序列可以是DNA或RNA序列,例如mRNA、siRNA、miRNA等。更特别地,目的核苷酸序列编码至少一种表型性状。优选地,由DNA或RNA赋予的表型性状可以选自对生物胁迫的抗性/耐受性,包括病原体抗性/耐受性,其中病原体可以是病毒、细菌、真菌或动物病原体,对非生物胁迫的抗性/耐受性,包括冷却抗性/耐受性、干旱胁迫抗性/耐受性、渗透抗性/耐受性、热胁迫抗性/耐受性、寒冷或霜冻胁迫抗性/耐受性、氧化胁迫抗性/耐受性、重金属胁迫抗性/耐受性、盐胁迫或水浸抗性/耐受性、倒伏抗性/耐受性、落粒抗性/耐受性、或一种或多种除草剂如草甘膦、草铵膦(glufosinate)、2,4-D、麦草畏、ALS抑制剂等的抗性/耐受性。至少一种目的表型性状还可以选自对另外的目的农学性状的修饰,包括产量增加、开花时间修饰、种子颜色修饰、胚乳组成修饰、营养含量修饰或目的途径的代谢工程改造。

[0027] 在本发明的上下文中,GRF5可作为包含编码GRF5多肽的多核苷酸的表达盒、作为编码GRF5多肽的mRNA(也包括前mRNA或前体mRNA)或作为GRF5多肽引入。用于引入核酸分子的示例性技术如上所述。或者,可以通过激活编码GRF5多肽的内源基因的表达在植物细胞中提供GRF5。这将导致内源GRF5基因的表达水平提高,即导致植物细胞中GRF5多肽的存在或出现量提高。内源基因表达的激活可通过修饰编码GRF5多肽的内源基因启动子的活性或结构来实现。例如,增强子元件可通过基因编辑引入启动子;或者调节启动子的增强子元件可进一步增强,或者调节启动子的沉默子元件可通过例如靶向诱变/修饰而弱化;或者修饰可以通过基因编辑工具如CRISPR系统(Hilton等人(2015). *Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. Nature biotechnology*, 33(5), 510-517)引入增强子相关的表观基因组中;或基于例如TALE激活剂或dCas9激活剂的合成转录因子可以引入细胞,其中它们能够结合启动子上或附近的靶向识别位点并激活GRF5基因的转录(Cheng等人(2013). *Multiplexed activation of endogenous genes by CRISPR-on, an RNA-guided transcriptional activator system. Cell research*, 23(10), 1163.);或者植物细胞中通过转录后抑制来调节GRF5基因表达的微RNA(miRNA)的量可通过例如敲除(无效突变体)或敲低来降低,以增加植物细胞中翻译的GRF5多肽的量,例如Rodriguez等人2010(上文)在拟南芥属中鉴定了拮抗GRF5表达的微RNA miR396,其因此代表影响植物细胞中GRF5多肽的量的合适靶标。

[0028] 根据植物物种以及细胞类型,需要不同水平的基因或表达激活,以便在再生发生时在植物细胞中具有足够量的GRF5多肽。本领域技术人员可以利用各种技术来测量内源或引入基因的实际表达水平,例如qPCR、RT-PCR、Northern印迹或微阵列。引入甜菜(*Beta vulgaris*)植物的AtGRF5基因表达水平的测量示于图4。这些方法允许本领域技术人员通过常规工作调节GRF5基因的表达水平,这实现了从多种组织或体细胞和生殖细胞(例如小孢子)的再生能力的改善。在一个优选实施方案中,在植物细胞中,编码GRF5多肽的内源基因的表达水平增加至少2倍、3倍或5倍,优选10倍、25倍或50倍,更优选100倍、200倍或500倍。

[0029] 如上文进一步描述的,通过应用一种或多种激活剂或其前体,可以在植物细胞中诱导内源基因的增强的表达水平。这些可以应用于培养植物细胞的培养基,然后被植物细胞主动或被动吸收。此外,一种或多种激活剂或其前体可以通过显微注射、电穿孔或生物射弹轰击直接引入植物细胞。除了上述合成转录激活剂,本领域已知许多其它激活剂可用于

增加内源基因的表达水平,特别是内源GRF5基因的表达水平:近年来,化学植物遗传学和化学植物生物学的技术领域出现,其中用小分子处理生物系统以特异性地扰乱细胞功能。小分子在商业上用作不同系统中的药物、除草剂和杀真菌剂,但近年来,它们也越来越多地用作遗传调节的工具。例如,化学遗传学涉及通过化合物文库的筛选发现各种细胞功能的小分子效应物(Dejonghe&Russinova(2017).Plant Chemical Genetics:From Phenotype-Based Screens to Synthetic Biology.Plant Physiology,pp-01805;Kawasumi,M.,&Nghiem,P.(2007).Chemical genetics:elucidating biological systems with small-molecule compounds.Journal of Investigative Dermatology,127(7),1577-1584.)。适于激活靶基因如GRF5表达的这种小分子效应物可通过按照不同策略的化学筛选来鉴定(Dejonghe&Russinova,2017)。可以获得全面的化合物文库,其允许简单筛选无数小分子和鉴定可用于激活基因如GRF5的基因表达的效应物。如上所述,另一种提高内源基因如GRF5表达水平的方法是应用所谓的合成转录激活剂。它们通常通过识别结构域和至少一个激活结构域的融合来设计。识别结构域可以来源于已知系统,如锌指、TAL效应子或CRISPR;对于激活,例如将单纯疱疹病毒衍生的VP-16或VP-64激活结构域与识别结构域融合可以引起转录增加。较弱的活化域如人NF- κ B的AD增加了基因活化的多种选择。此外,如内源启动子上所示,激活剂的组合可用于引入协同效应(Moore等人(2014)。“Transcription activator-like effectors:a toolkit for synthetic biology.”ACS synthetic biology,3(10),708-716.;US 2002/0046419A1;Lowder等人(2017)。“Multiplexed transcriptional activation or repression in plants using CRISPR-dCas9-based systems.”Plant Gene Regulatory Networks:Methods and Protocols,167-184.)。合成转录激活剂可以被递送到植物细胞或也作为前体,即作为编码这种人工或合成转录激活剂或其结构域的DNA或RNA分子或作为转录激活剂的失活形式被引入植物细胞,所述转录激活剂的失活形式随后在细胞中或在细胞的特定区室中被激活。最后,通过上游负调节因子(即miR396)的失活或通过产生对这样的负调节因子有抗性的GRF基因的突变形式,也可以实现GRF基因表达的增强。

[0030] 优选地,本发明的GRF5多肽包含选自SEQ ID NO:2、4、6、8、10、12、14、16、18、20、22、24、26、28、30、32、106、108、110、112或209的氨基酸序列,或与SEQ ID NO:2、4、6、8、10、12、14、16、18、20、22、24、26、28、30、32、106、108、110、112或209具有至少70%同一性的氨基酸序列,优选与SEQ ID NO:2、4、6、8、10、12、14、16、18、20、22、24、26、28、30、32、106、108、110、112或209具有至少80%、至少85%、至少90%、更优选至少95%、至少98%或至少99%同一性的氨基酸序列,且优选包含本文所述的指示物基序,特别优选由SEQ ID NO:41至SEQ ID NO:104或SEQ ID NO:113至SEQ ID NO:176的任何氨基酸序列组成的指示物基序。例如由内源基因编码的GRF5多肽可包含选自SEQ ID NO:2(拟南芥(*Arabidopsis thaliana*)), SEQ ID NO:4(甜菜),SEQ ID NO:6(玉蜀黍(*Zea mays*)),SEQ ID NO:8(普通小麦(*Triticum aestivum*)),SEQ ID NO:10(欧洲油菜(*Brassica napus*)),SEQ ID NO:12(芜菁(*Brassica rapa*)),SEQ ID NO:14(甘蓝(*Brassica oleracea*)),SEQ ID NO:16(萝卜(*Raphanus sativus*)),SEQ ID NO:18(两色蜀黍(*Sorghum bicolor*)),SEQ ID NO:20(葵花(*Helianthus annuus*)),SEQ ID NO:22(马铃薯(*Solanum tuberosum*)),SEQ ID NO:24(大麦(*Hordeum vulgare*)),SEQ ID NO:26(黑麦(*Secale cereal*)),SEQ ID NO:28(大豆

(*Glycine max*)), SEQ ID NO:30 (陆地棉 (*Gossypium hirsutum*)), SEQ ID NO:32 (稻 (*Oryza sativa*)), SEQ ID NO:106 (大豆), SEQ ID NO:108 (欧洲油菜), SEQ ID NO:110 (葵花), SEQ ID NO:112 (玉蜀黍) 或 SEQ ID NO:209 (*Zea mays*) 的氨基酸序列。

[0031] 编码本发明GRF5多肽的外源(异源)或内源多核苷酸包含

[0032] (i) 包含SEQ ID NO:1、3、5、7、9、11、13、15、17、19、21、23、25、27、29、31、105、107、109、111、207、208或210的核苷酸序列;

[0033] (ii) 包含与包含SEQ ID NO:1、3、5、7、9、11、13、15、17、19、21、23、25、27、29、31、105、107、109、111、207、208或210的核苷酸序列至少70%、优选至少80%、至少85%、至少90%、更优选至少95%、至少98%或至少99%同一的序列的核苷酸序列;

[0034] (iii) 编码如上定义的GRF5多肽的核苷酸序列或在遗传密码简并性范围内编码由(i)和/或(ii)编码的多肽的核苷酸序,;

[0035] (iv) 与(i)、(ii)或(iii)的核苷酸序列互补的核苷酸序列;和/或

[0036] (v) 在严格条件下与(iv)的核苷酸序列杂交的核苷酸序列。

[0037] 编码GRF5多肽的多核苷酸,特别是编码GRF5多肽的多核苷酸,可包含选自以下序列的核苷酸序列:SEQ ID NO:1(拟南芥);SEQ ID NO:3(甜菜)、SEQ ID NO:5(玉蜀黍)、SEQ ID NO:7(普通小麦)、SEQ ID NO:9(欧洲油菜)、SEQ ID NO:11(芜菁)、SEQ ID NO:13(甘蓝)、SEQ ID NO:15(萝卜)、SEQ ID NO:17(两色蜀黍)、SEQ ID NO:19(葵花)、SEQ ID NO:21(马铃薯)、SEQ ID NO:23(大麦)、SEQ ID NO:25(黑麦)、SEQ ID NO:27(大豆)、SEQ ID NO:29(陆地棉)、SEQ ID NO:31(稻)、SEQ ID NO:105(大豆)、SEQ ID NO:107(欧洲油菜)、SEQ ID NO:109(葵花)、SEQ ID NO:111(玉蜀黍)、SEQ ID NO:207(合成的)、SEQ ID NO:208(合成的)或合成的NO:210(合成的)。

[0038] 为了本发明的目的,两个相关核苷酸或氨基酸序列的“序列同一性”,以百分比表示,是指两个最佳比对序列中具有相同残基的位置数(x100)除以所比较的位置数。缺口,即在比对中残基存在于一个序列中而不存在于另一个序列中的位置,被认为是具有不相同残基的位置。通过Needleman和Wunsch算法(Needleman和Wunsch1970)进行两个序列的比对。上述计算机辅助序列比对可以方便地使用标准软件程序进行,例如欧洲分子生物学开放软件套装软件(EMBOSS)中实施的程序NEEDLE,例如6.3.1.2版(Trends in Genetics 16(6), 276(2000)),其缺省参数例如蛋白质矩阵=EBL0SUM62, gapopen=10.0和gapextend=0.5。

[0039] 术语“严格条件”或“在严格条件下杂交”是指彼此具有足够互补性的核苷酸序列通常保持杂交的条件。这些严格条件是本领域技术人员已知的,并且描述于例如Current Protocols in Molecular Biology, John Wiley&Sons, NY(1989)6.3.1-6.3.6中。技术人员知道如何根据例如Sambrook等人, Molecular Cloning, Cold Spring Harbour Laboratory, 1989确定所需的杂交条件。术语“杂交条件”在这方面不仅指核酸实际凝聚过程中的主要实际条件,而且指随后洗涤步骤中的主要条件。严格杂交条件的实例是这样的条件,在该条件下,主要仅那些具有至少80%,优选至少85%,至少90%,至少95%,至少98%或至少99%序列同一性的核酸分子进行杂交。严格杂交条件是,例如:4×SSC在65℃下洗涤,随后在0.1×SSC在65℃下洗涤约1小时。如本文所用的术语“严格杂交条件”也可以指:在68℃下,在0.25M磷酸钠,pH7.2,7%SDS,1mM EDTA和1%BSA中杂交16小时,随后在68℃下用2×SSC和0.1%SDS洗涤两次,优选地,杂交在严格条件下进行。

[0040] 表述“可操作地连接”是指嵌合基因的所述元件以其功能被协调并允许编码序列表达的方式彼此连接,即它们是功能性连接的。例如,当启动子能够确保另一核苷酸序列的转录和最终表达时,启动子与所述另一核苷酸序列功能性连接。如果两个蛋白编码核苷酸序列以可以形成第一和第二蛋白或多肽的融合蛋白的方式连接,则它们彼此功能性或可操作性连接。

[0041] 当基因导致表达产物的形成时,该基因被认为是表达的。表达产物表示由编码这种产物的核酸、DNA或RNA,例如本文所述的第二核酸的转录和任选的翻译产生的中间产物或终产物。在转录过程中,受调节区,特别是启动子控制的DNA序列被转录成RNA分子。RNA分子可以自身形成表达产物,或者当其能够被翻译成肽或蛋白质时,其可以是中间产物。当作为基因表达的终产物的RNA能够例如与另一种核酸或蛋白质相互作用时,该基因被认为编码作为表达产物的RNA分子。RNA表达产物的实例包括抑制性RNA,例如有义RNA(共抑制)、反义RNA、核酶、miRNA或siRNA、mRNA、rRNA和tRNA。当基因表达的终产物是蛋白质或肽时,该基因被认为编码作为表达产物的蛋白质。

[0042] 本文所用的核酸(分子)或核苷酸(序列)或多核苷酸是指DNA和RNA。DNA还包括cDNA和基因组DNA。核酸分子可以是单链或双链的,并且可以化学合成或通过体外或甚至体内生物表达产生。

[0043] 很明显,当RNA分子的核苷酸序列通过参照相应DNA分子的核苷酸序列来定义时,核苷酸序列中的胸腺嘧啶(T)应该被尿嘧啶(U)替代。从本申请的上下文中将清楚是否涉及RNA分子还是DNA分子。

[0044] 如本文所用,“包括”等应被解释为指定所提及的所述特征、整数、步骤或组分的存在,但不排除一个或多个特征、整数、步骤或组件或其群组的存在或添加。因此,例如,包含核苷酸或氨基酸序列的核酸或蛋白质可以包含比实际引用的更多的核苷酸或氨基酸,即,被嵌入更大的核酸或蛋白质中。包含功能或结构上定义的DNA区的嵌合基因可以包含另外的DNA区等。

[0045] 通过GRF5,可提供具有改善的再生能力的植物细胞或其它植物前体组织。这对于基因修饰的植物细胞,特别是具有编辑的基因组的植物细胞特别有用。

[0046] 根据本发明的优选实施方案,在植物细胞瞬时转化中,进行引入至少一个目的核苷酸序列和GRF5的步骤(a1)或引入至少一个目的核苷酸序列并诱导编码GRF5的内源基因的增强的表达水平的步骤(a2)。就本发明而言,“瞬时转化”是指插入序列未(稳定地)整合到植物细胞的基因组中。在另一个实施方案中,实现稳定的转化,其中将本文公开的转化方法的步骤(a1)和(a2)中的目的核苷酸序列和/或本文公开的修饰基因组的方法的步骤(a1)中的编码GRF5多肽的多核苷酸插入植物细胞的基因组中。根据本发明特别优选的实施方案,将编码GRF5的核苷酸序列瞬时转化入细胞,同时将目的核苷酸序列稳定转化入细胞基因组。

[0047] 修饰植物细胞的基因组可以通过双链DNA断裂(DSB)诱导酶来完成,所述酶优选识别所述细胞基因组中的预定位点。

[0048] 因此,本发明的另一个实施方案是修饰植物细胞基因组的方法,包括以下步骤

[0049] (a1)将如下引入植物细胞,包含编码GRF5多肽的多核苷酸的表达盒、编码GRF5多肽的mRNA(包括前mRNA)或GRF5多肽;或

[0050] (a2) 在植物细胞中诱导编码GRF5多肽的内源基因的增强的表达水平;以及
[0051] (b) 在一定条件下培养 (a1) 或 (a2) 的植物细胞或源自 (a1) 或 (a2) 的植物细胞的植物细胞,在所述条件中在所述植物细胞中,GRF5多肽由所述表达盒表达,GRF5多肽由所引入的mRNA翻译,GRF5多肽由所述内源基因增强/增加表达,或GRF5多肽存在,优选与野生型植物细胞或其中包含编码GRF5多肽的多核苷酸的表达盒、编码GRF5多肽的mRNA或GRF5多肽未根据步骤 (a1) 引入或其中编码GRF5多肽的内源基因的增强表达水平未根据步骤 (a2) 诱导的植物细胞中的量相比,所述植物细胞中的量增加;

[0052] (c) 通过双链DNA断裂 (DSB) 诱导酶和任选地通过修复核酸分子修饰 (b) 的植物细胞的基因组,所述诱导酶,所述酶优选地识别所述细胞的基因组中的预定位点,

[0053] 其中在所述预定位点的所述基因组的修饰选自

[0054] i. 至少一个核苷酸的置换;

[0055] ii. 至少一个核苷酸的缺失;

[0056] iii. 至少一个核苷酸的插入;或

[0057] iv. i-iii的任何组合;以及

[0058] 其中步骤 (c) 与步骤 (a1)/(a2) 和/或 (b) 同时进行,在步骤 (a1)/(a2) 之前进行,在步骤 (a1)/(a2) 或 (b) 之间进行或在步骤 (b) 之后进行。

[0059] 如本文所用,“双链DNA断裂诱导酶”或“DSBI酶”是能够在特定核苷酸序列,称为“识别位点”处诱导双链DNA断裂的酶。双链DNA断裂 (DSB) 诱导酶可以例如选自大范围核酸酶、TAL效应物核酸酶、锌指核酸酶、CRISPR系统如CRISPR/Cas9、CRISPR/Cpf1、CRISPR/CasX、CRISPR/CasY、CRISPR/Csm1或CRISPR/MAD7。稀有切割的内切核酸酶是DSBI酶,其具有优选约14至70个连续核苷酸的识别位点,因此具有非常低的切割频率,甚至在较大的基因组如大多数植物基因组中。归巢内切核酸酶,也称为大范围核酸酶,构成了这类稀有切割内切核酸酶的家族。它们可以由内含子、独立基因或间插序列编码,并呈现明显的结构和功能特性,这些特性使它们区别于更经典的限制性酶,通常区别于细菌限制修饰II型系统。它们的识别位点具有一般的不对称性,这与大多数限制酶识别位点的特征性二分体对称性形成对比。已经表明,由内含子或内含肽编码的几种归巢内切核酸酶促进它们各自的遗传元件归巢至等位无内含子或无内含肽位点。通过无内含子或无内含子的等位基因中产生位点特异性双链断裂,这些核酸酶产生重组末端,其参与基因转化过程,该过程复制编码序列并导致在DNA水平插入内含子或间插序列。W003/004659 (17-20页) 的表I中提供了其它罕见切割性大范围核酸酶及其各自的识别位点的列表 (引入本文作为参考)。

[0060] 此外,可用于设计基本上识别任何所选靶核苷酸序列的定制的罕见切割核酸内切酶的方法。简言之,嵌合限制酶可用设计用于识别特异性核苷酸序列的锌指结构域与来自天然限制酶如FokI的非特异性DNA切割结构域之间的杂合体制备。这些方法已经描述在例如W003/080809、W094/18313或W095/09233和Isalan等 (2001) 中。一种快速的、普遍适用的工程化锌指的方法,通过靶向HIV-1启动子来说明。Nature biotechnology, 19(7), 656; Liu等人 (1997)。设计用于在复杂基因组内独特寻址的聚乙酰基锌指蛋白。Proceedings of the National Academy of Sciences, 94(11), 5525-5530.)。

[0061] 定制设计的内切核酸酶的另一个实例包括TALE核酸酶 (TALEN), 其基于来自黄单胞菌属的与核酸酶 (例如FokI或其变体) 的催化结构域融合的转录激活子样效应物 (TALE)。

这些TALE的DNA结合特异性由串联排列的34/35个氨基酸重复单元的重复可变二残基(RVD)定义,使得一个RVD特异性识别靶DNA中的一个核苷酸。可以装配重复单元以基本上识别任何靶序列,并与核酸酶的催化结构域融合以产生序列特异性内切核酸酶Boch等人(2009). Breaking the code of DNA binding specificity of TAL-type III effectors. *Science*, 326(5959), 1509-1512; Moscou & Bogdanove (2009). A simple cipher governs DNA recognition by TAL effectors. *Science*, 326(5959), 1501-1501; 和 WO 2010/079430, WO 2011/072246, WO 2011/154393, WO 2011/146121, WO 2012/001527, WO 2012/093833, WO 2012/104729, WO 2012/138927, WO 2012/138939)。WO 2012/138927还描述了具有各种催化结构域的单体(紧密)TALEN和TALE及其组合。

[0062] 最近,已经描述了一种新型的可定制的内切核酸酶系统;所谓的CRISPR/Cas系统。在其天然环境中的CRISPR系统描述了一种分子复合物,该分子复合物包括至少一个小的且单独的非编码RNA与Cas核酸酶或另一种CRISPR核酸酶(如Cpf1核酸酶(Zetsche等人, , , Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System", *Cell*, 163, pp. 1-13, 10月2015))的组合,该复合物可以产生特定的DNA双链断裂。目前,CRISPR系统被分为2类,包括五种类型的CRISPR系统,II型系统,例如使用Cas9作为效应物,和V型系统,使用Cpf1作为效应物分子(Makarova等人, *Nature Rev. Microbiol.*, 2015)。在人工CRISPR系统中,合成的非编码RNA和CRISPR核酸酶和/或任选地经修饰以充当切口酶或缺乏任何核酸酶功能的经修饰的CRISPR核酸酶可以与至少一种组合crRNA和/或tracrRNA的功能的合成的或人工的引导RNA或gRNA组合使用(Makarova等人, 2015, 同上)。在天然系统中由CRISPR/Cas介导的免疫应答需要CRISPR-RNA(crRNA),其中控制CRISPR核酸酶的特异性激活的该指导RNA的成熟在迄今为止已表征的各种CRISPR系统之间显著变化。首先,入侵DNA,也称为间隔区,整合在CRISPR基因座近端的两个相邻重复区之间。II型CRISPR系统编码Cas9核酸酶作为干扰步骤的关键酶,该系统含有crRNA以及反式激活RNA(tracrRNA)作为指导基序。这些杂交并形成双链(ds)RNA区,其被RNase III识别并可被切割以形成成熟的crRNA。然后这些依次与Cas分子结合以便将核酸酶特异性地引导至靶核酸区域。重组gRNA分子可以包含可变DNA识别区和Cas相互作用区,并且因此可以独立于特定靶核酸和所需Cas核酸酶而被特异性设计。作为进一步的安全机制,PAM(原间隔区邻近基序)必须存在于靶核酸区域中;这些是直接从Cas9/RNA复合物识别的DNA继续的DNA序列。化脓链球菌(*Streptococcus pyogenes*)的Cas9的PAM序列已被描述为"NGG"或"NAG"(标准IUPAC核苷酸编码)(Jinek等人, "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity", *Science* 2012, 337:816-821)。金黄色葡萄球菌(*Staphylococcus aureus*)的Cas9的PAM序列是"NGRRT"或"NGRR(N)"。进一步的变体CRISPR/Cas9系统是已知的。因此,脑膜炎奈瑟球菌(*Neisseria meningitidis*)Cas9在PAM序列NNNGATT处切割。嗜热链球菌(*Streptococcus thermophilus*)Cas9在PAM序列NNAGAAW处切割。最近,已经描述了弯曲杆菌属(*Campylobacter*) CRISPR系统的进一步PAM基序NNNNRYAC(WO 2016/021973A1)。对于Cpf1核酸酶,已经描述了没有tracrRNA的Cpf1-crRNA复合物有效识别和切割靶DNA,其通过短的富含T的PAM进行,这与通常由Cas9系统识别的富含G的PAM相反(Zetsche等, 同上)。此外,通过使用修饰的CRISPR多肽,可以获得特异性单链断裂。Cas切口酶与各种重组gRNA的组合使用也可以通过双DNA切口诱导高度特异性的DNA双链断裂。此

外,通过使用两种gRNA,可以优化DNA结合的特异性,从而优化DNA切割。同时,其他CRISPR效应物,如最初针对细菌描述的CasX和CasY效应物是可用的,并且代表其他效应物,其可用于基因组工程目的(Burstein等人,“New CRISPR-Cas systems from uncultivated microbes”,Nature,2017,542,237-241)。

[0063] DSBI酶的切割位点涉及DNA上诱导双链DNA断裂的精确位置。切割位点可以包含在DSBI酶的识别位点中(或不包含在DSBI酶的识别位点中)(与DSBI酶的识别位点重叠),因此可以说DSBI酶的切割位点位于其识别位点处或附近。DSBI酶的识别位点,有时也称为结合位点,是由DSBI酶(特异性地)识别并决定其结合特异性的核苷酸序列。例如,TALEN或ZNF单体具有分别由其RVD重复或ZF重复决定的识别位点,而其切割位点由其核酸酶结构域(例如FokI)决定,并且通常位于识别位点之外。在二聚体TALEN或ZFN的情况下,切割位点位于各自单体的两个识别/结合位点之间,发生切割的该间插DNA区域称为间隔区。

[0064] 本领域技术人员能够选择识别某一识别位点并在预选/预定位点或其附近的切割位点诱导DSB的DSBI酶,或者对这种DSBI酶进行工程化。或者,可以使用任何常规转化方法或通过与基因组中具有DSBI酶识别位点的生物杂交将DSBI酶识别位点引入到靶基因组中,然后将任何所需的DNA引入到DSBI酶的切割位点处或其附近。

[0065] 在该实施方案的特别优选的方面,将修复核酸分子另外引入植物细胞中。如本文所用,“修复核酸分子”是单链或双链DNA分子或RNA分子,其用作模板用于在切割位点附近或切割位点处的预选位点处修饰基因组DNA。如本文所用,“用作修饰基因组DNA的模板”是指修复核酸分子通过侧翼区域和预选位点侧翼的靶基因组中相应同源区域之间的同源重组,任选地与修复核酸分子两个末端之一处的非同源末端连接(NHEJ)组合(例如,在仅存在一个侧翼区域的情况下),在预选位点处拷贝或整合。通过同源重组的整合将允许修复核酸分子与靶基因组精确连接直至核苷酸水平,而NHEJ可能在修复核酸分子与基因组DNA之间的连接处导致小的插入/缺失。

[0066] 如本文所用,“基因组的修饰”是指基因组已经改变了至少一个核苷酸。这可以通过置换至少一个核苷酸和/或缺失至少一个核苷酸和/或插入至少一个核苷酸来进行,只要与修饰前的预选基因组靶位点的核苷酸序列相比,它导致至少一个核苷酸的总变化,从而允许鉴定修饰,例如通过技术人员熟知的例如测序或PCR分析等的技术。

[0067] 如本文所用,“预选位点”、“预确定位点”或“预定位点”表示基因组(例如核基因组或叶绿体基因组)中的特定核苷酸序列,在该位置上期望插入、置换和/或缺失一个或多个核苷酸。这可以是例如内源基因座或在先前引入的外源DNA或转基因中的或与其连接的特定核苷酸序列。预选位点可以是特定的核苷酸位置,在该位置(之后)其意图进行一个或多个核苷酸的插入。预选位点还可以包含待交换(置换)或缺失的一个或多个核苷酸的序列。

[0068] 如本申请上下文中所用,术语“约”是指所述值的 $\pm 10\%$,优选所述值的 $\pm 5\%$ 。例如,约100个核苷酸(nt)应理解为90至110nt之间的值,优选95至105之间的值。

[0069] 如本文所用,“侧翼区”是修复核酸分子的区域,其具有与预选位点侧翼(即上游或下游)的DNA区的核苷酸序列同源的核苷酸序列。很明显,应当选择侧翼区域的长度和序列同一性百分比,以便能够在所述侧翼区域和它们在预选位点上游或下游的相应DNA区域之间进行同源重组。侧翼于预选位点的与修复核酸分子的侧翼DNA区具有同源性的DNA区也称为基因组DNA中的同源区。

[0070] 为了具有足够的重组同源性,修复核酸分子的侧翼DNA区域的长度可以变化,并且长度应至少为约10nt,约15nt,约20nt,约25nt,约30nt,约40nt或约50nt。然而,侧翼区域可以尽实际可能的长(例如,高达约100-150kb,例如完整的细菌人工染色体(BAC)。优选地,侧翼区域将是约50nt至约2000nt,例如约100nt,200nt,500nt或1000nt,此外,目标DNA侧翼的区域不必与同源区域(预选位点侧翼的DNA区域)相同,并且可以具有约80%至约100%的序列同一性,优选与预选区域侧翼的DNA区域具有约95%至约100%的序列同一性,侧翼区域越长,对同源性的要求越不严格;此外,在不改变相邻DNA序列的DNA序列的情况下实现在预选位点的靶DNA序列的交换,侧翼DNA序列应优选与在预选位点侧翼的上游和下游DNA区域相同。

[0071] 如本文所用,“上游”表示核酸分子上更靠近所述核酸分子5'端的位置。同样,术语“下游”是指核酸分子上更靠近所述核酸分子3'末端的位置。为了避免疑惑,核酸分子及其序列通常以其5'至3'方向(从左到右)表示。

[0072] 为了靶向预选位点的序列修饰,必须选择侧翼区,使得上游侧翼区的3'端和/或下游侧翼区的5'端与预定位点的末端对齐。这样,上游侧翼区的3'端决定了预定位点的5'端,而下游侧翼区的5'端决定了预定位点的3'端。

[0073] 如本文所用,所述预选位点位于所述切割(和/或识别)位点之外或远离所述切割(和/或识别)位点,是指意图进行基因组修饰的位点(预选位点)不包含DSBI酶的切割位点和/或识别位点,即预选位点不与切割(和/或识别)位点重叠。因此,在这方面,之外/远离是指切割(和/或识别)位点的上游或下游。

[0074] 已经根据本发明的方法转化或基因编辑的并且可能具有修饰的基因组的修饰的植物细胞可以再生为完整(能育)植物。由于在植物细胞中存在额外的GRF5,它们再生的能力显着改善。因此,在本发明的一个优选方面,植物细胞的转化或植物细胞基因组的修饰分别在再生植物的步骤之后。因此,本发明提供了一种产生转基因植物的方法,包括

[0075] (a) 如上所述转化植物细胞,和

[0076] (b) 从(a)的植物细胞或从(a)的植物细胞衍生的植物细胞再生包含至少一种植物细胞的植物,所述植物细胞包含作为转基因的所述至少一种目的核苷酸序列。

[0077] 此外,本发明还提供了产生遗传修饰植物的方法,包括

[0078] (a) 如上文所述修饰植物细胞的基因组,和

[0079] (b) 从(a)的植物细胞或从衍生自(a)的植物细胞再生在至少一个细胞中包含所述基因组修饰或所述经修饰的植物细胞的植物。

[0080] 再生技术依赖于对组织培养生长培养基中某些植物激素的操作,有时依赖于可与所需目的核苷酸序列一起引入的杀生物剂和/或除草剂标记物。Evans等人,Protoplasts Isolation and Culture,Handbook of Plant Cell Culture,pp.124-176,MacMillan Publishing Company,New York,1983和Binding,Regeneration of Plants,Plant Protoplasts,pp.21-73,CRC Press,Boca Raton,1985中描述了从培养的原生质体再生植物。再生也可以从植物愈伤组织、外植体、原生质体、未成熟或成熟胚、胚组织、分生组织、器官或其部分获得。这种再生技术一般描述于Klee(1987)Ann.Rev.of Plant Phys.38:467486。为了从转基因组织如未成熟胚获得完整植物,可以在受控的环境条件下在一系列含有营养物和激素的培养基中培养它们,这是一种称为组织培养的方法。一旦产生完整植

株并产生种子,就开始评价后代。

[0081] 根据本发明,不仅可以改善转化的或遗传修饰的植物细胞的再生能力,而且可以改善其它类型的具有差再生能力的敏感细胞的再生能力。特别地,通过GRF5可以改善从前体如未成熟雄性配子体或小孢子产生单倍体植物胚。

[0082] 因此,本发明的另一方面是产生单倍体植物胚的方法,包括步骤

[0083] (a1) 将如下引入未成熟雄配子体或小孢子中: 包含编码GRF5多肽的多核苷酸的表达盒、编码GRF5多肽的mRNA或GRF5多肽的mRNA; 或

[0084] (a2) 在未成熟雄配子体或小孢子中诱导编码GRF5多肽的内源基因的增强的表达水平; 以及

[0085] (b) 在一定条件下培养(a)的未成熟雄配子体或小孢子,其中在未成熟雄配子体或小孢子中,GRF5多肽从表达盒表达,GRF5多肽从引入的mRNA翻译,GRF5多肽从内源基因增强表达,或GRF5多肽存在,优选与野生型植物细胞或其中包含编码GRF5多肽的多核苷酸的表达盒、编码GRF5多肽的mRNA或GRF5多肽未根据步骤(a1)引入或其中编码GRF5多肽的内源基因的增强表达水平未根据步骤(a2)诱导的植物细胞中的量相比,所述植物细胞中的量增加; 以及

[0086] (c) 选择来源于步骤(b)的未成熟雄性配子体或小孢子的单倍体植物胚。

[0087] 本发明还包括产生单倍体幼苗的方法,其包括将单倍体植物材料暴露于包含编码GRF5多肽的多核苷酸的表达盒、编码GRF5多肽的mRNA或GRF5多肽以产生单倍体胚,然后将单倍体胚转化(即萌发)为幼苗。因此,本发明包括一种制备单倍体植物的方法,包括培养按照上述方法产生的幼苗。本发明还提供了产生双单倍体植物的方法,包括在GRF5存在下培养单倍体植物材料一段时间,刺激或允许自发染色体加倍,和使双单倍体植物材料生长成幼苗、小植株或植物。在某些实施方案中,单倍体胚胎发生和染色体加倍可以基本上同时发生。在其它实施方案中,在单倍体胚胎发生和染色体加倍之间可能存在时间延迟。如果单倍体幼苗、植物或小植株的生长不涉及自发染色体加倍事件,则可以使用化学染色体加倍剂,例如秋水仙素。许多方法涉及使植物细胞与秋水仙素、抗微管剂或抗微管除草剂如拿草特(pronamide)、一氧化二氮或任何有丝分裂抑制剂接触。结果是纯合的加倍单倍体细胞。当使用秋水仙素时,培养基中的浓度通常可以是0.01% - 0.2%。秋水仙素浓度的范围可以是约400-600mg/L。

[0088] 当小孢子暴露于GRF5多肽时,则可形成愈伤组织,其可经历器官发生以形成胚。因此,本发明包括产生单倍体植物愈伤组织的方法,包括将未成熟雄性配子体或小孢子暴露于GRF5多肽,或将包含编码GRF5多肽的多核苷酸的表达盒、编码GRF5多肽的mRNA或GRF5多肽引入其中。

[0089] 在培养步骤中,未成熟雄性配子体或小孢子优选暴露于GRF5一段时间,所述时间足以诱导单倍体胚形成。所需的时间阶段可取决于所涉及的植物的种类,并且这些都是本领域普通技术人员容易确定的。GRF5暴露的优选范围为约1至约20小时;更优选约2-约20小时。

[0090] 在本发明的某些方面,在引入包含编码GRF5多肽的多核苷酸的表达盒、编码GRF5多肽的mRNA或GRF5多肽之前,对单倍体植物材料施加物理胁迫。物理胁迫可以是例如温度、黑暗、光或电离辐射、饥饿或渗透胁迫中的任何一种。光可以是全光谱日光,或者是选自可

见、红外或UV光谱的一个或多个频率。胁迫可以是连续的或间断的(周期性的);随时间的推移是规则的或随机的。

[0091] 本发明适用于任何植物物种,无论是单子叶植物还是双子叶植物。优选地,可进行本发明的方法和用途的植物是选自以下属的植物:大麦属(*Hordeum*)、蜀黍属(*Sorghum*)、甘蔗属(*Saccharum*)、玉蜀黍属(*Zea*)、狗尾草属(*Setaria*)、稻属(*Oryza*)、小麦属(*Triticum*)、黑麦属(*Secale*)、黑小麦属(*Triticale*)、苹果属(*Malus*)、短柄菜属(*Brachypodium*)、*Aegilops*、胡萝卜属(*Daucus*)、甜菜属(*Beta*)、桉树属(*Eucalyptus*)、烟草属(*Nicotiana*)、茄属(*Solanum*)、咖啡属(*Coffea*)、葡萄属(*Vitis*)、*Erythranthe*、*Genlisea*、香瓜属(*Cucumis*)、*Marus*、拟南芥属(*Arabidopsis*)、*Crucihimalaya*、*Cardamine*、独行菜属(*Lepidium*)、*Capsella*、*Olmrabidopsis*、*Arabis*、芸苔属(*Brassica*)、*Eruca*、萝卜属(*Raphanus*)、柑橘属(*Citrus*)、麻风树属(*Jatropha*)、杨属(*Populus*)、苜蓿属(*Medicago*)、鹰嘴豆属(*Cicer*)、*Cajanus*、菜豆属(*Phaseolus*)、大豆属(*Glycine*)、棉属(*Gossypium*)、黄芪属(*Astragalus*)、莲属(*Lotus*)、*Torenia*、葱属(*Allium*)或向日葵属(*Helianthus*)。更优选的,植物选自大麦、*Hordeum bulbosum*、两色蜀黍、*Saccharum officinarum*、玉蜀黍物种(*Zea spp.*) (包括玉蜀黍)、谷子(*Setaria italica*)、*Oryza minuta*、稻、*Oryza australiensis*、*Oryza alta*、普通小麦、硬粒小麦(*Triticum durum*)、黑麦、黑小麦(*Triticale*)、*Malus domestica*、*Brachypodium distachyon*、海滨大麦(*Hordeum marinum*)、*Aegilops tauschii*、*Daucus glochidiatus*、甜菜属物种(包括甜菜)、小胡萝卜(*Daucus pusillus*)、*Daucus muricatus*、野胡萝卜(*Daucus carota*)、*Eucalyptus grandis*、*Nicotiana sylvestris*、*Nicotiana tomentosiformis*、烟草(*Nicotiana tabacum*)、*Nicotiana benthamiana*、*Solanum lycopersicum*、马铃薯(*Solanum tuberosum*)、*Coffea canephora*、葡萄(*Vitis vinifera*)、*Erythranthe guttata*、*Genlisea aurea*、*Cucumis sativus*、*Marus notabilis*、*Arabidopsis arenosa*、*Arabidopsis lyrata*、拟南芥、*Crucihimalaya himalaica*、*Crucihimalaya wallichii*、*Cardamine nexuosa*、*Lepidium virginicum*、*Capsella bursa pastoris*、*Olmrabidopsis pumila*、*Arabis hirsute*、欧洲油菜、甘蓝、芜菁、萝卜、*Brassica juncacea*、黑芥(*Brassica nigra*)、*Eruca vesicaria subsp. sativa*、甜橙(*Citrus sinensis*)、*Jatropha curcas*、*Populus trichocarpa*、*Medicago truncatula*、*Cicer yamashitae*、*Cicer bijugum*、鹰嘴豆(*Cicer arietinum*)、*Cicer reticulatum*、*Cicer judaicum*、*Cajanus cajanifolius*、*Cajanus scarabaeoides*、菜豆(*Phaseolus vulgaris*)、大豆、棉属物种(*Gossypium sp.*)、紫云英(*Astragalus sinicus*)、*Lotus japonicas*、*Torenia fournieri*、洋葱(*Allium cepa*)、葱(*Allium fistulosum*)、*Allium sativum*、葵花、菊芋(*Helianthus tuberosus*)和/或*Allium tuberosum*。特别优选的是甜菜、玉蜀黍、普通小麦、大麦、黑麦、葵花、马铃薯、两色蜀黍、芜菁、欧洲油菜、*Brassica juncacea*、甘蓝、萝卜、稻、大豆、和/或棉属物种(*Gossypium sp.*)。

[0092] 本发明的合适植物细胞尤其是愈伤组织,优选脆性愈伤组织,分生组织,再生组织(例如小孢子)或胚组织以及原生质体的细胞。

[0093] 植物的部分可以附着于完整的整体植物或与完整的整体植物分离。植物的这些部分包括但不限于植物的器官、组织和细胞,优选种子。

[0094] 本发明的主题还是通过上述方法获得的或可通过上述方法获得的植物。因此,本

发明的一个实施方案是通过上述转化植物细胞并从所述细胞再生植物的方法获得的或可获得转基因植物,以及其后代或部分,其中所述后代或部分包含至少一种作为转基因的目的核苷酸序列。本发明的另一个实施方案是通过修饰植物细胞基因组并从所述细胞再生植物的上述方法获得的或可获得的遗传修饰植物以及其后代或部分,其中所述后代或部分包含通过上述修饰方法引入的基因组中的修饰。

[0095] 本发明的进一步主题是源自上述转基因植物或遗传修饰植物的植物细胞或种子。这种植物细胞优选包含瞬时或稳定整合的编码GRF5多肽的多核苷酸和双链DNA断裂(DSB)诱导酶,其优选识别所述细胞基因组中的预定位点,和任选地包含修复核酸分子。编码GRF5多肽的多核苷酸优选可操作地连接到合适的调节序列,以使植物细胞能够表达GRF5多肽。调节序列意指例如“启动子”,其是指通常在其编码序列上游(5')的核苷酸序列,其通过提供对RNA聚合酶和正确转录所需的其它因子的识别来控制编码序列的表达。“组成型启动子”是指在几乎所有组织中和在所有时间指导基因表达的启动子。组成型启动子的实例包括CaMV35S启动子、双CaMV35S启动子(70S启动子)、胭脂碱合酶(nos)启动子、BdEF1启动子或遍在蛋白启动子如PcUbi4或ZmUbi1。“调节启动子”是指非组成型地、而是以时间和/或空间调节方式指导基因表达的启动子,包括组织特异性和诱导型启动子。它包括天然和合成序列以及可以是合成和天然序列的组合的序列。不同的启动子可以指导基因在不同组织或细胞类型中、或在不同发育阶段、或响应于不同环境条件的表达。用于植物细胞的各种类型的新启动子正被持续地发现,并且是本领域技术人员公知的。“组织特异性启动子”指不在所有植物细胞中表达,而仅在特定器官(如叶或种子)、特定组织(如胚或子叶)或特定细胞类型(如叶实质或种子储存细胞)中的一种或多种细胞类型中表达的调节启动子。这些启动子还包括在发育种子或果实中果实成熟期间、在完全分化的叶中或在衰老开始时被时间调节(例如在早期或晚期胚胎发生中)的启动子。“诱导型启动子”是指那些受调节的启动子,其可以在一种或多种细胞类型中通过外部刺激(例如化学、光、激素、胁迫或病原体)而开启。诱导型启动子的实例是可由蜕皮激素、地塞米松、乙醇诱导的启动子。这些启动子是本领域公知的(例如Samalova等人(2005). pOp6/LhGR: a stringently regulated and highly responsive dexamethasone-inducible gene expression system for tobacco. *The Plant Journal*, 41 (6), 919-935; Gatz&Lenk(1998). Promoters that respond to chemical inducers. *Trends in Plant Science*, 3(9), 352-358.)。

[0096] 本发明的另一个主题是通过本发明的方法获得的或可获得的单倍体植物胚。

[0097] 本发明的另一个主题是植物细胞,其包含瞬时或稳定整合的编码GRF5多肽的多核苷酸,和优选识别所述细胞基因组中预定位点的双链DNA断裂(DSB)诱导酶,和任选的修复核酸分子,其中优选编码GRF5多肽的多核苷酸与合适的调节序列可操作连接,以使植物细胞能够表达GRF5多肽。当进行上述修饰植物细胞基因组的方法时,可以获得这样的植物细胞。

[0098] 本发明的另一方面是编码GRF5多肽的多核苷酸、编码GRF5多肽的mRNA、GRF5多肽或编码GRF5多肽的内源基因的表达激活蛋白在植物细胞的转化方法中,优选在上述转化方法中,在修饰植物细胞基因组的方法中,优选在上述修饰基因组的方法中,在产生植物或单倍体植物胚的方法中,优选在上述产生植物或单倍体植物胚的方法中,或在植物再生的方法中,优选在上述植物再生的方法中的用途。

[0099] 除非在实施例中另有说明,所有重组DNA技术都按照如下所述的标准技术进行: Sambrook等人(1989) *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, NY and in Volumes 1 and 2 of Ausubel等人(1994) *Current Protocols in Molecular Biology*, Current Protocols, USA。植物分子工作的标准材料和方法描述于 *Plant Molecular Biology Labfax* (1993), R.D.D.Cray, BIOS Scientific Publications Ltd (UK) 和 Blackwell Scientific Publications, UK 联合发行。其他标准分子生物学技术的参考文献包括 Sambrook 和 Russell (2001) *Molecular Cloning: A Laboratory Manual*, Third Edition, Cold Spring Harbor Laboratory Press, NY, Volumes I and II of Brown (1998) *Molecular Biology LabFax*, Second Edition, Academic Press (UK)。聚合酶链式反应的标准磁疗和方法见于 Dieffenbach 和 Dveksler (1995) *PCR Primer: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 以及 McPherson 等人 (2000) *PCR-Basics: From Background to Bench*, First Edition, Springer Verlag, Germany.。

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[0101] 将参考以下附图和本文所述的实施例进一步描述本发明。然而,应当理解,本发明并不限于这些实施例。

[0102] 附图

[0103] 图1: 在每个选择步骤中发育的芽的总数(S1-S4)。从用对照构建体 pZFN-tdT-nptII 或用 pZFN-nptII-GRF5 进行的5个独立转化实验获得的数据(比较表3)。

[0104] 图2: 在对照实验(A)和用 pZFN-nptII-GRF5 进行的实验(B)上,在选择步骤3开始时(S3)芽再生14个独立转化的愈伤组织。箭头指示发育中的芽。按照2.1的方法进行实验。

[0105] 图3: 用 pZFN-nptII-GRF5 (A) 和对照质粒 pABM70S-TurboYFP (B) 轰击后10天,甜菜愈伤组织中的芽再生。用 pUbi4-tdT 质粒共轰击愈伤组织,以通过红色荧光控制瞬时表达水平。按照方法2.2进行实验。显示了三个独立轰击的愈伤组织。

[0106] 图4: 十一个独立的甜菜转基因事件中GRF5的表达水平。

[0107] 图5: A: 来自16个不同植物物种的GRF5多肽序列的序列比较和GRF5特异性指示物基序(SEQ ID NO:177)的推导;如所示的GRF5多肽序列的部分序列列在SEQ ID NO:178-206; B: 通过对来自16个不同植物物种的完整蛋白质序列进行序列比对/比较进行基序分析,允许多至10个错配。“起始”和“终止”列表示GRF5多肽序列片段对应于指示物基序的起始和终止位置;“错配”列显示各个GRF5多肽和指示物基序之间的错配数目;如所示的GRF5多肽序列的部分序列列在SEQ ID NO:178-184和SEQ ID NO:186-206。AtGRF1_AT2G22840.1 (SEQ ID NO:33); AtGRF2_AT4G37740.1 (SEQ ID NO:34); AtGRF3_AT2G36400.1 (SEQ ID NO:35); AtGRF4_AT3G52910.1 (SEQ ID NO:36); AtGRF6_AT2G06200.1 (SEQ ID NO:37); AtGRF7_AT5G53660.1 (SEQ ID NO:38); AtGRF8_AT4G24150.1 (SEQ ID NO:39); AtGRF9_AT2G45480.1 (SEQ ID NO:40)。

[0108] 图6: 用如下获得的糖用甜菜中的转化频率: 对照构建体 70S::tdT, 过表达 AtGRF5 (cDNA-SEQ ID NO:1; 氨基酸序列-SEQ ID NO:2), 和甜菜 GRF5 直系同源物 BvGRF5 (合成 DNA-SEQ ID NO:207; 氨基酸序列-SEQ ID NO:4) 的构建体。

[0109] 图7:通过使用过表达td番茄(tDT)(对照)和AtGRF5(cDNA-SEQ ID NO:1;氨基酸序列-SEQ ID NO:2)的构建体,在转化实验中获得的每接种甜菜愈伤组织的转基因芽数目。

[0110] 图8:从用对照构建体70S-tDT(对照)和过表达AtGRF5的构建体(70S-GRF5)的转化实验获得的每个甜菜愈伤组织的转基因芽平均数。使用两种顽拗基因型(recalcitrant genotype)A和B。基因型A的不顺应再生枝条水平非常高,而基因型B显示比A更温和的不顺应。

[0111] 图9:在甜菜中过表达AtGRF5的转基因株系的愈伤组织再生实验。通过使用Kischenko等人2005(也参见实施例1)所述的愈伤组织诱导培养基和芽再生培养基,对愈伤组织形成频率(A)和芽再生频率(B)进行评分。测定了总共5个过表达AtGRF5的独立事件(AtGRF5-36,AtGRF5-41,AtGRF5-14,AtGRF5-94,AtGRF5-50)。作为对照,使用非转基因甜菜芽(WT1和WT2)和过表达tDT报道蛋白的转基因芽(tDT-事件)。通过qRT-PCR测定从用于测定(C)的GRF5转基因事件分离的样品中AtGRF5的表达水平。

[0112] 图10:或者用对照构建体70S::tDT的序列,或用过表达AtGRF5(cDNA-SEQ ID NO:1;氨基酸序列-SEQ ID NO:2),两个GRF5玉米直向同源物[ZmGRF5版本A(合成DNA-SEQ ID NO:208;氨基酸序列-SEQ ID NO:209)和ZmGRF5版本B(合成DNA-SEQ ID NO:210;氨基酸序列-SEQ ID NO:112)的构建体的玉米中的转化频率。转化效率计算为转基因事件的数目除以接种的不成熟胚的数目。

[0113] 图11:转化21天后大豆外植体上芽发育和芽的DsRed荧光。DsRed对照、AtGRF5和GmGRF5外植体的代表性图片显示(A)在初生节的芽发育和(B)芽的DsRed荧光。DsRed图片是多个图片的合成,并且出于定向目的而被定位在临近的位置。

[0114] 图12:选择后16至22天,'良好'芽生长的形成持续迅速增加。在两个时间点评分外植体的百分比,以突出针对用三种构建体转化的外植体选择时芽的快速产生。

[0115] 图13:在用和不用GRF5变体转化的大豆外植体上形成芽。显示了三种处理(构建体)的从一次重复的顶视图和侧视图中获得的图片。在用AtGRF5(中图)和GmGRF5(下图)相对于DsRed对照(上图)转化的外植体中,芽生长更明显。

[0116] 图14:盒须图显示在选择时第16和22天,用AtGRF5或GmGRF5转化的外植体中转基因芽再生的显著增加。

[0117] 图15:转化21天后,卡诺拉油菜外植体上芽发育和芽的DsRed荧光。来自DsRed对照、AtGRF5和BnGRF5的外植体的代表性图片显示(A)下胚轴节段的愈伤组织发育和(B)外植体上的DsRed荧光。

[0118] 图16:在用AtGRF5、BnGRF5和DsRed对照转化的五个欧洲油菜实验中表达DsRed的外植体的百分比在盒须图中描述。与对照构建体相比,AtGRF5和BnGRF5中表达DsRed的外植体百分比显著更高,平均值增加了接近3倍。

[0119] 图17:用对照构建体70S-tDT和70S-AtGRF5共转化甜菜愈伤组织。共转化实验中愈伤组织的接种是用各自含有每个构建体的农杆菌菌株的1:1混合物分别进行的。

实施例

[0120] 甜菜试验

[0121] 二元质粒的制备:

[0122] 二元载体pZFN-nptII-GRF5通过以下标准克隆方法产生。在该载体的T-DNA中,将编码GRF5 (At3g13960) 的cDNA克隆在双CaMV35S启动子和胭脂碱合酶 (NOS) 终止子之间,以确保GRF5蛋白的高异位表达水平。T-DNA还含有赋予对一系列氨基糖苷抗生素(例如卡那霉素或巴龙霉素) 的抗性的新霉素磷酸转移酶II (nptII) 基因,并用于选择转基因植物细胞和组织。NOS启动子和pAG7终止子位于nptII基因的侧翼。二元载体的骨架含有分别用于在大肠杆菌 (*Escherichia coli*) 和根癌农杆菌 (*Agrobacterium tumefaciens*) 中复制质粒的colE1和pVS1起点;和为细菌选择赋予链霉素/壮观霉素抗性的aadA基因。通过标准方法将pZFN-nptII-GRF5质粒转化到AGL-1农杆菌菌株中。

[0123] 微繁殖芽的转化:

[0124] 通过不同的方法转化甜菜的芽:

[0125] a) 农杆菌介导的转化基于Kischenko等,2005Cell Biology International:

[0126] 1. 基因型S706的微繁殖的芽用作起始材料。在补充了30g/1蔗糖和0.25mg/1苄基腺嘌呤 (BAP) 的MS盐中增殖芽。

[0127] 2. 为了诱导脆弱愈伤组织,将叶外植体在30°C左右在包括15g/1蔗糖和2mg/1BAP的MS盐中孵育几周。

[0128] 3. 收获易碎愈伤组织。

[0129] 4. 使含有载体pZFN-nptII-GRF5的农杆菌AGL-1在补充了适当抗生素的合适培养基中生长。

[0130] 5. 用农杆菌悬液接种愈伤组织。愈伤组织和农杆菌的共培养在含有440mg/1 $\text{CaCl}_2 \times 2\text{H}_2\text{O}$ 、170mg/1 KH_2PO_4 、1900mg/1 KNO_3 、370mg/1 MgSO_4 、1650mg/1 NH_4NO_3 、2mg/1 BAP、40 μg /1乙酰丁香酮、20g/1蔗糖和2g/1葡萄糖的培养基中进行至少2天。

[0131] 6. 将愈伤组织继代培养至补充了30g/1蔗糖、1mg/1 GA3、1mg/1 TDZ和500mg/1特美汀的MS盐,并在黑暗中孵育用于培养1周。

[0132] 7. 为了选择转基因细胞,将愈伤组织转移至补充了100mg/1巴龙霉素的步骤6的培养基中,并在光照下温育数周。

[0133] 8. 选择转基因愈伤组织并在相同的培养基和条件下传代培养若干次。

[0134] 9. 分离再生芽并在MS盐中繁殖,MS盐包括30g/1蔗糖、0.25mg/1 BAP和100mg/1卡那霉素。

[0135] 10. 将绿色的芽转移到补充了30g/1蔗糖、0.1mg/1 BAP、250mg/1特美汀和200mg/1巴龙霉素的MS盐中以完成选择阶段,并在该培养基中定期扩增数次。

[0136] 11. 从绿色生长的芽中分离叶外植体,用于DNA提取和PCR分析,以便证实推定的转基因株系。

[0137] 12. 将选择的芽在补充了0.5mg/1 IBA、100mg/1头孢噻肟和10mg/1 PPT的MS盐中生根,并转移到温室中进行种子生产。

[0138] b) 甜菜愈伤组织的粒子轰击

[0139] 1. 如先前在2.1的方法中所述产生易碎愈伤组织。

[0140] 2. 渗透处理进行若干小时。

[0141] 3. 按照PDS-1000/He使用手册中的标准方法制备金颗粒并对其进行DNA包被。质粒pZFN-nptII-GRF5和pUbi4-tDT (含有红色荧光报道分子) 与金颗粒共沉淀。作为对照,用

pUbi4tDT和pABM70STurboYFP(含有黄色荧光报道分子)包被金颗粒。

[0142] 4.用PDS-1000/He装置(Bio-Rad)轰击愈伤组织,每次发射使用30ng包被有500ng DNA的金颗粒。

[0143] 5.为了评价GRF5瞬时表达对芽再生频率的影响,将轰击的愈伤组织在补充了30g/1蔗糖、1mg/1 GA3和1mg/1噻苯隆的MS盐中在光照条件下孵育用于培养2周。通过使用标准双目镜对芽数进行评分。

[0144] 结果:

[0145] 用构建体pZFN-nptII-GRF5对来自甜菜微繁殖芽的愈伤组织进行根癌农杆菌介导的转化,在选择步骤结束时显著增加了再生芽的数目(表2)。作为对照,使用了含有红色荧光报道分子的构建体pZFN-tdT-nptII(对照)。五个独立实验显示平均转化频率从5.0%增加到33.9%。此外,通过PCR证实的转基因事件的数目平均从每个转化实验的4.8个事件增加到25.4个事件。

[0146] 表2.通过a)的转化方法用对照构建体或用pZFN-nptII-GRF5进行的5个独立实验的转化频率。还显示了选择阶段结束时的芽总数和通过PCR证实的转基因事件总数。粗体表示发育中的芽、转基因事件和转化频率的平均数。

实验名称	# 选择结束时芽	# 确认的转基因株系	转化频率(%)
对照_Rep01	9	3	5.5
对照_Rep02	8	5	8.5
对照_Rep03	11	5	3.3
对照_Rep04	9	4	3.1
对照_Rep05	13	7	4.7
对照 平均	10	4.8	5.0
GRF5_Rep01	108	23	11.5
GRF5_Rep02	61	28	35.0
GRF5_Rep03	27	13	21.7
GRF5_Rep04	167	35	38.9
GRF5_Rep05	86	28	62.2
GRF5 平均	89.8	25.4	33.9

[0147] 在愈伤组织转化实验中,也已经确定了每个单独选择步骤的芽再生。在每个选择步骤中再生芽的定量显示,在甜菜愈伤组织中GRF5的过量表达加速芽器官发生(表3,图1和2)。在另一个愈伤组织转化实验中,已经确定了两种顽拗甜菜基因型的芽再生(图8):用构建体pZFN-tdT-nptII(对照)转化,对于基因型A没有转基因芽,对于基因型B每个愈伤组织平均仅产生0.3个芽。在70S启动子下使用AtGRF5过表达,转基因芽的平均数量对于基因型A从0增加到1.67个转基因芽,对于基因型B从每个愈伤组织的0.3增加到4.82个芽。这令人印象深刻地证明了AtGRF5的潜力,并开辟了使用标准转化方法以基因型独立的方式工作的可能性。

[0148] 进一步的实验证明,通过使用用于在甜菜愈伤组织中过表达AtGRF5的构建体,每

个接种的愈伤组织的转基因事件的数目可以增加9倍(图9)。作为对照,使用了含有红色荧光报道分子的构建体pZFN-tdT-nptII(对照)。GRF5基因以及报道基因处于组成型70S启动子(来自花椰菜花叶病毒的双重增强的组成型35S启动子)的控制之下。

[0149] 已经在十一个随机选择的独立的甜菜转基因事件中测定了GRF5的表达水平。用结合NOS终止子的3'-UTR的引物进行表达分析。在所有分析的转基因事件中,检测到高水平的GRF5表达(见图4)。

[0150] 表3. 在5个独立愈伤组织转化实验的每个选择步骤中再生芽的定量,所述实验用pZFN-tdT-nptII(Ctrl)或pZFN-nptII-GRF5(RB)进行。每个步骤中发育的芽的总数以粗体表示。

实验 ID	过表达的基因	选择步骤			
		S1	S2	S3	S4
Ctrl-1	td 番茄	0	0	3	6
Ctrl-2	td 番茄	0	1	3	4
Ctrl-3	td 番茄	0	0	0	11
Ctrl-4	td 番茄	0	0	4	5
Ctrl-5	td 番茄	0	0	7	6
Ctrl-总	td 番茄	0	1	17	32
RB-1	GRF5	0	40	41	27
RB-2	GRF5	0	11	20	30
RB-3	GRF5	0	9	11	7
RB-4	GRF5	0	65	77	25
RB-5	GRF5	2	27	23	34
RB-总	GRF5	2	152	172	123

[0152] 在愈伤组织再生的进一步实验中,已经分析了在甜菜中过表达AtGRF5的五个不同的转基因事件。作为对照,一方面使用两种非转基因甜菜系(WT1和WT2),另一方面使用过表达tdT报道蛋白的转基因系。图9A显示对照系和转基因AtGRF5事件中平均愈伤组织形成频率几乎是相当的,在转基因事件中可能略有提高。图9B显示了转基因芽的平均数量。五个AtGRF5事件中的四个与两个对照相比显示芽形成的显著增加。从图9C明显看出,对芽形成的积极作用与转基因事件中AtGRF5的表达水平有关。

[0153] 在用对照构建体的共转化实验中,70S-tDT和70S-AtGRF5已经稳定整合到甜菜愈伤组织细胞的基因组中。共转化实验中愈伤组织的接种是用含有每个构建体的农杆菌菌株的1:1混合物分别进行的。平均共转化频率为31.5%。如图17所示,在共转化中红色荧光(tDT阳性)事件的数目比在单一转化实验中高约8倍。

[0154] 根据2.2的方法通过粒子轰击进行转化,显示GRF5的瞬时(过)表达甚至也导致转化频率显著增加。经轰击的愈伤组织显示出改善的再生能力。图3显示用构建体pZFN-nptII-GRF5生物射弹转化的愈伤组织与用对照构建体转化的愈伤组织相比,再生芽数增加。

[0155] 在另外的愈伤组织转化实验中,还使用构建体pZFN-nptII-AtGRF5确定了甜菜中的转化频率,与相同但表达来自甜菜的GRF同源物(BvGRF5)的构建体进行了比较。作为对照,使用了含有红色荧光报道分子的构建体pZFN-tdT-nptII(对照)。GRF基因以及报道基因在组成型70S启动子的控制下。图6中所示的实验在早期选择阶段停止。这解释了为什么tdT构建体的转化效率是0%。实现了对于构建体AtGRF5,平均转化频率为70%,对于构建体BvGRF5,平均转化频率为32.5%。

[0156] 2. 稻实验

[0157] 用于二元质粒的构建体:

[0158] 通过标准克隆方法产生二元载体。在该载体的T-DNA中,将编码GRF5(At3g13960)的cDNA克隆在合适的启动子和终止子之间,以确保GRF5蛋白在稻中有足够的异位表达水平(=单一构建体)。T-DNA还含有用于选择转基因植物细胞和组织的GFP基因。另外,产生了二元载体,其除了携带编码GRF5(At3g13960)的cDNA外还包括合适的启动子和终止子,确保GRF5蛋白在稻中足够的异位表达水平,以及在启动子和终止子(=双(堆叠)构建体)控制下的另一目的基因。

[0159] 种子灭菌播种:

[0160] 野生型绿色种子在70%乙醇中孵育并摇动大约1分钟。除去乙醇后,用无菌mQ水洗涤种子一次。然后加入30ml16%次氯酸钠溶液,并将种子摇动40-60分钟。在除去次氯酸钠溶液后,用无菌mQ水洗涤种子3-5次。

[0161] 在完成灭菌(0-3小时)后,将种子在无菌滤纸上干燥,并放置在诱导培养基R001的表面上。在32°C下在连续光(3000lux)下进行6天的温育。

[0162] 转化和共培养:

[0163] 对于外植体制备,选择适于转化的野生型种子的膨胀胚(盾片衍生愈伤组织),并转移到含有用掺入到植物细胞中的质粒转化的根癌农杆菌的液体感染培养基(R002)中1.5分钟,然后转移到共培养平板(R003)上。将平板在25°C黑暗中孵育3天。抗性组织的选择

[0164] 选择:

[0165] 共培养3天后,从种子上除去愈伤组织,用无菌mQ水洗涤几次,用含有250mg/l头孢噻肟的无菌mQ水洗涤一次,并转移到R004选择培养基中。在32°C下在连续光(3000lux)下进行温育2周。

[0166] 微愈伤组织的分离和再生

[0167] 通过无菌镊子将微愈伤组织转移到R005中,并在32°C的连续光(3000lux)下孵育1周。然后在暗室中检查用作选择标记的GFP。将GFP阳性的健康愈伤组织转移到R006上,并在32°C的连续光照(3000lux)下进一步孵育1周。为了连续再生,将愈伤组织转移到R007上,在32°C的连续光照(3000lux)下进行3周,在将小植株带到温室中之前,用无菌镊子将健康的小植株拔出,并在32°C的连续光照(3000lux)下转移到R008中进行2周。

[0168] 表4. 培养基R001至R008的组成。

[0169]

R001 (固体)诱导培养基			
成分	供应商/货码	最终浓度	1.00 l
N6 盐	Sigma - C1416		4.00 g
N6 维生素	Duchefa - C0401	1x	1.00 ml
L-脯氨酸	Sigma -P5607	2878 mg/l	2.88 g
酪蛋白氨基酸	BD - 223050	300 mg/l	0.30 g
蔗糖	Duchefa - S0809	30 g/l	30.00 g
pH			5.80
Gelrite	Duchefa - G1101.5	4 g/l	4.00 g
2,4-D (1mg/ml)	Sigma - D7299	2 mg/l	2000.00 µl
R002 (液体) 感染培养基 (灭菌的滤器)			
成分	供应商/货码	最终浓度	1.00 l

N6 盐	Sigma - C1416	1x	4.00 g
N6 维生素	Duchefa - C0401	1x	1.00 ml
酪蛋白氨基酸	BD - 223050	300 mg/l	0.30 g
蔗糖	Duchefa - S0809	68.5 g/l	68.50 g
D+-葡萄糖单水合物	VWR - MERC1.08342.1000	36 g/l	36.00 g
pH			5.20
乙酰丁香酮(2M)	Sigma Aldrich 2478-38-8	100 µM	66.00 µl
乙酰丁香酮的制备: 2M=392 mg, 在 1 ml DMSO 中 dus: 0.04 g 在 100 µl DMSO 中			
R003 (固体) 共培养培养基			
成分	供应商/货码	最终浓度	1.00 l
N6 盐	Sigma - C1416		4.00 g
N6 维生素	Duchefa - C0401	1x	1.00 ml
酪蛋白氨基酸	BD - 223050	300 mg/l	0.30 g
蔗糖	Duchefa - S0809	30 g/l	30.00 g
D+-葡萄糖单水合物	VWR - MERC1.08342.1000	10 g/l	10.00 g
pH			5.20
Gelrite	Duchefa - G1101.5	4 g/l	4.00 g
乙酰丁香酮 (2M)	Sigma Aldrich 2478-38-8	100 µM	66.00 µl
2,4-D (1mg/ml)	Sigma - D7299	2 mg/l	2000.00 µl
乙酰丁香酮的制备: 2M=392 mg, 在 1 ml DMSO 中 dus: 0.04 g 在 100 µl DMSO 中			
R004 选择培养基 (LBA nptII)			
成分	供应商/货码	最终浓度	1.00 l
N6 盐	Sigma - C1416		4.00 g
N6 维生素	Duchefa - C0401	1x	1.00 ml
L-脯氨酸	Duchefa - P0717	2878 mg/l	2.88 g
酪蛋白氨基酸	BD - 223050	300 mg/l	0.30 g
蔗糖	Duchefa - S0809	30 g/l	30.00 g
pH			5.80
琼脂糖类型 1	Sigma - A6013	7 g/l	7.00 g
2,4-D (1mg/ml)	Sigma - D7299	2 mg/l	2000.00 µl
头孢噻肟(200 mg/ml)	Duchefa - C0111	100 mg/l	500.00 µl

[0170]

万古霉素 (100 mg/ml)	Duchefa - V0155	100 mg/l	1000.00 μ l
G418 硫酸氢盐 (100 mg/ml)	Sigma - G1279	35 mg/l	350.00 μ l
R005 预再生培养基			
成分	供应商/货码	最终浓度	1.00 l
N6 盐	Sigma - C1416		4.00 g
N6 维生素	Duchefa - C0401	1x	1.00 ml
L-脯氨酸	Duchefa - P0717	500 mg/l	0.50 g
酪蛋白氨基酸	BD - 223050	300 mg/l	0.30 g
蔗糖	Duchefa - S0809	30 g/l	30.00 g
pH			5.80
琼脂糖类型 1	Sigma - A6013	7 g/l	7.00 g
激动素(Kinetin) (1mg/ml)	Sigma - K0753	2 mg/l	2000.00 μ l
NAA (1mg/ml)	Duchefa - N0903	1 mg/l	1000.00 μ l
ABA	Sigma - A1049	5 mg/ml	1000.00 μ l
头孢噻肟(200 mg/ml)	Duchefa - C0111	100 mg/l	500.00 μ l
万古霉素 (100 mg/ml)	Duchefa - V0155	100 mg/l	1000.00 μ l
G418 硫酸氢盐 (100 mg/ml)	Sigma - G1279	35 mg/l	350.00 μ l
R006 再生培养基, 其具有 10 g/l 琼脂糖			
成分	供应商/货码	最终浓度	1.00 l
MS 盐	Duchefa - M0221	1x	4.30 g
MS 维生素	Duchefa - 1000x /M0409	1x	1.00 ml
酪蛋白氨基酸	BD - 223050	2000 mg/l	2.00 g
蔗糖	Duchefa - S0809	30 g/l	30.00 g
山梨糖醇	Duchefa S0807	30 g/l	30.00 g
pH			5.80
琼脂糖类型 1	Sigma - A6013	10 g/l	10.00 g
激动素 (1mg/ml)	Sigma - K0753	2 mg/l	2000.00 μ l
NAA (1mg/ml)	Duchefa - N0903	0.02 mg/l	20.00 μ l
头孢噻肟(200 mg/ml)	Duchefa - C0111	100 mg/l	500.00 μ l
万古霉素(100 mg/ml)	Duchefa - V0155	100 mg/l	1000.00 μ l
G418 硫酸氢盐(100 mg/ml)	Sigma - G1279	20 mg/l	200.00 μ l

[0171]

R007 再生培养基			
成分	供应商/货码	最终浓度	1.00 l
MS 盐	Duchefa - M0221	1x	4.30 g
MS 维生素	Duchefa - 1000x /M0409	1x	1.00 ml
蔗糖	Duchefa - S0809	30 g/l	30.00 g
pH			5.80
琼脂糖类型 1	Sigma - A6013	7 g/l	7.00 g
头孢噻肟(200 mg/ml)	Duchefa - C0111	100 mg/l	500.00 µl
万古霉素(100 mg/ml)	Duchefa - V0155	100 mg/l	1000.00 µl
G418 硫酸氢盐(100 mg/ml)	Sigma - G1279	20 mg/l	200.00 µl
R008 发育培养基			
成分	供应商/货码	最终浓度	1.00 l
MS 盐	Duchefa - M0221	2.151045 g/l	2.15 g
B5 维生素 (1000x stock)	Duchefa - G0415	0.5x	0.50 ml
蔗糖	Duchefa - S0809	10 g/l	10.00 g
NAA	Duchefa - N0903	0.05 mg/l	50.00 µl
MgCl ₂ ·6H ₂ O	VWR - MERC1	0.75 g/l	0.75 g
pH			5.80
Gelrite	Duchefa - G1101.5	2.5 g/l	2.50 g

[0172]

[0173] 结果:

[0174] 用含有GRF5的不同构建体对来源于稻不成熟胚的愈伤组织进行根癌农杆菌介导的转化,导致平均转化频率显著增加。作为对照或比较,使用28个随机选择的其它不含GRF5的构建体观察到的转化效率,显示平均转化效率为63%。对于‘单’构建体,转化效率平均可以从63%提高到78%,对于‘双’构建体,甚至可以从63%提高到84%(表5)。

[0175] 表5.独立实验的转化频率,在稻中具有含GRF5的单构建体或具有与另一基因XY堆叠的GRF5。总共,测试了与来自拟南芥的GRF5堆叠中的12种不同基因(“GRF5”,如SEQ ID NO:1中所示的核苷酸序列,SEQ ID NO:2的氨基酸序列),其中重复3个实验两次(由Rep01和Rep02表示)。另外,单独和以堆叠的方式测试来自两色蜀黍的GRF5(“GRF5-蜀黍”,核苷酸序列如SEQ ID NO:17所示,氨基酸序列SEQ ID NO:18)。平均转化频率以粗体表示。

单/堆叠	基因 #1	基因 #2	转化频率 [%]
单	GRF5		72
单	GRF5		62
单	GRF5		73
单	GRF5		88
单	GRF5		93
		平均:	78

双	GRF5	基因 XY-01	77
双	GRF5	基因 XY-02	86
双	GRF5	基因 XY-03_Rep01	91
双	GRF5	基因 XY-03_Rep02	92
双	GRF5	基因 XY-04	92
双	GRF5	基因 XY-05_Rep01	75
双	GRF5	基因 XY-05_Rep02	100
双	GRF5	基因 XY-06	79
双	GRF5	基因 XY-07	89
双	GRF5	基因 XY-08	79
双	GRF5	基因 XY-09	81
双	GRF5	基因 XY-10_Rep01	80
双	GRF5	基因 XY-10_Rep02	82
双	GRF5	基因 XY-11	76
双	GRF5	基因 XY-12	77
		平均:	84

[0176]

单	GRF5-高粱		67
		平均:	67

双	GRF5-高粱	基因 XY-13	78
		平均:	78

对照:		平均:	63
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[0177] 3. 玉蜀黍实验

[0178] 在二元质粒中使用的构建体:

[0179] 通过标准克隆方法产生二元载体。在这些载体的T-DNA中,将a) 编码AtGRF5的cDNA (SEQ ID NO:1), b) 编码ZmGRF5的合成DNA (版本A) (SEQ ID NO:208), 和c) 编码ZmGRF5的合成DNA (版本B) SEQ ID NO:210) 克隆在合适的启动子 (例如BdEF1启动子) 和终止子之间, 以确保GRF5蛋白在玉米中足够的异位表达水平。作为对照, 使用了含有在具有ZmUbi内含子的

70S启动子控制下的tDT报道基因的构建体。

[0180] 转化:

[0181] 根癌农杆菌介导的转化通过使用不成熟胚的盾片转化单子叶植物的标准方法(例如W095/06722)进行。

[0182] 结果:

[0183] 如图10所示,用含有来源于拟南芥的GRF的构建体进行根癌农杆菌介导的不成熟胚转化,导致平均转化频率显著增加,从8%增加到14%。与此相反,使用来源于不同玉蜀黍基因型的两种ZmGRF5版本增强了转化效率,强得多。对于ZmGRF5的版本A,效率从8%提高到52%,对于ZmGRF5的版本B,效率从8%提高到48%。

[0184] 4. *Glycine max* (大豆) 实验

[0185] 大豆转化:

[0186] 大豆转化使用发根农杆菌 (*Agrobacterium rhizogenes*) 进行,用于将T-DNA递送到位于栽培品种Jake的大豆幼苗的初级节点处的上胚轴腋生分生组织细胞中(根据Olhoft P.M., Bernal L.M., Grist L.B., Hill D.S., Mankin S.L., Shen Y., Kalogerakis M., Wiley H., Toren E., Song H.-S., Hillebrand H., and Jones T. 2007, A novel *Agrobacterium rhizogenes*-mediated transformation method soybean [*Glycine max* (L.) Merrill] using primary-node explants from seedlings, *In Vitro Cell. Dev. Biol. - Plant* 43:536-549; US 2014237688, WO 2006024509, WO 2005121345)。

[0187] 二元质粒的产生:

[0188] 通过以下标准克隆方法产生三个二元质粒。第一个二元质粒是用于实验的对照质粒(称为DsRed对照)和用于其它载体的基础载体。其在T-DNA中含有用于转基因植物细胞和组织的表型评分的DsRed基因和用于转基因细胞的优先选择的AtAHAS基因。两个基因都用合适的启动子和终止子克隆,所述启动子和终止子确保足够的异位表达以用于上述目的。第二个二元质粒含有具有编码AtGRF5 (SEQ ID NO.2)的cDNA的基础载体,所述cDNA克隆在合适的启动子和终止子之间,确保GRF5蛋白在大豆中足够的异位表达水平。第三个二元质粒含有基础载体,其具有克隆在合适的启动子和终止子之间的编码GmGRF5 (SEQ ID NO.106)的cDNA,确保GRF5蛋白在大豆中足够的异位表达水平。

[0189] 种子灭菌和萌发:

[0190] 将'Jake'栽培种的大豆种子在具有氯气的室中灭菌,所述氯气通过将3.5ml 12N HCl加入100ml漂白剂中而产生。灭菌后,将约65粒种子铺在PlantCons中的固体萌发培养基(1×B5盐和和维生素,2%蔗糖,0.8% Noble琼脂(A5431Sigma-Aldrich); pH5.8)上。幼苗在26°C光照($150\mu\text{m}^{-2}\text{s}^{-2}$)中萌发7天,并用作转化的外植体材料。

[0191] 农杆菌制备:

[0192] 用下列载体之一转化发根农杆菌(WO2006024509),所述载体包含:(1) pSUPER: DsRed和pPcUBI: AHAS选择标记作为对照,或对照载体加上(2) pPcUbi-AtGRF5或(3) pPcUBI-GmGRF5。使发根农杆菌生长并在Falcon试管中重悬于50ml液体接种培养基(1/10th B5盐(G768Phytotetech)、3%蔗糖、20mM MES、1X Gamborg维生素、200 μM 乙酰丁香酮、1.44 μM 赤霉素、5.0 μM 激动素;pH5.4)中至OD₆₀₀为1.5。然后将农杆菌悬浮液置于深培养皿中以接收制备的外植体。

[0193] 外植体的制备与转化:

[0194] 通过除去根和大部分下胚轴、一个子叶、子叶节处的腋生组织生长和包括所有预形成叶的初生节上方的上胚轴,从8日龄幼苗制备幼苗外植体。在制备外植体后,将大约45-50个外植体与农杆菌悬浮液在培养皿中孵育30分钟。然后将外植体置于培养皿中的湿滤纸上,所述滤纸含有共培养基(1/10th B5盐(G768Phytotech)、3%蔗糖、20mM MES、0.5% Noble琼脂(A5431 Sigma-Aldrich[®])、1X Gamborg维生素、200 μ M乙酰丁香酮、1.44 μ M赤霉素、5.0 μ M激动素、4.1mM L-半胱氨酸、0.5mM二硫苏糖醇、0.5mM硫代硫酸钠;pH5.4),并在室温下密封于容器中5天。

[0195] 芽的发育和选择:

[0196] 5天后,将外植体转移到选择培养基(1X B5盐和维生素(G398Phytotech)、3%蔗糖、3mM MES、1 μ M 6-苄基-氨基嘌呤、5 μ M激动素、250mg/l特美汀STK、3 μ M灭草烟(imazapyr)、0.8% Noble琼脂(A5431Sigma-Aldrich);pH5.6),每个平板上五个,并在26 $^{\circ}$ C培养。选择16天后,外植体在初生节腋生分生组织处具有显著生长,并首先对芽发育(再生)评分。在选择22天后(选择结束),外植体从固体培养基中移出,并在(1)芽形成的质量和(2)在初生节处发育的芽上的DsRed荧光评分之前置于Oasis[®]生长培养基上。

[0197] 实验设计和结果:

[0198] 一个实验在四个研究者和三个构建体之间进行(表6)。用DsRed对照、AtGRF5和GmGRF5转化大豆初生节腋生分生组织。总共转化524个幼苗外植体,并在选择16天后(转化后21天)和选择22天后(转化后27天)评分。选择16天后,芽在初生节处快速生长,并且是小的、紧凑的芽盘和更大的、伸长的芽的组合(图11)。在靶组织(初生节)处的再生[(具有芽的外植体的数目)/总外植体*100]对于所有构建体为80-100%,并且在选择时在16天时固定。在16至22天之间,外植体上的芽继续快速生长。对于两个时间点,对外植体主观评分健康的、伸长的芽生长的存在,其形态("良好")预示着成功形成生根的转基因植物(图12)。用来自两个时间点的所有三种构建体转化的外植体上的"良好"芽形成增加,尤其是用AtGRF5和GmGRF5转化的外植体。在四次重复中,当与DsRed对照相比时,用GRF5的任一种形式转化的外植体倾向于具有更晚期的芽形成(更大的芽,更伸长)(图13)。

[0199] 为了获得转化效率的早期测量,在选择的第16和22天对外植体进行评分,针对在初生节处是否存在DsRed发荧光的芽,这是由于转基因细胞表达DsRed蛋白。在两个时间点,DsRed表达在含有AtGRF5或GmGRF5的构建体中比DsRed对照明显更强;然而在选择时的16天更是如此(图11)。表征为具有"良好"芽形态的外植体通常具有有DsRed表达的芽(图11)。在两个时间点,与用DsRed对照转化的外植体相比,用AtGRF5或GmGRF5转化的外植体中具有表达DsRed的芽的外植体的百分比显著更高($\alpha=0.05$)(表6;图14)。在用DsRed对照构建体转化的外植体中,在选择22天后,54.5%显示DsRed表达的芽,相较而言AtGRF5和GmGRF5分别为70.2%和74.6%(表6)。数据表明在转化后27天,用AtGRF5或GmGRF5转化的大豆外植体比未用GRF5形式转化的外植体在初生节再生的转基因芽明显更多。

[0200] 表6. 在选择时第16天和第22天,用AtGRF5或GmGRF5转化的外植体中转化的芽的再生显著增加。显示了再生和DsRed发荧光的芽的平均值和范围。显著不同(在 $\alpha=0.05$)的构建体后跟有不同的字母;¹=[(在初生节处具有芽的外植体的数目/接种的外植体的总数{n}) \times 100];²=[(在初生节具有DsRed芽的外植体的数目/接种的外植体的总数{n}) \times

100]

构建体	n	再生 (%) ¹		具有 DsRed 芽的外植体 (%) ²			
		选择 16d		16d 选择		22d 选择	
		平均	范围 (%)	平均	范围 (%)	平均	范围 (%)
[0201] DsRed 对照	162	89.8 a	81.8-95.2	31.4 a	20.5-40.5	54.5 a	50.0-57.1
AtGRF5	180	88.4 a	82.6-91.3	58.0 b	52.2-66.7	70.2 b	65.2-78.6
GmGRF5	182	94.6 a	85.4-100	53.3 b	45.2-61.9	74.6 b	62.5-86.0

[0202] 5. 欧洲油菜 (卡诺拉油菜) 实验

[0203] 卡诺拉油菜转化:

[0204] 使用发根农杆菌进行欧洲油菜转化,用于将T-DNA递送到基因型BNS3的欧洲油菜幼苗的下胚轴区段中。

[0205] 种子灭菌和萌发:

[0206] 通过将200-300粒种子置于50-mL Falcon管中的70%乙醇中2分钟,对种子进行表面灭菌。在轻轻摇动2分钟后,除去乙醇,加入40-50ml30%Clorox漂白剂和1滴Tween。将种子在偶尔混合下孵育10分钟。除去液体,然后用无菌水冲洗种子三次,之后将种子置于PlantCon盒中的萌发培养基(1/2×MS盐/维生素,10g l⁻¹蔗糖,Phyto琼脂7g l⁻¹;pH5.8)上。将这些盒置于23℃的黑暗中4至5天。

[0207] 农杆菌制备:

[0208] 用下列载体之一转化发根农杆菌:(1) pSUPER: DsRed和PcUBI: AHAS选择性标记作为对照,或对照载体加上(2) PcUbi -AtGRF5或(3) PcUBI -BnGRF5 (SEQ ID NO.108)。使发根农杆菌生长至OD₆₀₀为1.0,随后用液体感染培养基(1×MS盐/维生素,30g l⁻¹蔗糖;pH5.8)稀释至0.1。然后将农杆菌悬浮液置于用于接收制备的下胚轴外植体的皿中。

[0209] 外植体的制备与转化:

[0210] 通过从萌发盒中取出并放置在用无农杆菌的感染培养基润湿的无菌滤纸上以保持幼苗肿胀,从四至五天黄化的幼苗制备下胚轴外植体。除去根、子叶和上胚轴后,制备长度为7-10mm的下胚轴节段。切割后,将外植体浸入农杆菌悬液中,印迹在干燥的无菌滤纸上,最后置于平板中共培养基(1x MS盐/维生素,30g l⁻¹蔗糖,0.6g l⁻¹MES,18g l⁻¹甘露醇,7g l⁻¹Phyto琼脂,1mg l⁻¹2,4-D,100mg l⁻¹乙酰丁香酮,200mg l⁻¹L-半胱氨酸,pH5.6)顶部的滤纸上。一旦铺上~50个外植体,用微孔胶带密封平板,在23℃光照下,16小时光照/8小时黑暗光周期培养3天。

[0211] 愈伤组织发育和芽起始:

[0212] 3天后,将所有外植体转移到恢复培养基(1×MS盐/维生素,30g l⁻¹蔗糖,0.6g l⁻¹MES,18g l⁻¹甘露醇,7g l⁻¹Phyto琼脂,1mg l⁻¹2,4-D,300mg l⁻¹特美汀;pH5.6)中,用微孔胶带密封,并在23℃培养7天。一周后将外植体转移到选择培养基#1(1×MS盐/维生素,30g l⁻¹蔗糖,0.5g l⁻¹MES,7g l⁻¹Phyto琼脂,3mg l⁻¹BAP,0.1mg l⁻¹NAA,0.1mg l⁻¹GA₃,2.5mg l⁻¹AgNO₃,100nM咪唑乙烟酸,300mg l⁻¹特美汀;pH5.8)中,在23℃下在16h光/8h黑暗光周期下培养14天。选择两周后,将外植体转移到选择培养基2(1x MS盐/维生素,30g l⁻¹蔗糖,0.5g l⁻¹MES,7g l⁻¹Phyto琼脂,0.5mg l⁻¹BAP,0.1mg l⁻¹GA₃,2.5mg l⁻¹AgNO₃,100nM咪唑乙烟酸,

300mg l⁻¹特美汀;pH5.8)中。

[0213] 实验设计和结果:

[0214] 在3名研究者中进行五个随时间的实验,每个研究者最少一个重复(表卡诺拉油菜-1)。在实验1中,仅对于实验1用DsRed对照和BnGRF5构建体转化欧洲油菜下胚轴;在其它实验中,外植体用DsRed对照、AtGRF5和BnGRF5构建体转化。总共接种3,156个外植体,在16-22天后进行DsRed荧光评分。在这个时间点,外植体迅速地发育愈伤组织,尤其是在下胚轴的切断端。对于外植体,评分外植体上是否存在DsRed荧光,而不考虑单个外植体的大小、强度和频率。DsRed表达部分的频率是处理的转化效率的早期指标。

[0215] DsRed表达在含有AtGRF5或BnGRF5的构建体中比DsRed对照明显更强(图15)。与用DsRed对照转化的外植体相比,用AtGRF5或BnGRF5转化的外植体中具有DsRed表达芽的外植体的百分比显著更高(表7;图16)。在用DsRed对照构建体转化的外植体中,20.1%显示DsRed表达的芽,相较而言AtGRF5和GmGRF5分别为56.6%和58.5%。0数据表明在转化后21天,用AtGRF5或BnGRF5转化的欧洲油菜外植体比未用GRF5任一形式转化的外植体具有多3倍的转基因愈伤组织部分(统计学上显著, $\alpha=0.05$)。在处理(构建体)之间没有检测到研究者和实验之间的显著差异。

[0216] 表7.在接种后21天,转化的愈伤组织在用AtGRF5或BnGRF5转化的外植体上显著增加。显示了再生和DsRed发荧光愈伤组织的平均值和范围。显著不同(在 $\alpha=0.05$)的构建体后跟有不同的字母;¹=[(具有DsRed愈伤组织的外植体的数目/接种的外植体的总数{n}) $\times 100$]。

构建体	Exp.	研究者	重复	接种的外植体	外植体(%), 其具有 DsRed ¹	
					平均	范围
[0217] 对照	5	3	20	1202	20.1 a	13 - 28.7
AtGRF5	4	3	15	822	56.6 b	40.8 - 70.3
BnGRF5	5	3	20	1132	58.5 b	44.0 - 78.4

序列表

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 巴斯夫欧洲公司(BASF SE)

<120> 遗传修饰植物的再生

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Ile Arg Arg Ser Leu Asp Thr Ser Leu Val Ser Arg Leu Leu Pro His
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Gln Ser Leu Gly Trp Gly Cys Tyr Gln Met Gly Phe Gly Arg Lys Pro
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Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
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Asn Pro Ser Pro Thr Leu Ser Ser Ser Ser Ser Ser Asn Ser Ser Ser
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Asn Ser His Ser Leu Asp Tyr Pro Tyr Pro Ser Thr Ser Pro Lys Gln
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Gln Gln Gln Thr Leu His His Ala Ser Ala Leu Ser Leu His Gln Asn
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Lys Asp Phe Arg Tyr Phe Gln Gly Ile Gly Glu Arg Val Gly Gly Val
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Gly Glu Arg Thr Phe Phe Pro Glu Ala Ser Arg Ser Phe Gln Asp Ser
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Pro Tyr His His His Gln Gln Pro Leu Ala Thr Val Met Asn Asp Pro
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Tyr His His Cys Ser Thr Asp His Asn Lys Ile Asp His His His Thr
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Tyr Ser Ser Ser Ser Ser Ser Gln His Leu His His Asp His Asp His
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tttacagcaa cacaatggca agaactgaa catcaagctc taattataa gtatatggct 120

gctggtgttc ctattctctc tgatcttctt ttaccatca aacgtagtct tgactcttct 180

ctctctcca agctcttcc ttaccaacct tctcctttgg gatggaacc atatcaatg 240

gggtatggaa aaaagataga cccagaacca ggaaggtgca gaagaacaga tgggaaaaaa 300

tggagggtgt caaagaagc ataccagat tcaaagtact gtgagaggca catgcataga 360
 ggcaaaaacc gttcaagaaa gctctggaa tcaccttga ctacaactc aactactgtt 420
 tctaataaca acaataataa taataataat aataattctg ctgcaaattc atcactgaca 480
 gtagcagctg ctgctgctgc agcatctttg accaatcaat cactgcttaa taagaatcct 540
 tctctgttt caacttctct ctttctctt ccttctctg attcctctg taattctcac 600
 ctctttatc cccattcttc ttacaatcac aaggactata gggaaaggta ttatcaagga 660
 ttaaagagg aggtgggaga gcatgcattt ttacagaga gctcaggatc aagtatgaga 720
 ggtttttcag gttcatccat ggatgaaagt tggcaaattg gtggtggaag taacatagat 780
 catcatcaac aacaacaaca acaatcaaaa caaagtggg ggtaccctaa ttactgcag 840
 cagcttcaaa gtaatagtac aactagtaat aatggtacat cagcaaagca ggagaagcaa 900
 tgctacattt ggggaagaga ttttaactgt gatttatcaa tgaaggttga agaagaaaga 960
 gaaaacttct atgaaaaaac caccaccat ttctttgatg aatggcctat taaaagtgg 1020
 ggaagaggag gaagagattc tcatggcat gattcttct caactactca actttccata 1080
 tccattcctt ctctacttc tcatcatcat gacttttcc tcacaaattc tagggactcc 1140
 taa 1143

[0004]

<210> 4
 <211> 380
 <212> PRT
 <213> 甜菜
 <400> 4

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

Ser Lys Phe Pro Phe Thr Ala Thr Gln Trp Gln Glu Leu Glu His Gln
 20 25 30

Ala Leu Ile Tyr Lys Tyr Met Ala Ala Gly Val Pro Ile Pro Pro Asp
 35 40 45

Leu Leu Phe Thr Ile Lys Arg Ser Leu Asp Ser Ser Leu Ser Ser Lys
 50 55 60

Leu Phe Pro Tyr Gln Pro Ser Pro Leu Gly Trp Asn Pro Tyr Gln Met
 65 70 75 80

Gly Tyr Gly Lys Lys Ile Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr
 85 90 95

Asp Gly Lys Lys Trp Arg Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys
 100 105 110

Tyr Cys Glu Arg His Met His Arg Gly Lys Asn Arg Ser Arg Lys Pro
 115 120 125

Val Glu Ser Pro Leu Thr Thr Thr Ser Thr Thr Val Ser Asn Asn Asn
 130 135 140

Asn Asn Asn Asn Asn Asn Asn Asn Ser Ala Ala Asn Ser Ser Leu Thr
 145 150 155 160

Val Ala Ala Ala Ala Ala Ala Ala Ser Leu Thr Asn Gln Ser Leu Leu
 165 170 175

Asn Lys Asn Pro Ser Ser Val Ser Thr Ser Leu Phe Ser Leu Pro Ser
 180 185 190

Ser Asp Ser Ser Cys Asn Ser His Leu Leu Tyr Pro His Ser Ser Tyr
 195 200 205

Asn His Lys Asp Tyr Arg Glu Arg Tyr Tyr Gln Gly Leu Lys Glu Glu
 210 215 220

Val Gly Glu His Ala Phe Phe Thr Glu Ser Ser Gly Ser Ser Met Arg
 225 230 235 240

[0005]

Gly Phe Ser Gly Ser Ser Met Asp Glu Ser Trp Gln Ile Gly Gly Gly
 245 250 255

Ser Asn Ile Asp His His Gln Gln Gln Gln Gln Ser Lys Gln Ser
 260 265 270

Gly Gly Tyr Pro Asn Tyr Leu Gln Gln Leu Gln Ser Asn Ser Thr Thr
 275 280 285

Ser Asn Asn Gly Thr Ser Ala Lys Gln Glu Lys Gln Cys Tyr Ile Trp
 290 295 300

Gly Arg Asp Phe Asn Cys Asp Leu Ser Met Lys Val Glu Glu Glu Arg
 305 310 315 320

Glu Asn Phe His Glu Lys Thr Thr His His Phe Phe Asp Glu Trp Pro
 325 330 335

Ile Lys Ser Gly Gly Arg Gly Gly Arg Asp Ser Ser Trp His Asp Ser
 340 345 350

Ser Ser Thr Thr Gln Leu Ser Ile Ser Ile Pro Ser Ser Thr Ser His
 355 360 365

His His Asp Phe Phe Leu Thr Asn Ser Arg Asp Ser
370 375 380

<210> 5

<211> 1197

<212> DNA

<213> 人工序列

<220>

<223> ZmGRF5的cDNA

<400> 5

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acggcgtcgc agtggcagga gctggagcac caggcgtca tctacaagt cctggcgtcc 120

ggcaagccca tccctccta cctcatgcc ccgctccgc gcatcctga ctccgcctc 180

gccacgtcgc cgtcctcgc ctaccgccg caaccctcac tgggctggg ctgctcggg 240

atgggcttca cccggaaggc cgacgaggac ccggagccc ggcggtgcc ggcacggac 300

ggcaagaagt ggcgctgct caaggaggc taccggact ccaagtact cgagaagcac 360

atgcaccggg gcaagaacc ttcaagaaag cctgtgaaa tgtcctggc cagccggcc 420

ccggcggcgg cccccggc cgccacaacc gccaccgcca cctcatccc ggcgctcc 480

taccaccgcc cggcccacga cgccacggc tctccgtacc acgcgctga tggaggcggc 540

[0006]

ggcgggcg gcgtagccc ttactcggc tcggcacgcc caggagcaac cggaggcggc 600

ggcgctacc accacgcga gcatgtgagc ccctccacc tccacctga gaccaccac 660

ccgacccgc cgccgccta caactactc gccgaccaga gggactacg gtacggcac 720

gcgccgcca aggaggtcgg cgagcacgcc ttctctcgg acggcgggg cgagcgggtc 780

gaccggcagg ccgcggggg gcagtggcag ttcaggcagc tcggggtgga gacgaagccg 840

ggccccacgc cgctgtccc cgtcggggg tacgggcacg gcgcggcgtc gccgtacggc 900

gtggagatgg gcaaggacga cgacgagcag gaggagagg gccgacgca ctgctcgtt 960

cttgagcgg acctcggct ggagcggcc tcgtcgggccc atggccatgg ccatgacat 1020

gacgacggc ccgcccgca gaagcggctc cggccctct tcgacgagt gccgcaccag 1080

aagggggaca aggccgggtc gtggatggg ctcgacggc agacgcagct ctccatgtc 1140

atccccatgg ccgccaccga cctccccgtc acctcccgt tccgtaacga cgagtga 1197

<210> 6

<211> 398

<212> PRT

<213> 玉蜀黍

<400> 6

Met Met Met Met Ser Ser Gly Arg Ala Gly Gly Ala Thr Ala Gly

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 Arg Tyr Pro Phe Thr Ala Ser Gln Trp Gln Glu Leu Glu His Gln Ala
 20 25 30
 Leu Ile Tyr Lys Cys Leu Ala Ser Gly Lys Pro Ile Pro Ser Tyr Leu
 35 40 45
 Met Pro Pro Leu Arg Arg Ile Leu Asp Ser Ala Leu Ala Thr Ser Pro
 50 55 60
 Ser Leu Ala Tyr Pro Pro Gln Pro Ser Leu Gly Trp Gly Cys Phe Gly
 65 70 75 80
 Met Gly Phe Thr Arg Lys Ala Asp Glu Asp Pro Glu Pro Gly Arg Cys
 85 90 95
 Arg Arg Thr Asp Gly Lys Lys Trp Arg Cys Ser Lys Glu Ala Tyr Pro
 100 105 110
 Asp Ser Lys Tyr Cys Glu Lys His Met His Arg Gly Lys Asn Arg Ser
 115 120 125
 [0007] Arg Lys Pro Val Glu Met Ser Leu Ala Thr Pro Ala Pro Ala Pro Ala
 130 135 140
 Pro Ala Ala Ala Thr Thr Ala Thr Ala Thr Ser Ser Pro Ala Pro Ser
 145 150 155 160
 Tyr His Arg Pro Ala His Asp Ala Thr Pro Ser Pro Tyr His Ala Leu
 165 170 175
 Tyr Gly Gly Gly Gly Gly Gly Gly Gly Ser Pro Tyr Ser Ala Ser Ala
 180 185 190
 Arg Pro Gly Ala Thr Gly Gly Gly Gly Ala Tyr His His Ala Gln His
 195 200 205
 Val Ser Pro Phe His Leu His Leu Glu Thr Thr His Pro His Pro Pro
 210 215 220
 Pro Pro Tyr Asn Tyr Ser Ala Asp Gln Arg Asp Tyr Ala Tyr Gly His
 225 230 235 240
 Ala Ala Ala Lys Glu Val Gly Glu His Ala Phe Phe Ser Asp Gly Ala
 245 250 255
 Gly Glu Arg Val Asp Arg Gln Ala Ala Ala Gly Gln Trp Gln Phe Arg

260 265 270
 Gln Leu Gly Val Glu Thr Lys Pro Gly Pro Thr Pro Leu Phe Pro Val
 275 280 285
 Ala Gly Tyr Gly His Gly Ala Ala Ser Pro Tyr Gly Val Glu Met Gly
 290 295 300
 Lys Asp Asp Asp Glu Gln Glu Glu Arg Arg Arg Gln His Cys Phe Val
 305 310 315 320
 Leu Gly Ala Asp Leu Arg Leu Glu Arg Pro Ser Ser Gly His Gly His
 325 330 335
 Gly His Asp His Asp Asp Ala Ala Ala Ala Gln Lys Pro Leu Arg Pro
 340 345 350
 Phe Phe Asp Glu Trp Pro His Gln Lys Gly Asp Lys Ala Gly Ser Trp
 355 360 365
 Met Gly Leu Asp Gly Glu Thr Gln Leu Ser Met Ser Ile Pro Met Ala
 370 375 380

[0008] Ala Thr Asp Leu Pro Val Thr Ser Arg Phe Arg Asn Asp Glu
 385 390 395

<210> 7
 <211> 1245
 <212> DNA
 <213> 人工序列
 <220>
 <223> TaGRF5的cDNA
 <400> 7
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 ccgttcacgg cgacgcagtg gcaggagctt gagcaccagg cactcatcta caagtacatg 120
 gcctccggcg tgccatccc ctccgacctc ctctcccgc tccgccgcag cttctctctc 180
 gactccgccc tcgccacctc ccctccctc gccttcctc cccaggccgc acttggtggg 240
 ggttgctttg gcatgggggtt cgccggaag gcggaggacc cggagccggg gcggtgccgg 300
 cggacggacg gcaagaagtg gcgctgctcc aaggaggcgt acccggactc caagtactgc 360
 gagaagcaca tgaccgcgg caagaacctg tcaagaaagc ctgtgaaat gtccttgccc 420
 agccccccgc cgccgccttc ctctcggccc tctctctct cctccaactg cactccgcc 480
 gtcaactgctg ccaccaccac ctctcccc gcgccgtct accaccgcca cgccgccgcg 540
 actcaagaca cgacgccta ccacgcgctc tacggcggcc cctactctc cgccggccc 600

cagcagcag ctagcgcta ccaccagcc gcgagggtca gcccgttcca cctgcacctc 660
 gacaccacc acccgaccc gccgccgtcc tactactcca gcatggacca cagcaaggac 720
 agctacgctt acgggcacag cgtaaggag gtgcacggcg gcggcgagca cgccttcttc 780
 tcctccgagc tcaccaccga caggaccat caccaccacc accatcagca ccaacaccac 840
 gtagcgcggc gggcaacgg ccagtggcag ttcaagcagc tcggcggcat ggagccgaag 900
 cagcataacc caacgtcgtc cttccccggc tgcggcggct acggcaacaa cgcggcctac 960
 gccatcgacc tgtccagcaa agaagaggac gaggagaagg agaggcggca gcagcagcag 1020
 cactgcttc tgtggggcgc cgacctgagg ctgcacaagc cgtcgtcggg gcacggcgac 1080
 tccgccgacc agaagcctct ccggcccttc ttcgacgagt ggccgcacga gaagaccggg 1140
 agcaaggggt cgtggatggg gctcgagggg gagacgcagc tctcatctc catcgccaac 1200
 gaactcccca tcaccaccac ctcccgtac caccatggtg aatga 1245

<210> 8
 <211> 414
 <212> PRT
 <213> 普通小麦

<400> 8

[0009]

Met Met Met Met Gly Gly Arg Ala Gly Ala Gly Gly Val Gly Ala Gly
 1 5 10 15

Gly Gly Arg Cys Pro Phe Thr Ala Thr Gln Trp Gln Glu Leu Glu His
 20 25 30

Gln Ala Leu Ile Tyr Lys Tyr Met Ala Ser Gly Val Pro Ile Pro Ser
 35 40 45

Asp Leu Leu Leu Pro Leu Arg Arg Ser Phe Leu Leu Asp Ser Ala Leu
 50 55 60

Ala Thr Ser Pro Ser Leu Ala Phe Pro Pro Gln Ala Ala Leu Gly Trp
 65 70 75 80

Gly Cys Phe Gly Met Gly Phe Gly Arg Lys Ala Glu Asp Pro Glu Pro
 85 90 95

Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg Cys Ser Lys Glu
 100 105 110

Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met His Arg Gly Lys
 115 120 125

Asn Arg Ser Arg Lys Pro Val Glu Met Ser Leu Ala Thr Pro Pro Pro
 130 135 140

Pro Pro Ser Ser Ser Ala Ser Ser Ser Ser Ser Asn Val His Ser Ala
145 150 155 160

Val Asn Val Ala Thr Thr Thr Ser Ser Pro Ala Pro Ser Tyr His Arg
165 170 175

His Ala Ala Ala Thr His Asp Thr Thr Pro Tyr His Ala Leu Tyr Gly
180 185 190

Gly Pro Tyr Ser Ser Ala Gly Arg Gln Gln His Ala Ser Ala Tyr His
195 200 205

His Ala Ala Gln Val Ser Pro Phe His Leu His Leu Asp Thr Thr His
210 215 220

Pro His Pro Pro Pro Ser Tyr Tyr Ser Ser Met Asp His Ser Lys Asp
225 230 235 240

Ser Tyr Ala Tyr Gly His Ser Val Lys Glu Val His Gly Gly Gly Glu
245 250 255

His Ala Phe Phe Ser Ser Asp Val Thr Thr Asp Arg Asp His His His
260 265 270

[0010]

His His His Gln His Gln His His Ala Ser Ala Gly Gly Asn Gly Gln
275 280 285

Trp Gln Phe Lys Gln Leu Gly Gly Met Glu Pro Lys Gln His Asn Pro
290 295 300

Thr Ser Leu Phe Pro Gly Cys Gly Gly Tyr Gly Asn Asn Ala Ala Tyr
305 310 315 320

Ala Ile Asp Leu Ser Ser Lys Glu Glu Asp Glu Glu Lys Glu Arg Arg
325 330 335

Gln Gln Gln Gln His Cys Phe Leu Leu Gly Ala Asp Leu Arg Leu Asp
340 345 350

Lys Pro Ser Ser Gly His Gly Asp Ser Ala Asp Gln Lys Pro Leu Arg
355 360 365

Pro Phe Phe Asp Glu Trp Pro His Glu Lys Thr Gly Ser Lys Gly Ser
370 375 380

Trp Met Gly Leu Glu Gly Glu Thr Gln Leu Ser Ile Ser Ile Ala Asn
385 390 395 400

Glu Leu Pro Ile Thr Thr Thr Ser Arg Tyr His His Gly Glu
 405 410

<210> 9
 <211> 1170
 <212> DNA
 <213> 人工序列

<220>
 <223> BnGRF5的cDNA

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 caatggcaag aactggagaa tcaagccta attacaagt acatggtctc aggagttcct 120
 gtccacctg agtcatctt ctccattaga agaagcttg actcttctt ggtctctaga 180
 ctctccctc accaatccat tgggtgggga tgctatcaga tggggtttg tagaaaacca 240
 gatccagaac caggaaggtg cagaagaaca gatggtgaaga aatggagatg ctcaagagaa 300
 gcataccag attcaaagta ctgtgaaaa cacatgcaca gaggaaggaa ccgtgccaga 360
 aaatctattg atcagaatca gacaactgct ccttaacat caccatctct ctctttcccc 420
 aacaacaaca acccaagccc tacctgtct tcttctctct ctactattc agctgcttct 480
 tcatctctt ccattgatgc ttacagtaat atcaatagc ttgggttgg tagtagtaac 540
 [0011] agtagagggt acttcaaca ccattccctt gactatcctt atcctttgtc ctcacctaaa 600
 cagcaacaac aacagactct tcatcatgct tctgcttgt ctctcacca aaacacatct 660
 actgattctc agtcaatgc cttagcttct gcaactgacc ataaagactt cagatacttt 720
 caagggattg gggagagagc tggagtggga gctggggaga ggacttttt tccagaagct 780
 tctagaagct ttcaagattc tccataccat caccaacgac cgtagcaac agtaatgaat 840
 gaccctgacc actctggtac tgatcataag gttgatcatc atcatcacac atactcatcc 900
 gtatcatcat catctcagca tgatcaagat catcatcgac aacaacagca gcaatgtttt 960
 gttatgggcg ctgacatggt caacaaccc acaagaactg tcttcgaaa cacatcgagg 1020
 caagatcatc aagaagagga ggagaaagat tcatcagaaa caaagaagtc tctacatcat 1080
 ttcttgggtg aggactgggc gcagaacaaa aacaattcag attcttggct tgacctttct 1140
 tccattcaa gactcgacac tggtagttga 1170

<210> 10
 <211> 389
 <212> PRT
 <213> 欧洲油菜

<400> 10

Met Met Ser Leu Ser Gly Asn Gly Gly Arg Thr Ile Glu Arg Pro Pro

1 5 10 15
 Phe Thr Pro Thr Gln Trp Gln Glu Leu Glu Asn Gln Ala Leu Ile Tyr
 20 25 30
 Lys Tyr Met Val Ser Gly Val Pro Val Pro Pro Glu Leu Ile Phe Ser
 35 40 45
 Ile Arg Arg Ser Leu Asp Ser Ser Leu Val Ser Arg Leu Leu Pro His
 50 55 60
 Gln Ser Ile Gly Trp Gly Cys Tyr Gln Met Gly Phe Gly Arg Lys Pro
 65 70 75 80
 Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 85 90 95
 Cys Ser Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 100 105 110
 His Arg Gly Arg Asn Arg Ala Arg Lys Ser Ile Asp Gln Asn Gln Thr
 115 120 125
 [0012] Thr Ala Pro Leu Thr Ser Pro Ser Leu Ser Phe Pro Asn Asn Asn Asn
 130 135 140
 Pro Ser Pro Thr Leu Ser Ser Ser Ser Ser Thr Tyr Ser Ala Ala Ser
 145 150 155 160
 Ser Ser Pro Ser Ile Asp Ala Tyr Ser Asn Ile Asn Arg Leu Gly Val
 165 170 175
 Gly Ser Ser Asn Ser Arg Gly Tyr Phe Asn Asn His Ser Leu Asp Tyr
 180 185 190
 Pro Tyr Pro Leu Ser Ser Pro Lys Gln Gln Gln Gln Gln Thr Leu His
 195 200 205
 His Ala Ser Ala Leu Ser Leu His Gln Asn Thr Ser Thr Asp Ser Gln
 210 215 220
 Phe Asn Ala Leu Ala Ser Ala Thr Asp His Lys Asp Phe Arg Tyr Phe
 225 230 235 240
 Gln Gly Ile Gly Glu Arg Ala Gly Val Gly Ala Gly Glu Arg Thr Phe
 245 250 255
 Phe Pro Glu Ala Ser Arg Ser Phe Gln Asp Ser Pro Tyr His His Gln

260 265 270
 Arg Pro Leu Ala Thr Val Met Asn Asp Pro Tyr His Ser Gly Thr Asp
 275 280 285

 His Lys Val Asp His His His His Thr Tyr Ser Ser Val Ser Ser Ser
 290 295 300

 Ser Gln His Asp Gln Asp His His Arg Gln Gln Gln Gln Cys Phe
 305 310 315 320

 Val Met Gly Ala Asp Met Phe Asn Lys Pro Thr Arg Thr Val Phe Ala
 325 330 335

 Asn Thr Ser Arg Gln Asp His Gln Glu Glu Glu Glu Lys Asp Ser Ser
 340 345 350

 Glu Thr Lys Lys Ser Leu His His Phe Phe Gly Glu Asp Trp Ala Gln
 355 360 365

 Asn Lys Asn Asn Ser Asp Ser Trp Leu Asp Leu Ser Ser His Ser Arg
 370 375 380

[0013] Leu Asp Thr Gly Ser
385

<210> 11
 <211> 1173
 <212> DNA
 <213> 人工序列

<220>
 <223> BrGRF5的cDNA

<400> 11
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 caatggcaag aactggagaa tcaagcccta attacaagt acatggtctc aggagttcct 120
 gtcccacctg agctcatctt ctccattaga agaagcttgg actcttcctt ggtctctaga 180
 ctctccctc accaatccat tgggtgggga tgctatcaga tggggtttg tagaaaacca 240
 gatccagaac caggaaggtg cagaagaaca gatgtaaga aatggagatg ctcaagagaa 300
 gcacatccag attctaagta ctgtgaaaaa cacatgcaca gaggaaggaa ccgtgccaga 360
 aaatctattg atcagaatca gacaactgct cctttaacat caccatctct ctcttcccc 420
 aacaacaaca acccaagccc tacctgtct tcttctctct ctacttattc agcttctct 480
 tcatctctt ccatgatgc ttacagtaat atcaataggc ttggtgttgg taatagtaac 540
 agtagaggtt actcaacaa ccattccctt gactatcctt atcctttgtc ctcacctaaa 600

cagcaacaac aacaacagac tcttcatcat gcttctgctt tgtctcttca ccaaaacgca 660
 tctactgctt ctcaagtcaa tgccttagct tctgcaactg accataaaga cttcagatac 720
 tttaagggga ttggggagag agttggagtt ggagctgggg agaggacttt tttccagaa 780
 gcttctagaa gctttcaaga ttctccatac catcaccaac aaccgtagc tacagtaatg 840
 aatgaccctg tccactctgg tactgatcat aaggttgatc atcagcatca cacatactca 900
 tccgatcat catcatctca gcatgatcaa gatcatcatc gacaacaaca gcagcaatgt 960
 ttgttatgg gcgctgacat gttaacaaaa cccacaagaa ctgtcttcgc aaactcatct 1020
 agacaagatc atcaagaaga ggaggagaaa gattcatcag aaacaagaa gtctctacat 1080
 catttcttg gtgaggactg ggcacagaac aaaaacagtt cagattcttg gcttgacctt 1140
 tcttccatt caagactgga cactgtagt tga 1173

<210> 12
 <211> 390
 <212> PRT
 <213> 芫菁
 <400> 12

Met Met Ser Leu Ser Gly Asn Gly Gly Arg Thr Ile Glu Arg Pro Pro
 1 5 10 15

[0014]

Phe Thr Pro Thr Gln Trp Gln Glu Leu Glu Asn Gln Ala Leu Ile Tyr
 20 25 30

Lys Tyr Met Val Ser Gly Val Pro Val Pro Pro Glu Leu Ile Phe Ser
 35 40 45

Ile Arg Arg Ser Leu Asp Ser Ser Leu Val Ser Arg Leu Leu Pro His
 50 55 60

Gln Ser Ile Gly Trp Gly Cys Tyr Gln Met Gly Phe Gly Arg Lys Pro
 65 70 75 80

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 85 90 95

Cys Ser Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 100 105 110

His Arg Gly Arg Asn Arg Ala Arg Lys Ser Ile Asp Gln Asn Gln Thr
 115 120 125

Thr Ala Pro Leu Thr Ser Pro Ser Leu Ser Phe Pro Asn Asn Asn Asn
 130 135 140

Pro Ser Pro Thr Leu Ser Ser Ser Ser Ser Thr Tyr Ser Ala Ser Ser
145 150 155 160

Ser Ser Pro Ser Ile Asp Ala Tyr Ser Asn Ile Asn Arg Leu Gly Val
 165 170 175

Gly Asn Ser Asn Ser Arg Gly Tyr Phe Asn Asn His Ser Leu Asp Tyr
 180 185 190

Pro Tyr Pro Leu Ser Ser Pro Lys Gln Gln Gln Gln Gln Thr Leu
 195 200 205

His His Ala Ser Ala Leu Ser Leu His Gln Asn Ala Ser Thr Ala Ser
 210 215 220

Gln Phe Asn Ala Leu Ala Ser Ala Thr Asp His Lys Asp Phe Arg Tyr
225 230 235 240

Phe Gln Gly Ile Gly Glu Arg Val Gly Val Gly Ala Gly Glu Arg Thr
 245 250 255

Phe Phe Pro Glu Ala Ser Arg Ser Phe Gln Asp Ser Pro Tyr His His
 260 265 270

[0015]

Gln Gln Pro Leu Ala Thr Val Met Asn Asp Pro Phe His Ser Gly Thr
 275 280 285

Asp His Lys Val Asp His Gln His His Thr Tyr Ser Ser Val Ser Ser
 290 295 300

Ser Ser Gln His Asp Gln Asp His His Arg Gln Gln Gln Gln Cys
305 310 315 320

Phe Val Met Gly Ala Asp Met Phe Asn Lys Pro Thr Arg Thr Val Phe
 325 330 335

Ala Asn Ser Ser Arg Gln Asp His Gln Glu Glu Glu Lys Asp Ser
 340 345 350

Ser Glu Thr Lys Lys Ser Leu His His Phe Phe Gly Glu Asp Trp Ala
 355 360 365

Gln Asn Lys Asn Ser Ser Asp Ser Trp Leu Asp Leu Ser Ser His Ser
 370 375 380

Arg Leu Asp Thr Gly Ser
385 390

<210> 13
 <211> 1173
 <212> DNA
 <213> 人工序列

<220>
 <223> BoGRF5的cDNA

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 caatggcaag aactggagaa tcaagcccta atttacaagt acatggtctc aggagttcct 120
 gtcccacctg agctcatctt ctccattaga agaagcttgg actcttcctt ggtctctaga 180
 ctctccctc accaatccat tgggtgggga tgctatcaga tggggtttgg tagaaaacca 240
 gatccagaac caggaaggtg cagaagaaca gatggtaaga aatggagatg ctcaagagaa 300
 gcataccctg attcaaagta ctgtgaaaaa cacatgcaca gaggaaggaa cctgtccaga 360
 aaatctattg atcagaatca gacaactgct cctctaactt caccatctct ctcttcccc 420
 aacaacaaca acccaagccc tacctgtgcc tcttctctct ctactattc agcttcttct 480
 tcattctctt ccattgatgc ttacagtaat atcaataggc ttggtgttgg tagtagtaac 540
 agtagagggt acttcaacaa ccattccctt gagtatcctt atcctttgtc ctcacctaaa 600
 cagcaacaac aacaacagac tcttcatcat gtttctgctt tgtctcttca ccaaacaca 660
 [0016] tctactgctt ctcagttcaa tgccttagct tctgcaaccg accataaaga cttcagatat 720
 tttaagggga ttggggagag agttggagtt ggagctgggg agagaacttt tttccagaa 780
 gcttctagaa gctttcaaga ttctccatac catcaccaac aaccgtagc aacagtaatg 840
 agtgaccctg accactctgg tactgatcat aagggtgac atcatcctca cacatactca 900
 tccgtatcat catcatctca gcatgatcaa gatcatcctc gacaacaaca gcagcaatgt 960
 ttgttatgg gcgctgacat gttaacaaa cccacaagaa ctggcttcgc aaacacatcg 1020
 aggcaagatc atcaagaaga ggaggagaaa gattcatcag aaacaagaa gtctctacat 1080
 catttctttg gtgaggactg ggcgcagaac aaaaacaatt cagattcttg gcttgacctt 1140
 tcttccatt caagactcga cactgtagt tga 1173

<210> 14
 <211> 390
 <212> PRT
 <213> 甘蓝

<400> 14

Met Met Ser Leu Ser Gly Asn Gly Gly Arg Thr Ile Glu Arg Pro Pro
 1 5 10 15

Phe Thr Pro Thr Gln Trp Gln Glu Leu Glu Asn Gln Ala Leu Ile Tyr
 20 25 30

Lys Tyr Met Val Ser Gly Val Pro Val Pro Pro Glu Leu Ile Phe Ser
 35 40 45

Ile Arg Arg Ser Leu Asp Ser Ser Leu Val Ser Arg Leu Leu Pro His
 50 55 60

Gln Ser Ile Gly Trp Gly Cys Tyr Gln Met Gly Phe Gly Arg Lys Pro
 65 70 75 80

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 85 90 95

Cys Ser Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 100 105 110

His Arg Gly Arg Asn Arg Ala Arg Lys Ser Ile Asp Gln Asn Gln Thr
 115 120 125

Thr Ala Pro Leu Thr Ser Pro Ser Leu Ser Phe Pro Asn Asn Asn Asn
 130 135 140

Pro Ser Pro Thr Leu Ser Ser Ser Ser Ser Thr Tyr Ser Ala Ser Ser
 145 150 155 160

[0017]

Ser Ser Pro Ser Ile Asp Ala Tyr Ser Asn Ile Asn Arg Leu Gly Val
 165 170 175

Gly Ser Ser Asn Ser Arg Gly Tyr Phe Asn Asn His Ser Leu Glu Tyr
 180 185 190

Pro Tyr Pro Leu Ser Ser Pro Lys Gln Gln Gln Gln Gln Thr Leu
 195 200 205

His His Ala Ser Ala Leu Ser Leu His Gln Asn Thr Ser Thr Ala Ser
 210 215 220

Gln Phe Asn Ala Leu Ala Ser Ala Thr Asp His Lys Asp Phe Arg Tyr
 225 230 235 240

Phe Gln Gly Ile Gly Glu Arg Val Gly Val Gly Ala Gly Glu Arg Thr
 245 250 255

Phe Phe Pro Glu Ala Ser Arg Ser Phe Gln Asp Ser Pro Tyr His His
 260 265 270

Gln Gln Pro Leu Ala Thr Val Met Ser Asp Pro Tyr His Ser Gly Thr
 275 280 285

Asp His Lys Val Asp His His Pro His Thr Tyr Ser Ser Val Ser Ser
 290 295 300

Ser Ser Gln His Asp Gln Asp His His Arg Gln Gln Gln Gln Cys
 305 310 315 320

Phe Val Met Gly Ala Asp Met Phe Asn Lys Pro Thr Arg Thr Gly Phe
 325 330 335

Ala Asn Thr Ser Arg Gln Asp His Gln Glu Glu Glu Lys Asp Ser
 340 345 350

Ser Glu Thr Lys Lys Ser Leu His His Phe Phe Gly Glu Asp Trp Ala
 355 360 365

Gln Asn Lys Asn Asn Ser Asp Ser Trp Leu Asp Leu Ser Ser His Ser
 370 375 380

Arg Leu Asp Thr Gly Ser
 385 390

<210> 15
 <211> 1197
 <212> DNA
 <213> 人工序列

<220>
 <223> RsGRF5的cDNA

<400> 15
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 caatggcaag aactggagag tcaagcccta attacaagt acatggtctc aggggttctc 120
 gtccacctg agctcatct ctccattaga agaagcttg actcttctt ggtctctaga 180
 ctctccctc accaatctct tggctgggga tgctatcaga tgggatttg tagaaaacca 240
 gatccggaac caggaagtg cagaagaaca gatggaaga aatggagatg ctcaagagaa 300
 gcataccag attcaaagta ctgtgaaaa cacatgcaca gaggaaggaa ccgtgccagg 360
 aaatctattg atcagaatca gacaactgct ctttgacat caccatctct ctctttcccc 420
 aacaaccaa gcctacctt gtcttcttct tcttctgct ctactattc tgctgcatct 480
 tcatctctt ctattgatgc tttcagtaat atcaataggc ctggtgttg tagtagcatc 540
 agcagaggtt acttcaacaa ccattccctt gactatcctt atccttgtc ctacacataa 600
 caacaacagc aacaacagac tcttcatcat gcttctgctt tgtcacttca ccaaaacaca 660
 tctactgctt ctcagttcaa tgtcttagcc tcttcaactg accataaaga cttcagatac 720
 tttaagga ttggggagag agttggagtt ggagttgggg agagaacatt tttccagaa 780

[0018]

gcttctagaa gctttcaaga ttctccatac catcaccaac aaccgttggc aacagtaatg 840
aatgaccctg accactgtag tactgatcac aagggtgatc atcatcacac atactcatcc 900
gcatcatcat cgtctcaaca tcaacatgat caagatcatg agcatagaca acaacagcag 960
caatgtttcg ttatgggtgc tgacatgttc aacaaacca cacgaactgt cttcgcaaac 1020
acatcgagac aagatcaaga agaggaggag aaagattcat ctgaaacca gaagtctcta 1080
catcatttct ttggtgagga ctgggacgag aacaagaaca gttcagattc ttggcttgat 1140
ctttctccc actcaagact cgaccctggt agtaacctac attctgatct atgctaa 1197

<210> 16

<211> 398

<212> PRT

<213> 萝卜

<400> 16

Met Met Ser Leu Ser Gly Asn Gly Gly Arg Thr Ile Glu Arg Pro Pro
1 5 10 15

Phe Thr Pro Thr Gln Trp Gln Glu Leu Glu Ser Gln Ala Leu Ile Tyr
20 25 30

Lys Tyr Met Val Ser Gly Val Pro Val Pro Pro Glu Leu Ile Phe Ser
35 40 45

[0019]

Ile Arg Arg Ser Leu Asp Ser Ser Leu Val Ser Arg Leu Leu Pro His
50 55 60

Gln Ser Leu Gly Trp Gly Cys Tyr Gln Met Gly Phe Gly Arg Lys Pro
65 70 75 80

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
85 90 95

Cys Ser Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
100 105 110

His Arg Gly Arg Asn Arg Ala Arg Lys Ser Ile Asp Gln Asn Gln Thr
115 120 125

Thr Ala Pro Leu Thr Ser Pro Ser Leu Ser Phe Pro Asn Asn Pro Ser
130 135 140

Pro Thr Leu Ser Ser Ser Ser Ala Ser Thr Tyr Ser Ala Ala Ser
145 150 155 160

Ser Ser Pro Ser Ile Asp Ala Phe Ser Asn Ile Asn Arg Pro Gly Val
165 170 175

Gly Ser Ser Ile Ser Arg Gly Tyr Phe Asn Asn His Ser Leu Asp Tyr
 180 185 190

Pro Tyr Pro Leu Ser Ser Pro Lys Gln Gln Gln Gln Gln Thr Leu
 195 200 205

His His Ala Ser Ala Leu Ser Leu His Gln Asn Thr Ser Thr Ala Ser
 210 215 220

Gln Phe Asn Val Leu Ala Ser Ser Thr Asp His Lys Asp Phe Arg Tyr
 225 230 235 240

Phe Gln Gly Ile Gly Glu Arg Val Gly Val Gly Val Gly Glu Arg Thr
 245 250 255

Phe Phe Pro Glu Ala Ser Arg Ser Phe Gln Asp Ser Pro Tyr His His
 260 265 270

Gln Gln Pro Leu Ala Thr Val Met Asn Asp Pro Tyr His Cys Ser Thr
 275 280 285

Asp His Lys Val Asp His His His Thr Tyr Ser Ser Ala Ser Ser Ser
 290 295 300

[0020]

Ser Gln His Gln His Asp Gln Asp His Glu His Arg Gln Gln Gln Gln
 305 310 315 320

Gln Cys Phe Val Met Gly Ala Asp Met Phe Asn Lys Pro Thr Arg Thr
 325 330 335

Val Phe Ala Asn Thr Ser Arg Gln Asp Gln Glu Glu Glu Lys Asp
 340 345 350

Ser Ser Glu Thr Lys Lys Ser Leu His His Phe Phe Gly Glu Asp Trp
 355 360 365

Ala Gln Asn Lys Asn Ser Ser Asp Ser Trp Leu Asp Leu Ser Ser His
 370 375 380

Ser Arg Leu Asp Pro Gly Ser Asn Leu His Ser Asp Leu Cys
 385 390 395

<210> 17

<211> 1182

<212> DNA

<213> 人工序列

<220>

<223> SbGRF5的cDNA

<400> 17

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aagccatcc  cgctctacct catgccgccc ctccgccga tctcgactc cgccctcgcc    180
acgtcggcgt ccctcgctt cccgcccaa ccctcgttg ggtggggctg ttcgggatg    240
ggcttcagca ggaagcccga cgaggaccgg gagcccggcc ggtgccggcg gacggacggc    300
aagaagtggc gctgctcaa ggaggcgtac ccggactcca agtactgca gaagcacatg    360
caccggggca agaaccgtc aagaagcct gtgaaatgt ccttgccac accggcgccg    420
gcctctcggg tgcctccgc cacaagcgc acagccggc cgcgccgcg caccaccacc    480
acctcgtgc cagcaccgtc ctaccgcca gcgccacct cgacgacgc ctgccgtac    540
cacgcgctgt acggcggcgg cagtcctac tcggcgtcgg cgcgtcccgc cgttgcccc    600
ggccccacc atcatccgc gcaggtgagc ccctccacc tccacctga gaccaccac    660
ccgacccgc cgccgtcta ctactccga gaccagggg actacgcga cgggcacgc    720
accaaggagg tcgtcggcga gcacgcctt ttctccgat gcgcggcga gcgggaccgc    780
cagcatgccg cggccagtg gcagttcaag cagctcggg tggacacga gccgagcccc    840
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ttcgttctg gtgccacct gcggtggag cggccgtcgt cgggcatga cgccgccacc   1020
gcgcagaagc cgctccggc cttcttgac gaggggcgc acgagaaggg aaacaagggt   1080
gggcatgga tggggcttg cggcgagacg cagctctcca tgcctatccc catggccgcc   1140
agcgacctcc ccgtcacct cgcctaccgt aatgatgagt ga                    1182
    
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<210> 18

<211> 393

<212> PRT

<213> 两色蜀黍

<400> 18

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

Tyr Pro Phe Thr Ala Ser Gln Trp Gln Glu Leu Glu His Gln Ala Leu
 20 25 30

Ile Tyr Lys Cys Leu Ala Ser Gly Lys Pro Ile Pro Ser Tyr Leu Met
 35 40 45

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

50 55 60
 Leu Ala Phe Pro Pro Gln Pro Ser Leu Gly Trp Gly Cys Phe Gly Met
 65 70 75 80
 Gly Phe Ser Arg Lys Pro Asp Glu Asp Pro Glu Pro Gly Arg Cys Arg
 85 90 95
 Arg Thr Asp Gly Lys Lys Trp Arg Cys Ser Lys Glu Ala Tyr Pro Asp
 100 105 110
 Ser Lys Tyr Cys Glu Lys His Met His Arg Gly Lys Asn Arg Ser Arg
 115 120 125
 Lys Pro Val Glu Met Ser Leu Ala Thr Pro Ala Pro Ala Ser Ala Val
 130 135 140
 Ser Ser Ala Thr Ser Ala Thr Ala Ala Ala Ala Ala Thr Thr Thr
 145 150 155 160
 Thr Ser Ser Pro Ala Pro Ser Tyr Arg Pro Ala Pro Thr Ser His Asp
 165 170 175
 [0022] Ala Ser Pro Tyr His Ala Leu Tyr Gly Gly Gly Ser Pro Tyr Ser Ala
 180 185 190
 Ser Ala Arg Pro Ala Gly Gly Pro Gly Pro Tyr His His Pro Ala Gln
 195 200 205
 Val Ser Pro Phe His Leu His Leu Glu Thr Thr His Pro His Pro Pro
 210 215 220
 Pro Ser Tyr Tyr Ser Val Asp Gln Arg Asp Tyr Ala Tyr Gly His Ala
 225 230 235 240
 Thr Lys Glu Val Val Gly Glu His Ala Phe Phe Ser Asp Gly Ala Ala
 245 250 255
 Glu Arg Asp Arg Gln His Ala Ala Gly Gln Trp Gln Phe Lys Gln Leu
 260 265 270
 Gly Met Asp Thr Lys Pro Ser Pro Thr Ser Leu Phe Pro Val Ala Gly
 275 280 285
 Tyr Gly Asn Gly Ala Gly Ala Ser Pro Tyr Gly Val Asp Leu Gly Ala
 290 295 300
 Lys Glu Asp Asp Glu Glu Glu Arg Arg Arg Gln Gln Gln Gln His Cys

305 310 315 320
 Phe Val Leu Gly Ala Asp Leu Arg Leu Glu Arg Pro Ser Ser Gly His
 325 330 335
 Asp Ala Ala Thr Ala Gln Lys Pro Leu Arg Pro Phe Phe Asp Glu Trp
 340 345 350
 Pro His Glu Lys Gly Asn Lys Gly Gly Ser Trp Met Gly Leu Asp Gly
 355 360 365
 Glu Thr Gln Leu Ser Met Ser Ile Pro Met Ala Ala Ser Asp Leu Pro
 370 375 380
 Val Thr Ser Arg Tyr Arg Asn Asp Glu
 385 390

<210> 19
 <211> 1002
 <212> DNA
 <213> 人工序列
 <220>
 <223> HaGRF5的cDNA

[0023]

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 atgcctatcc cccctgatct gcttttcacc atcaaaacaa gtctagattc ttctacaaag 180
 ctctctcttc accaccagcc acctcatccc tcttcaattg gatggaactg ctccagatg 240
 ggatttggga gaaaaataga tccagaacca ggaagatgca gaagaacaga tggtaaagaaa 300
 tggaggtggt caaaagaagc ctatctgat tcaaaatact gtgaaaggca catgcacaga 360
 ggcagaaacc gttcaagaaa gcctgtggaa gtcaaatgt cgtaacacc aacacccaaa 420
 acaccaccaa ccgccattcc aatgatccca tcttcatct gtaccaaatc ccaaattac 480
 ccttctccca attcacatcc actctcttct tcttctctg actattacca taataataac 540
 accaccacc ttcatctta ttctagacct tcttctaag ttttcacaca agaccacttc 600
 ttgttgatt ctagccata catgagaaag gggatggga tgaagagggt gatagatgag 660
 cactcatttt ttcagaatc ttctggaacc atcaagactg attcttgga attggaacca 720
 ctggctatga acaattcatc ctcaaagcaa acaacttttt ctgattatca tcaaaacaga 780
 tactcatatc aacaacagca agatccaggc tattatgatc aacaactggc ttgaaaatt 840
 gacagaaatg atgaaccca gaaagtaag caccatttct ttgatgaatg gccaccaaat 900
 gatgataata acaagattc ttcttccact actcagctct caatatccat cccagttct 960

gctcgtgatt tcttctctc acataatgct ggtgataaat ga 1002

<210> 20
 <211> 333
 <212> PRT
 <213> 葵花

<400> 20

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

Tyr Pro Phe Thr Ala Thr Gln Trp Gln Glu Leu Glu His Gln Ala Leu
 20 25 30

Val Tyr Lys Tyr Met Ile Ser Gly Met Pro Ile Pro Pro Asp Leu Leu
 35 40 45

Phe Thr Ile Lys Thr Ser Leu Asp Ser Ser Thr Lys Leu Leu Leu His
 50 55 60

His Gln Pro Pro His Pro Ser Ser Ile Gly Trp Asn Cys Phe Gln Met
 65 70 75 80

[0024]

Gly Phe Gly Arg Lys Ile Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr
 85 90 95

Asp Gly Lys Lys Trp Arg Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys
 100 105 110

Tyr Cys Glu Arg His Met His Arg Gly Arg Asn Arg Ser Arg Lys Pro
 115 120 125

Val Glu Val Asn Met Ser Ser Thr Pro Thr Pro Lys Thr Pro Pro Thr
 130 135 140

Ala Ile Pro Met Ile Pro Ser Ser Ile Cys Thr Lys Ser Pro Asn Tyr
 145 150 155 160

Pro Ser Pro Asn Ser His Pro Leu Ser Ser Ser Ser Asp Tyr Tyr
 165 170 175

His Asn Asn Asn Thr Thr His Leu Pro Ser Tyr Ser Arg Pro Ser Ser
 180 185 190

Asn Val Phe Thr Gln Asp His Phe Leu Leu Asp Ser Ser Pro Tyr Met
 195 200 205

Arg Lys Gly Tyr Gly Met Lys Glu Val Ile Asp Glu His Ser Phe Phe
 210 215 220

Ser Glu Ser Ser Gly Thr Ile Lys Thr Asp Ser Trp Gln Leu Glu Pro
 225 230 235 240

Leu Ala Met Asn Asn Ser Ser Ser Lys Gln Thr Thr Phe Ser Asp Tyr
 245 250 255

His Gln Asn Arg Tyr Ser Tyr Gln Gln Gln Gln Asp Pro Gly Tyr Tyr
 260 265 270

Asp Gln Gln Leu Ala Leu Lys Ile Asp Arg Asn Asp Glu Pro Gln Lys
 275 280 285

Val Met His His Phe Phe Asp Glu Trp Pro Pro Asn Asp Asp Asn Asn
 290 295 300

Lys Asp Ser Ser Ser Thr Thr Gln Leu Ser Ile Ser Ile Pro Ser Ser
 305 310 315 320

Ala Arg Asp Phe Phe Leu Ser His Asn Ala Gly Asp Lys
 325 330

[0025]

<210> 21

<211> 1053

<212> DNA

<213> 人工序列

<220>

<223> StGRF5的cDNA

<400> 21

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gtacctatac gacgtagctt tgaaccatt tcagcgaggt tctttcatca tcttagcttg 180

ggttattgct cctattatgg gaagaagttt gatcctgagc caggaaggtg tagaaggaca 240

gatggaaaaga agtgagggtg cgccaagat gcatacctg actcaaagta ttgcgagcgg 300

cacatgcatc gaggccgcaa ccgttcaaga aagcatgtgg aatctcaatc gactgcccag 360

tccttggtga ctgatgtgc acataatact actgggagca gaaaacaag tggaaacttc 420

caacgtagca gtatgggcaa tttccaactg agcagcagtg aaagcttcca gaacacgcca 480

ctatattctg ctgctaatac tgaaggacca agttatggaa gtgccacaac aaagatgcag 540

atggagcctg ccacatgac agtagatagc aaggggtatt tccatggaat gactgctgat 600

gctgatgagc agaatttctc tctcgaagct tcggcaggca cgagaagttt agggatggga 660

tctaacacag acagcatgtg gtgtctgatg cctcctccac aacttcctc aagccccatg 720

gtgaaaccaa aaaatgattc acagttgcta gatagctcgc gacatatccg aatgcctaata 780

ccattcgagc ctatgaatga tacaactatt tcgggacaac accaacattg ctttttcagc 840
 agtgacatag gctctcccg gacagtaaag caggagcaac gttcaatgcg ccctttcttt 900
 gacgaatggc ctacaactaa ggaatcatgg tccaatcttg atgatgaggg atccaacaaa 960
 aataatttct ccactactca gctgtccata tccattccta tggctccttc tgacttctct 1020
 tcaaggagtt cttgttcccc aaacgatgct tga 1053

<210> 22
 <211> 350
 <212> PRT
 <213> 马铃薯
 <400> 22

Met Ala Ala Glu Asn Gly Tyr Arg Pro Pro Phe Thr Ala Val Gln Trp
 1 5 10 15

Gln Glu Leu Glu His Gln Ala Met Ile Tyr Lys Tyr Leu Val Ala Gly
 20 25 30

Ile Pro Val Pro Ala Asp Leu Val Val Pro Ile Arg Arg Ser Phe Glu
 35 40 45

[0026] Pro Ile Ser Ala Arg Phe Phe His His Pro Ser Leu Gly Tyr Cys Ser
 50 55 60

Tyr Tyr Gly Lys Lys Phe Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr
 65 70 75 80

Asp Gly Lys Lys Trp Arg Cys Ala Lys Asp Ala Tyr Pro Asp Ser Lys
 85 90 95

Tyr Cys Glu Arg His Met His Arg Gly Arg Asn Arg Ser Arg Lys His
 100 105 110

Val Glu Ser Gln Ser Thr Ala Gln Ser Leu Leu Thr Ser Met Ser His
 115 120 125

Asn Thr Thr Gly Ser Ser Lys Thr Ser Gly Asn Phe Gln Arg Ser Ser
 130 135 140

Ser Gly Asn Phe Gln Arg Ser Ser Ser Glu Ser Phe Gln Asn Thr Pro
 145 150 155 160

Leu Tyr Ser Ala Ala Asn Thr Glu Gly Pro Ser Tyr Gly Ser Ala Thr
 165 170 175

Thr Lys Met Gln Met Glu Pro Ala Thr Tyr Ala Val Asp Ser Lys Gly

180 185 190
 Tyr Phe His Gly Met Thr Ala Asp Ala Asp Glu Gln Asn Phe Ser Leu
 195 200 205

 Glu Ala Ser Ala Gly Thr Arg Ser Leu Gly Met Gly Ser Asn Thr Asp
 210 215 220

 Ser Met Trp Cys Leu Met Pro Pro Pro Gln Leu Pro Ser Ser Pro Met
 225 230 235 240

 Val Lys Pro Lys Asn Asp Ser Gln Leu Leu Asp Ser Ser Arg His Ile
 245 250 255

 Arg Met Pro Asn Pro Phe Glu Pro Met Asn Asp Thr Thr Ile Ser Gly
 260 265 270

 Gln His Gln His Cys Phe Phe Ser Ser Asp Ile Gly Ser Pro Gly Thr
 275 280 285

 Val Lys Gln Glu Gln Arg Ser Met Arg Pro Phe Phe Asp Glu Trp Pro
 290 295 300

 [0027] Thr Thr Lys Glu Ser Trp Ser Asn Leu Asp Asp Glu Gly Ser Asn Lys
 305 310 315 320

 Asn Asn Phe Ser Thr Thr Gln Leu Ser Ile Ser Ile Pro Met Ala Pro
 325 330 335

 Ser Asp Phe Ser Ser Arg Ser Ser Cys Ser Pro Asn Asp Ala
 340 345 350

 <210> 23
 <211> 1242
 <212> DNA
 <213> 人工序列

 <220>
 <223> HvGRF5的cDNA

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 gcctccggcg tgectatccc ctccgacctc ctctcccgc tccgccgag ctctctctc 180
 gactccgccc tcgccacctc cctctcctc gccttcctc cgcaggccac actgggctgg 240
 ggttgcttcg ggatggggtt cgccggaag ccggaggacc cggagccggg gcggtgccgg 300
 cggacggacg gcaagaagtg gcgctgctcc agggaggcgt accagactc caagtactgc 360

gagaagcaca tgcaccgcgg caagaaccgt tcaagaaagc ctgtggaaat gtccttggcc 420
 acgccccgc cgccgcttc ctctcggcc tctctctct cctccaacgt cactccgcc 480
 gtcaccgtcg ccaccaccac cacctcccc gcgccgtctt accaccgcca cgccgccag 540
 actcagcagc cggcacccta ccacgcgctc tacggcggcc cctacgcctc cgccggccgc 600
 cagcagcagc ggagcgcta ccaccagcc gcgcaggta gcccggtcca cctgcacctc 660
 gacaccacc acccgaccc gccgccgtcc tactactcca ccatggacca cagcaaggac 720
 agctacgctt acgggcacag cgtaaggag gtgcacggcg gcggcgagca cgccttttc 780
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 gggcacggcc agtggcagtt caagcagctc ggcggcatgg agccgaagca gcacaacccc 900
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 ctccccatca ccaccacctc ccgttaccac caggtgaat ga 1242

[0028]

<210> 24
 <211> 413
 <212> PRT
 <213> 大麦

<400> 24

Met Met Met Met Gly Gly Arg Ala Gly Ala Gly Gly Val Gly Ala Gly
 1 5 10 15

Gly Gly Arg Cys Pro Phe Thr Ala Thr Gln Trp Gln Glu Leu Glu His
 20 25 30

Gln Ala Leu Ile Tyr Lys Tyr Met Ala Ser Gly Val Pro Ile Pro Ser
 35 40 45

Asp Leu Leu Leu Pro Leu Arg Arg Ser Phe Leu Leu Asp Ser Ala Leu
 50 55 60

Ala Thr Ser Pro Ser Leu Ala Phe Pro Pro Gln Ala Thr Leu Gly Trp
 65 70 75 80

Gly Cys Phe Gly Met Gly Phe Gly Arg Lys Pro Glu Asp Pro Glu Pro
 85 90 95

Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg Cys Ser Arg Glu
 100 105 110

Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met His Arg Gly Lys
 115 120 125

Asn Arg Ser Arg Lys Pro Val Glu Met Ser Leu Ala Thr Pro Pro Pro
 130 135 140

Pro Pro Ser Ser Ser Ala Ser Ser Ser Ser Ser Asn Val His Ser Ala
 145 150 155 160

Val Thr Val Ala Thr Thr Thr Thr Ser Pro Ala Pro Ser Tyr His Arg
 165 170 175

His Ala Ala Thr Thr His Asp Ala Ala Pro Tyr His Ala Leu Tyr Gly
 180 185 190

Gly Pro Tyr Ala Ser Ala Gly Arg Gln Gln His Gly Ser Ala Tyr His
 195 200 205

His Ala Ala Gln Val Ser Pro Phe His Leu His Leu Asp Thr Thr His
 210 215 220

Pro His Pro Pro Pro Ser Tyr Tyr Ser Thr Met Asp His Ser Lys Asp
 225 230 235 240

[0029]

Ser Tyr Ala Tyr Gly His Ser Val Lys Glu Val His Gly Gly Gly Glu
 245 250 255

His Ala Phe Phe Ser Ser Asp Val Thr Thr Asp Arg Asp His Gln His
 260 265 270

His Gln His His Gly Ser Ala Gly Gly His Gly Gln Trp Gln Phe Lys
 275 280 285

Gln Leu Gly Gly Met Glu Pro Lys Gln His Asn Pro Thr Ser Leu Phe
 290 295 300

Pro Gly Cys Gly Gly Tyr Gly Asn Asn Ala Ala Tyr Ala Ile Asp Leu
 305 310 315 320

Ser Gly Lys Glu Glu Ala Glu Glu Lys Glu Arg Arg Gln Gln Gln Gln
 325 330 335

His Cys Phe Leu Leu Gly Ala Asp Leu Arg Leu Asp Lys Pro Ser Ser
 340 345 350

Gly His Gly Asp Ser Ala Asp Gln Lys Pro Leu Arg Pro Phe Phe Asp
 355 360 365

Glu Trp Pro His Glu Lys Thr Gly Asn Lys Gly Ser Trp Met Gly Leu
 370 375 380

Glu Gly Glu Thr Gln Leu Ser Ile Ser Ile Pro Met Thr Ala Asn Asp
 385 390 395 400

Leu Pro Ile Thr Thr Thr Ser Arg Tyr His His Gly Glu
 405 410

<210> 25

<211> 1236

<212> DNA

<213> 人工序列

<220>

<223> ScGRF5的cDNA

<400> 25

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 gcctccggcg tgcccatccc ctccgacctc ctctcccgc tccgccgag cttctcctc 180
 gactccgcc tcgccacctc cctctcctc gccttcctc cccaggccgc acttgatgg 240
 ggctgcttcg ggatgggggt cggccggaag gcggaggacc cggagccggg gcggtgccgg 300
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 gagaagcaca tgaccgcgg caagaacctg tcaagaaagc ctgtggaat gtccttggcc 420
 acgccccgc cgccgcttc ctctcggcc tctctctct cctccaagt cactccgcc 480
 gtcaacgtcg ccaccaccac ctctcccc gcgccatct accaccgcca cgccgccgg 540
 actcagaca cgacgccta ccacgcgctc tacggcgcc cctacgctc tgccggccgc 600
 cagcagcagc ccagcgcta ccaccagcc gcgcaggta gccgttcca cctgcacctc 660
 gacaccacc acccgaccc gcccgctcc tactactcca ccatggacca cagcaaggac 720
 agctacgct acgggcacag cgtcaaggag gtgcacggcg gcggcgagca cgccttctc 780
 gcctccgacg tcgccaccga cagggaccac caccaccacc accaacacca cgccggcgcc 840
 ggcggcaacg gccaatggca gttcaagcag ctccggcgca tggagcccaa gcagcataac 900
 cccagctgc tcttccccg ctccggcgcc tacggcaaca acgcgcgta cgccatcgac 960
 ctgtccagca aagaagagga cgaggagaag gagaggcggc agcagcagca gcaactctc 1020
 ctgtggggcg ccgacctgag gctcgacaag ccgtcgtcgg ggcacggcga ctccgccgac 1080
 cagaagcccc tccggcgtt ctctgacgag tggccgcag agaaggccgg gagcaagggg 1140
 tcgtgatgg ggctcaggg ggagacgcag ctctcatct ccatgccaa cgaactcccc 1200
 atcaccacca cctcccgcta ccacatggt gaatga 1236

<210> 26
 <211> 411
 <212> PRT
 <213> 黑麦

<400> 26

Met Met Met Met Gly Gly Arg Ala Gly Ala Gly Gly Val Gly Ala Gly
 1 5 10 15

Gly Gly Arg Cys Pro Phe Thr Ala Thr Gln Trp Gln Glu Leu Glu His
 20 25 30

Gln Ala Leu Ile Tyr Lys Tyr Met Ala Ser Gly Val Pro Ile Pro Ser
 35 40 45

Asp Leu Leu Leu Pro Leu Arg Arg Ser Phe Leu Leu Asp Ser Ala Leu
 50 55 60

Ala Thr Ser Pro Ser Leu Ala Phe Pro Pro Gln Ala Ala Leu Gly Trp
 65 70 75 80

Gly Cys Phe Gly Met Gly Phe Gly Arg Lys Ala Glu Asp Pro Glu Pro
 85 90 95

[0031]

Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg Cys Ser Lys Glu
 100 105 110

Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met His Arg Gly Lys
 115 120 125

Asn Arg Ser Arg Lys Pro Val Glu Met Ser Leu Ala Thr Pro Pro Pro
 130 135 140

Pro Pro Ser Ser Ser Ala Ser Ser Ser Ser Ser Asn Val His Ser Ala
 145 150 155 160

Val Asn Val Ala Thr Thr Thr Ser Ser Pro Ala Pro Ser Tyr His Arg
 165 170 175

His Ala Ala Ala Thr His Asp Thr Thr Pro Tyr His Ala Leu Tyr Gly
 180 185 190

Gly Pro Tyr Ala Ser Ala Gly Arg Gln Gln His Ala Ser Ala Tyr His
 195 200 205

His Ala Ala Gln Val Ser Pro Phe His Leu His Leu Asp Thr Thr His
 210 215 220

Pro His Pro Pro Pro Ser Tyr Tyr Ser Thr Met Asp His Ser Lys Asp
225 230 235 240

Ser Tyr Ala Tyr Gly His Ser Val Lys Glu Val His Gly Gly Gly Glu
 245 250 255

His Ala Phe Phe Ala Ser Asp Val Ala Thr Asp Arg Asp His His His
 260 265 270

His His Gln His His Ala Gly Ala Gly Gly Asn Gly Gln Trp Gln Phe
 275 280 285

Lys Gln Leu Gly Gly Met Glu Pro Lys Gln His Asn Pro Thr Ser Leu
 290 295 300

Phe Pro Gly Cys Gly Gly Tyr Gly Asn Asn Ala Ala Tyr Ala Ile Asp
305 310 315 320

Leu Ser Ser Lys Glu Glu Asp Glu Glu Lys Glu Arg Arg Gln Gln Gln
 325 330 335

Gln His Cys Phe Leu Leu Gly Ala Asp Leu Arg Leu Asp Lys Pro Ser
 340 345 350

[0032]

Ser Gly His Gly Asp Ser Ala Asp Gln Lys Pro Leu Arg Pro Phe Phe
 355 360 365

Asp Glu Trp Pro His Glu Lys Ala Gly Ser Lys Gly Ser Trp Met Gly
 370 375 380

Leu Glu Gly Glu Thr Gln Leu Ser Ile Ser Ile Ala Asn Glu Leu Pro
385 390 395 400

Ile Thr Thr Thr Ser Arg Tyr His His Gly Glu
 405 410

<210> 27

<211> 1083

<212> DNA

<213> 人工序列

<220>

<223> GmGRF5的cDNA

<400> 27

atgatgagtg caagtcagg tgcaagaaat aggtctccgt tcacacaaat tcagtggcaa 60

gagcttgagc aacaagtct tgttttaag tacatggta caggaacacc tatcccacca 120

gatctcatct actctattaa aagaagtcta gacactcaa tttctcaag gctctccca 180

catcatcaa ttgggtgggg atgtttgaa atgggattg gcagaaaagt agaccagag 240
 ccagggaggt gcagaagaac agatggcaag aatggagat gtcaaagga ggcatacca 300
 gactcaaagt actgtgaaag acacatgcac agaggcagaa accgttcaag aaagcctgtg 360
 gaagtttctt cagcaacaag caccgccaca aacacctccc aaacaatccc atcatctat 420
 accgaaacc ttccttgac caataacagt aaccccaaca taacaccacc accaccacc 480
 tcttcttcc tttctctca ttgccctct tctatgccta ttgatcagtc ccaaccctt 540
 tcccaatct accaaaactc ttcttcaat cccttctct actccaatc aacctctct 600
 agacccccag atgctgatt tccacccca gatgccacca cccaccacct atcatggac 660
 tctgtggct ctatttca tgatgaaaag aattatagca ggcatgtca tggaataagg 720
 gaagatgtg atgagagagc tttcttcca gaagcatcag gatcagctag gagctataca 780
 gactcgtacc aacaactatc aatgagctcc tacaagtct attcaaactc caacttcag 840
 aacattaata atgatgccac caccaacca agacagcaag agcagcaact acaacaaca 900
 caaactgtt ttgttttagg gacagactc aatcaaca ggccaagcaa agagaagaa 960
 gctgagaca caacaggtca gagaccctt caccgttct ttggggagt gccaccaaag 1020
 aacaacaaca cagattcctg gctagatctt gctccaact ccagaatcca aaccgatgaa 1080

[0033]

tga 1083

<210> 28
 <211> 360
 <212> PRT
 <213> 大豆

<400> 28

Met Met Ser Ala Ser Ala Gly Ala Arg Asn Arg Ser Pro Phe Thr Gln
 1 5 10 15

Ile Gln Trp Gln Glu Leu Glu Gln Gln Ala Leu Val Phe Lys Tyr Met
 20 25 30

Val Thr Gly Thr Pro Ile Pro Pro Asp Leu Ile Tyr Ser Ile Lys Arg
 35 40 45

Ser Leu Asp Thr Ser Ile Ser Ser Arg Leu Phe Pro His His Pro Ile
 50 55 60

Gly Trp Gly Cys Phe Glu Met Gly Phe Gly Arg Lys Val Asp Pro Glu
 65 70 75 80

Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg Cys Ser Lys
 85 90 95

Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met His Arg Gly
 100 105 110

Arg Asn Arg Ser Arg Lys Pro Val Glu Val Ser Ser Ala Thr Ser Thr
 115 120 125

Ala Thr Asn Thr Ser Gln Thr Ile Pro Ser Ser Tyr Thr Arg Asn Leu
 130 135 140

Ser Leu Thr Asn Asn Ser Asn Pro Asn Ile Thr Pro Pro Pro Pro
 145 150 155 160

Ser Ser Phe Pro Phe Ser His Leu Pro Ser Ser Met Pro Ile Asp Gln
 165 170 175

Ser Gln Pro Phe Ser Gln Ser Tyr Gln Asn Ser Ser Leu Asn Pro Phe
 180 185 190

Phe Tyr Ser Gln Ser Thr Ser Ser Arg Pro Pro Asp Ala Asp Phe Pro
 195 200 205

Pro Gln Asp Ala Thr Thr His His Leu Phe Met Asp Ser Ala Gly Ser
 210 215 220

[0034]

Tyr Ser His Asp Glu Lys Asn Tyr Ser Arg His Val His Gly Ile Arg
 225 230 235 240

Glu Asp Val Asp Glu Arg Ala Phe Phe Pro Glu Ala Ser Gly Ser Ala
 245 250 255

Arg Ser Tyr Thr Asp Ser Tyr Gln Gln Leu Ser Met Ser Ser Tyr Lys
 260 265 270

Ser Tyr Ser Asn Ser Asn Phe Gln Asn Ile Asn Asn Asp Ala Thr Thr
 275 280 285

Asn Pro Arg Gln Gln Glu Gln Gln Leu Gln Gln Gln His Cys Phe
 290 295 300

Val Leu Gly Thr Asp Phe Lys Ser Thr Arg Pro Ser Lys Glu Lys Glu
 305 310 315 320

Ala Glu Thr Thr Thr Gly Gln Arg Pro Leu His Arg Phe Phe Gly Glu
 325 330 335

Trp Pro Pro Lys Asn Thr Thr Thr Asp Ser Trp Leu Asp Leu Ala Ser
 340 345 350

Asn Ser Arg Ile Gln Thr Asp Glu
355 360

<210> 29

<211> 945

<212> DNA

<213> 人工序列

<220>

<223> GhGRF5的cDNA

<400> 29

atgattagtg ccagaaataa gtacctttc actccaaatc aatggcaaga gcttgaacac 60

caagctctca tcttcaaata catggtttca ggagttccta tcccacctca actcctttat 120

tctgtcaaaa caagctttga ttctctttg gcttcacacc tcttcctca ccaaccacaca 180

gggtggggct gtttcaggt gggttttggc agaaaaccag acccagagcc ggggaggtgc 240

aggagaactg atggaaaaa atggagatgc tccaagaag cttaccaga ctccaagtac 300

tgtgagaggc acatgcatag aggcaggaac cgttcaagaa agcctgtgga agctaattca 360

tcatcatcaa cagcaccacc agcaccacca acaacagcag cagcttccat cctttcacca 420

tctttcccat caatcaacag taaccttccc acttcaagtt ctctctctc tttttctct 480

atggctactg aaaactcac ccatttcgac ccctttctt attctcattc ttctacgaga 540

[0035] cttcatggtt caggcttatc agttccatct catcatttcc tagactctgg aactggaatt 600

gattaccctc agactgataa actttacagg tatgttcatg gaacaagga aggtgttgat 660

gaaagatctt tcttccccga agcttcagcg agtgaagag ttgtatctga ttctatcag 720

ccactgacaa tggccaag ctttggtgac aacaatggtt caaagcaggg gcagcaatgc 780

ttcgttttag gtactgatat caagtcagct aagccaatca agttagaaaa ggatgaagga 840

actcaaaaac cagtcacca attttaggg gattggacac aaggaacaa caatggttct 900

tggctgatc ttgcatcaa ttccagggtc caatcagata gttga 945

<210> 30

<211> 314

<212> PRT

<213> 陆地棉

<400> 30

Met Ile Ser Ala Arg Asn Lys Tyr Leu Phe Thr Pro Asn Gln Trp Gln
1 5 10 15

Glu Leu Glu His Gln Ala Leu Ile Phe Lys Tyr Met Val Ser Gly Val
20 25 30

Pro Ile Pro Pro Gln Leu Leu Tyr Ser Val Lys Thr Ser Phe Asp Ser
35 40 45

Ser Leu Ala Ser His Leu Phe Pro His Gln Pro Thr Gly Trp Gly Cys

50 55 60

Phe Gln Val Gly Phe Gly Arg Lys Pro Asp Pro Glu Pro Gly Arg Cys

65 70 75 80

Arg Arg Thr Asp Gly Lys Lys Trp Arg Cys Ser Lys Glu Ala Tyr Pro

85 90 95

Asp Ser Lys Tyr Cys Glu Arg His Met His Arg Gly Arg Asn Arg Ser

100 105 110

Arg Lys Pro Val Glu Ala Asn Ser Ser Ser Ser Thr Ala Pro Pro Ala

115 120 125

Pro Pro Thr Thr Ala Ala Ala Ser Ile Leu Ser Pro Ser Phe Pro Ser

130 135 140

Ile Asn Ser Asn Leu Pro Thr Ser Ser Ser Ser Leu Ser Phe Ser Pro

145 150 155 160

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

165 170 175

[0036]

Ser Ser Thr Arg Leu His Gly Ser Gly Leu Ser Val Pro Ser His His

180 185 190

Phe Leu Asp Ser Gly Thr Gly Ile Asp Tyr Pro Gln Thr Asp Lys Leu

195 200 205

Tyr Arg Tyr Val His Gly Thr Arg Glu Gly Val Asp Glu Arg Ser Phe

210 215 220

Phe Pro Glu Ala Ser Ala Ser Val Arg Val Val Ser Asp Ser Tyr Gln

225 230 235 240

Pro Leu Thr Met Val Gln Ser Phe Gly Asp Asn Asn Gly Ser Lys Gln

245 250 255

Gly Gln Gln Cys Phe Val Leu Gly Thr Asp Ile Lys Ser Ala Lys Pro

260 265 270

Ile Lys Leu Glu Lys Asp Glu Gly Thr Gln Lys Pro Val His Gln Phe

275 280 285

Leu Gly Asp Trp Thr Gln Gly Asn Asn Asn Gly Ser Trp Leu Asp Leu

290 295 300

Ala Ser Asn Ser Arg Val Gln Ser Asp Ser
305 310

<210> 31

<211> 1194

<212> DNA

<213> 人工序列

<220>

<223> OsGRF5的cDNA

<400> 31

atgatgatga tgagcggctg cccgagcggc ggcgccggcg gaggtcggtg cccgttcacg 60

gcgtcgcagt ggcaggagct ggagaccag gcgctcatct acaagtacat ggcgtccggg 120

actccatcc cctccgacct cctcctccc ctccgccga gcttctctct cgactccgcc 180

ctcgccacct ccccttctct cgccttcct cccaacctt cactggggtg ggggtgcttt 240

ggcatggggg ttgggcggaa ggcggaggac ccggagccag ggcgatgccg gcgtacggac 300

ggcaagaagt ggcgggtgct caaggaggcg taccgggact ccaagtactg cgagaagcac 360

atgcaccgtg gcaagaaccg ttcaagaag cctgtggaaa tgccttggc cacgccgccg 420

ccgccgtct cctccgccac ctccgcccg tgaacacct ccgccggcgt cgcctccacc 480

accaccacca cctcctccc ggcgcctcc tacagccgc cggcgccga cgacgcggcg 540

[0037] ccgtaccagg cgctctacgg cgggcccctac gcccgggcca ccgcgcgcac ccccgccgcc 600

gcggcgtacc acgcgcaggt gagcccgtc cacctccagc tcgacaccac ccaccgcac 660

ccgccgccgt cctactactc catggaccac aaggagtacg cgtacgggca cgccaccaag 720

gaggtgcacg gcgagcacgc cttcttctcc gatggcaccg agagggagca ccaccacgcc 780

gccgccggcg acggccagtg gcagttcaag cagctcggca tggagcccaa gcagagcacc 840

acgcctctct tcccggggcg cggctacggc cacaccgcgg cgtcgcgta cgccattgat 900

ctttcaaaag aggacgacga tgagaaagag aggcggcaac agcagcagca gcagcagcag 960

cagcactgct tctcctggg cgccgacctc cgtctggaga agccggcggg ccacgaccac 1020

gcggcggcgg cgcagaaacc tctccgccac ttcttcgacg agtgcccgca tgagaagaac 1080

agcaagggct cctggatggg gctcgaagc gagacgcagc tgtccatgct catcccatg 1140

gccccaacg acctcccgat caccaccacc tcccgtacc acaatgatga ttaa 1194

<210> 32

<211> 397

<212> PRT

<213> 稻

<400> 32

Met Met Met Met Ser Gly Arg Pro Ser Gly Gly Ala Gly Gly Gly Arg
1 5 10 15

Tyr Pro Phe Thr Ala Ser Gln Trp Gln Glu Leu Glu His Gln Ala Leu
 20 25 30

Ile Tyr Lys Tyr Met Ala Ser Gly Thr Pro Ile Pro Ser Asp Leu Ile
 35 40 45

Leu Pro Leu Arg Arg Ser Phe Leu Leu Asp Ser Ala Leu Ala Thr Ser
 50 55 60

Pro Ser Leu Ala Phe Pro Pro Gln Pro Ser Leu Gly Trp Gly Cys Phe
 65 70 75 80

Gly Met Gly Phe Gly Arg Lys Ala Glu Asp Pro Glu Pro Gly Arg Cys
 85 90 95

Arg Arg Thr Asp Gly Lys Lys Trp Arg Cys Ser Lys Glu Ala Tyr Pro
 100 105 110

Asp Ser Lys Tyr Cys Glu Lys His Met His Arg Gly Lys Asn Arg Ser
 115 120 125

Arg Lys Pro Val Glu Met Ser Leu Ala Thr Pro Pro Pro Pro Ser Ser
 130 135 140

[0038]

Ser Ala Thr Ser Ala Ala Ser Asn Thr Ser Ala Gly Val Ala Pro Thr
 145 150 155 160

Thr Thr Thr Thr Ser Ser Pro Ala Pro Ser Tyr Ser Arg Pro Ala Pro
 165 170 175

His Asp Ala Ala Pro Tyr Gln Ala Leu Tyr Gly Gly Pro Tyr Ala Ala
 180 185 190

Ala Thr Ala Arg Thr Pro Ala Ala Ala Ala Tyr His Ala Gln Val Ser
 195 200 205

Pro Phe His Leu Gln Leu Asp Thr Thr His Pro His Pro Pro Pro Ser
 210 215 220

Tyr Tyr Ser Met Asp His Lys Glu Tyr Ala Tyr Gly His Ala Thr Lys
 225 230 235 240

Glu Val His Gly Glu His Ala Phe Phe Ser Asp Gly Thr Glu Arg Glu
 245 250 255

His His His Ala Ala Ala Gly His Gly Gln Trp Gln Phe Lys Gln Leu
 260 265 270

Gly Met Glu Pro Lys Gln Ser Thr Thr Pro Leu Phe Pro Gly Ala Gly
275 280 285

Tyr Gly His Thr Ala Ala Ser Pro Tyr Ala Ile Asp Leu Ser Lys Glu
290 295 300

Asp Asp Asp Glu Lys Glu Arg Arg Gln Gln Gln Gln Gln Gln Gln
305 310 315 320

Gln His Cys Phe Leu Leu Gly Ala Asp Leu Arg Leu Glu Lys Pro Ala
325 330 335

Gly His Asp His Ala Ala Ala Ala Gln Lys Pro Leu Arg His Phe Phe
340 345 350

Asp Glu Trp Pro His Glu Lys Asn Ser Lys Gly Ser Trp Met Gly Leu
355 360 365

Glu Gly Glu Thr Gln Leu Ser Met Ser Ile Pro Met Ala Ala Asn Asp
370 375 380

Leu Pro Ile Thr Thr Thr Ser Arg Tyr His Asn Asp Asp
385 390 395

[0039]

<210> 33
<211> 530
<212> PRT
<213> 拟南芥

<400> 33

Met Asp Leu Gly Val Arg Val Ser Gly His Glu Thr Val Ser Ser Pro
1 5 10 15

Gly Gln Thr Glu Leu Gly Ser Gly Phe Ser Asn Lys Gln Glu Arg Ser
20 25 30

Gly Phe Asp Gly Glu Asp Cys Trp Arg Ser Ser Lys Leu Ser Arg Thr
35 40 45

Ser Thr Asp Gly Phe Ser Ser Ser Pro Ala Ser Ala Lys Thr Leu Ser
50 55 60

Phe His Gln Gly Ile Pro Leu Leu Arg Ser Thr Thr Ile Asn Asp Pro
65 70 75 80

Arg Lys Gly Gln Glu His Met Leu Ser Phe Ser Ser Ala Ser Gly Lys
85 90 95

Ser Asp Val Ser Pro Tyr Leu Gln Tyr Cys Arg Asn Ser Gly Tyr Gly
 100 105 110

Leu Gly Gly Met Met Asn Thr Ser Asn Met His Gly Asn Leu Leu Thr
 115 120 125

Gly Val Lys Gly Pro Phe Ser Leu Thr Gln Trp Ala Glu Leu Glu Gln
 130 135 140

Gln Ala Leu Ile Tyr Lys Tyr Ile Thr Ala Asn Val Pro Val Pro Ser
 145 150 155 160

Ser Leu Leu Leu Ser Leu Lys Lys Ser Phe Phe Pro Tyr Gly Ser Leu
 165 170 175

Pro Pro Asn Ser Phe Gly Trp Gly Ser Phe His Leu Gly Phe Ser Gly
 180 185 190

Gly Asn Met Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys
 195 200 205

Lys Trp Arg Cys Ser Arg Asp Ala Val Pro Asp Gln Lys Tyr Cys Glu
 210 215 220

[0040]

Arg His Ile Asn Arg Gly Arg His Arg Ser Arg Lys Pro Val Glu Gly
 225 230 235 240

Gln Asn Gly His Asn Thr Asn Ala Ala Ala Ala Ala Ser Ala Ala Ala
 245 250 255

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

Val Ala Met Arg Gly Ser Asp Asn Asn Asn Ser Leu Ala Ala Ala Val
 275 280 285

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

Arg Val Gln Asn Ser Arg Gly Ala Ser Val Phe Pro Ala Thr Met Asn
 305 310 315 320

Leu Gln Ser Lys Glu Thr His Pro Lys Gln Ser Asn Asn Pro Phe Glu
 325 330 335

Phe Gly Leu Ile Ser Ser Asp Ser Leu Leu Asn Pro Ser His Lys Gln
 340 345 350

Ala Ser Tyr Ala Thr Ser Ser Lys Gly Phe Gly Ser Tyr Leu Asp Phe
355 360 365

Gly Asn Gln Ala Lys His Ala Gly Asn His Asn Asn Val Asp Ser Trp
370 375 380

Pro Glu Glu Leu Lys Ser Asp Trp Thr Gln Leu Ser Met Ser Ile Pro
385 390 395 400

Met Ala Pro Ser Ser Pro Val Gln Asp Lys Leu Ala Leu Ser Pro Leu
405 410 415

Arg Leu Ser Arg Glu Phe Asp Pro Ala Ile His Met Gly Leu Gly Val
420 425 430

Asn Thr Glu Phe Leu Asp Pro Gly Lys Lys Thr Asn Asn Trp Ile Pro
435 440 445

Ile Ser Trp Gly Asn Asn Asn Ser Met Gly Gly Pro Leu Gly Glu Val
450 455 460

Leu Asn Ser Thr Thr Asn Ser Pro Lys Phe Gly Ser Ser Pro Thr Gly
465 470 475 480

[0041]

Val Leu Gln Lys Ser Thr Phe Gly Ser Leu Ser Asn Ser Ser Ser Ala
485 490 495

Ser Ser Thr Ile Ile Gly Asp Asn Asn Asn Lys Asn Gly Asp Gly Lys
500 505 510

Asp Pro Leu Gly Pro Thr Thr Leu Met Asn Thr Ser Ala Thr Ala Pro
515 520 525

Ser Leu
530

<210> 34

<211> 535

<212> PRT

<213> 拟南芥

<400> 34

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

Glu Ser Leu Gly Leu Lys Glu Leu Ile Gly Thr Lys Gln Asp Arg Ser
20 25 30

Gly Phe Ile Gly Glu Asp Cys Leu Gln Arg Ser Leu Lys Leu Ala Arg
35 40 45

Thr Thr Thr Arg Ala Glu Glu Glu Glu Asn Leu Ser Ser Ser Val Ala
50 55 60

Ala Ala Tyr Cys Lys Thr Met Ser Phe His Gln Gly Ile Pro Leu Met
65 70 75 80

Arg Ser Ala Ser Pro Leu Ser Ser Asp Ser Arg Arg Gln Glu Gln Met
85 90 95

Leu Ser Phe Ser Asp Lys Pro Asp Ala Leu Asp Phe Ser Lys Tyr Val
100 105 110

Gly Leu Asp Asn Ser Ser Asn Asn Lys Asn Ser Leu Ser Pro Phe Leu
115 120 125

His Gln Ile Pro Pro Pro Ser Tyr Phe Arg Ser Ser Gly Gly Tyr Gly
130 135 140

Ser Gly Gly Met Met Met Asn Met Ser Met Gln Gly Asn Phe Thr Gly
145 150 155 160

[0042]

Val Lys Gly Pro Phe Thr Leu Thr Gln Trp Ala Glu Leu Glu Gln Gln
165 170 175

Ala Leu Ile Tyr Lys Tyr Ile Thr Ala Asn Val Pro Val Pro Ser Ser
180 185 190

Leu Leu Ile Ser Ile Lys Lys Ser Phe Tyr Pro Tyr Gly Ser Leu Pro
195 200 205

Pro Ser Ser Phe Gly Trp Gly Thr Phe His Leu Gly Phe Ala Gly Gly
210 215 220

Asn Met Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys
225 230 235 240

Trp Arg Cys Ser Arg Asp Ala Val Pro Asp Gln Lys Tyr Cys Glu Arg
245 250 255

His Ile Asn Arg Gly Arg His Arg Ser Arg Lys Pro Val Glu Val Gln
260 265 270

Ser Gly Gln Asn Gln Thr Ala Ala Ala Ala Ser Lys Ala Val Thr Thr
275 280 285

Pro Gln Gln Pro Val Val Ala Gly Asn Thr Asn Arg Ser Asn Ala Arg
290 295 300

Ala Ser Ser Asn Arg Ser Leu Ala Ile Gly Ser Gln Tyr Ile Asn Pro
305 310 315 320

Ser Thr Glu Ser Leu Pro Asn Asn Arg Gly Val Ser Ile Tyr Pro Ser
325 330 335

Thr Val Asn Leu Gln Pro Lys Glu Ser Pro Val Ile His Gln Lys His
340 345 350

Arg Asn Asn Asn Asn Pro Phe Glu Phe Gly His Ile Ser Ser Asp Ser
355 360 365

Leu Leu Asn Pro Asn Thr Ala Lys Thr Tyr Gly Ser Ser Phe Leu Asp
370 375 380

Phe Ser Ser Asn Gln Glu Lys His Ser Gly Asn His Asn His Asn Ser
385 390 395 400

Trp Pro Glu Glu Leu Thr Ser Asp Trp Thr Gln Leu Ser Met Ser Ile
405 410 415

[0043]

Pro Ile Ala Ser Ser Ser Pro Ser Ser Thr His Asn Asn Asn Asn Ala
420 425 430

Gln Glu Lys Thr Thr Leu Ser Pro Leu Arg Leu Ser Arg Glu Leu Asp
435 440 445

Leu Ser Ile Gln Thr Asp Glu Thr Thr Ile Glu Pro Thr Val Lys Lys
450 455 460

Val Asn Thr Trp Ile Pro Ile Ser Trp Gly Asn Ser Leu Gly Gly Pro
465 470 475 480

Leu Gly Glu Val Leu Asn Ser Thr Thr Asn Ser Pro Thr Phe Gly Ser
485 490 495

Ser Pro Thr Gly Val Leu Gln Lys Ser Thr Phe Cys Ser Leu Ser Asn
500 505 510

Asn Ser Ser Val Ser Ser Pro Ile Ala Glu Asn Asn Arg His Asn Gly
515 520 525

Asp Tyr Phe His Tyr Thr Thr
530 535

<210> 35
 <211> 398
 <212> PRT
 <213> 拟南芥

<400> 35

Met Asp Leu Gln Leu Lys Gln Trp Arg Ser Gln Gln Gln Gln His
 1 5 10 15

Gln Thr Glu Ser Glu Glu Gln Pro Ser Ala Ala Lys Ile Pro Lys His
 20 25 30

Val Phe Asp Gln Ile His Ser His Thr Ala Thr Ser Thr Ala Leu Pro
 35 40 45

Leu Phe Thr Pro Glu Pro Thr Ser Ser Lys Leu Ser Ser Leu Ser Pro
 50 55 60

Asp Ser Ser Ser Arg Phe Pro Lys Met Gly Ser Phe Phe Ser Trp Ala
 65 70 75 80

Gln Trp Gln Glu Leu Glu Leu Gln Ala Leu Ile Tyr Arg Tyr Met Leu
 85 90 95

[0044]

Ala Gly Ala Ala Val Pro Gln Glu Leu Leu Leu Pro Ile Lys Lys Ser
 100 105 110

Leu Leu His Leu Ser Pro Ser Tyr Phe Leu His His Pro Leu Gln His
 115 120 125

Leu Pro His Tyr Gln Pro Ala Trp Tyr Leu Gly Arg Ala Ala Met Asp
 130 135 140

Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg Cys
 145 150 155 160

Ser Arg Asp Val Phe Ala Gly His Lys Tyr Cys Glu Arg His Met His
 165 170 175

Arg Gly Arg Asn Arg Ser Arg Lys Pro Val Glu Thr Pro Thr Thr Val
 180 185 190

Asn Ala Thr Ala Thr Ser Met Ala Ser Ser Val Ala Ala Ala Thr
 195 200 205

Thr Thr Thr Ala Thr Thr Thr Ser Thr Phe Ala Phe Gly Gly Gly Gly
 210 215 220

Gly Ser Glu Glu Val Val Gly Gln Gly Gly Ser Phe Phe Phe Ser Gly

225 230 235 240

 Ser Ser Asn Ser Ser Ser Glu Leu Leu His Leu Ser Gln Ser Cys Ser
 245 250 255

 Glu Met Lys Gln Glu Ser Asn Asn Met Asn Asn Lys Arg Pro Tyr Glu
 260 265 270

 Ser His Ile Gly Phe Ser Asn Asn Arg Ser Asp Gly Gly His Ile Leu
 275 280 285

 Arg Pro Phe Phe Asp Asp Trp Pro Arg Ser Ser Leu Gln Glu Ala Asp
 290 295 300

 Asn Ser Ser Ser Pro Met Ser Ser Ala Thr Cys Leu Ser Ile Ser Met
 305 310 315 320

 Pro Gly Asn Ser Ser Ser Asp Val Ser Leu Lys Leu Ser Thr Gly Asn
 325 330 335

 Glu Glu Gly Ala Arg Ser Asn Asn Asn Gly Arg Asp Gln Gln Asn Met
 340 345 350

 [0045] Ser Trp Trp Ser Gly Gly Gly Ser Asn His His His His Asn Met Gly
 355 360 365

 Gly Pro Leu Ala Glu Ala Leu Arg Ser Ser Ser Ser Ser Ser Pro Thr
 370 375 380

 Ser Val Leu His Gln Leu Gly Val Ser Thr Gln Ala Phe His
 385 390 395

 <210> 36
 <211> 380
 <212> PRT
 <213> 拟南芥

 <400> 36

 Met Asp Leu Gln Leu Lys Gln Trp Arg Ser Gln Gln Gln Asn Glu Ser
 1 5 10 15

 Glu Glu Gln Gly Ser Ala Ala Thr Lys Ile Ser Asn Phe Phe Phe Asp
 20 25 30

 Gln Ile Gln Ser Gln Thr Ala Thr Ser Ala Ala Ala Ala Pro Leu Pro
 35 40 45

 Leu Phe Val Pro Glu Pro Thr Ser Ser Ser Ser Phe Ser Cys Phe Ser
 50 55 60

Pro Asp Ser Ser Asn Ser Ser Ser Ser Arg Phe Leu Lys Met Gly
65 70 75 80

Asn Phe Phe Ser Trp Ala Gln Trp Gln Glu Leu Glu Leu Gln Ala Leu
 85 90 95

Ile Tyr Arg Tyr Met Leu Ala Gly Ala Ser Val Pro Gln Glu Leu Leu
 100 105 110

Leu Pro Ile Lys Lys Ser Leu Leu His Gln Ser Pro Met His Phe Leu
 115 120 125

His His Pro Leu Gln His Ser Phe Pro His His Gln Pro Ser Trp Tyr
 130 135 140

Trp Gly Arg Gly Ala Met Asp Pro Glu Pro Gly Arg Cys Lys Arg Thr
145 150 155 160

Asp Gly Lys Lys Trp Arg Cys Ser Arg Asp Val Val Ala Gly His Lys
 165 170 175

Tyr Cys Asp Arg His Ile His Arg Gly Arg Asn Arg Ser Arg Lys Pro
 180 185 190

[0046]

Val Glu Thr Ala Thr Thr Thr Ile Thr Thr Thr Ala Thr Thr Thr Ala
 195 200 205

Ser Ser Phe Val Leu Gly Glu Glu Leu Gly His Gly Pro Asn Asn Asn
 210 215 220

His Phe Phe Ser Ser Gly Ser Ser Gln Pro Leu His Leu Ser His Gln
225 230 235 240

Gln Ser Cys Ser Ser Glu Met Lys Gln Glu Ser Asn Asn Asn Lys Arg
 245 250 255

Pro Tyr Glu Ala Asn Ser Gly Phe Ser Asn Gly Arg Ser Asp Asp Gly
 260 265 270

His Ile Leu Arg His Phe Phe Asp Asp Trp Pro Arg Ser Ser Asp Ser
 275 280 285

Thr Ser Ser Pro Met Ser Ser Ser Thr Cys His Leu Ser Ile Ser Met
 290 295 300

Pro Gly Asn Asn Thr Ser Ser Asp Val Ser Leu Lys Leu Ser Thr Gly
305 310 315 320

Asn Glu Glu Glu Glu Glu Asn Met Arg Asn Asn Asn Asn Glu Arg Glu
325 330 335

Gln Met Asn Trp Trp Ser Asn Gly Gly Asn His His Asn Asn Met Gly
340 345 350

Gly Pro Leu Ala Glu Ala Leu Arg Ser Ala Ser Ser Thr Ser Ser Val
355 360 365

Leu His Gln Met Gly Ile Ser Thr Gln Val Phe His
370 375 380

<210> 37

<211> 244

<212> PRT

<213> 拟南芥

<400> 37

Met Ala Thr Arg Ile Pro Phe Thr Glu Ser Gln Trp Glu Glu Leu Glu
1 5 10 15

Asn Gln Ala Leu Val Phe Lys Tyr Leu Ala Ala Asn Met Pro Val Pro
20 25 30

[0047]

Pro His Leu Leu Phe Leu Ile Lys Arg Pro Phe Leu Phe Ser Ser Ser
35 40 45

Ser Ser Ser Ser Ser Ser Ser Ser Phe Phe Ser Pro Thr Leu Ser Pro
50 55 60

His Phe Gly Trp Asn Val Tyr Glu Met Gly Met Gly Arg Lys Ile Asp
65 70 75 80

Ala Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg Cys
85 90 95

Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met His
100 105 110

Arg Gly Lys Asn Arg Ser Ser Ser Arg Lys Pro Pro Pro Thr Gln Phe
115 120 125

Thr Pro Asn Leu Phe Leu Asp Ser Ser Ser Arg Arg Arg Arg Ser Gly
130 135 140

Tyr Met Asp Asp Phe Phe Ser Ile Glu Pro Ser Gly Ser Ile Lys Ser
145 150 155 160

Cys Ser Gly Ser Ala Met Glu Asp Asn Asp Asp Gly Ser Cys Arg Gly
 165 170 175

Ile Asn Asn Glu Glu Lys Gln Pro Asp Arg His Cys Phe Ile Leu Gly
 180 185 190

Thr Asp Leu Arg Thr Arg Glu Arg Pro Leu Met Leu Glu Glu Lys Leu
 195 200 205

Lys Gln Arg Asp His Asp Asn Glu Glu Glu Gln Gly Ser Lys Arg Phe
 210 215 220

Tyr Arg Phe Leu Asp Glu Trp Pro Ser Ser Lys Ser Ser Val Ser Thr
 225 230 235 240

Ser Leu Phe Ile

<210> 38

<211> 365

<212> PRT

<213> 拟南芥

<400> 38

[0048]

Met Asp Phe Leu Lys Val Ser Asp Lys Thr Thr Ile Pro Tyr Arg Ser
 1 5 10 15

Asp Ser Leu Phe Ser Leu Asn Gln Gln Gln Tyr Lys Glu Ser Ser Phe
 20 25 30

Gly Phe Arg Asp Met Glu Ile His Pro His Pro Thr Pro Tyr Ala Gly
 35 40 45

Asn Gly Leu Leu Gly Cys Tyr Tyr Tyr Tyr Pro Phe Thr Asn Ala Gln
 50 55 60

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

Ser Ile Pro Val Pro Phe Asp Leu Leu Val Ser Ser Pro Ser Ser Ala
 85 90 95

Ser Pro Cys Asn Asn Lys Asn Ile Ala Gly Asp Leu Glu Pro Gly Arg
 100 105 110

Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg Cys Ala Lys Glu Val Val
 115 120 125

Ser Asn His Lys Tyr Cys Glu Lys His Leu His Arg Gly Arg Pro Arg
 130 135 140

Ser Arg Lys His Val Glu Pro Pro Tyr Ser Arg Pro Asn Asn Asn Gly
 145 150 155 160

Gly Ser Val Lys Asn Arg Asp Leu Lys Lys Leu Pro Gln Lys Leu Ser
 165 170 175

Ser Ser Ser Ile Lys Asp Lys Thr Leu Glu Pro Met Glu Val Ser Ser
 180 185 190

Ser Ile Ser Asn Tyr Arg Asp Ser Arg Gly Ser Glu Lys Phe Thr Val
 195 200 205

Leu Ala Thr Thr Glu Gln Glu Asn Lys Tyr Leu Asn Phe Ile Asp Val
 210 215 220

Trp Ser Asp Gly Val Arg Ser Ser Glu Lys Gln Ser Thr Thr Ser Thr
 225 230 235 240

Pro Val Ser Ser Ser Asn Gly Asn Leu Ser Leu Tyr Ser Leu Asp Leu
 245 250 255

[0049]

Ser Met Gly Gly Asn Asn Leu Met Gly Gln Asp Glu Met Gly Leu Ile
 260 265 270

Gln Met Gly Leu Gly Val Ile Gly Ser Gly Ser Glu Asp His His Gly
 275 280 285

Tyr Gly Pro Tyr Gly Val Thr Ser Ser Leu Glu Glu Met Ser Ser Trp
 290 295 300

Leu Ala Pro Met Ser Thr Thr Pro Gly Gly Pro Leu Ala Glu Ile Leu
 305 310 315 320

Arg Pro Ser Thr Asn Leu Ala Ile Ser Gly Asp Ile Glu Ser Tyr Ser
 325 330 335

Leu Met Glu Thr Pro Thr Pro Ser Ser Ser Pro Ser Arg Val Met Lys
 340 345 350

Lys Met Thr Ser Ser Val Ser Asp Glu Ser Ser Gln Val
 355 360 365

<210> 39
 <211> 493
 <212> PRT
 <213> 拟南芥

<400> 39

Met Arg Met Leu Leu Gly Ile Pro Tyr Val Asp Lys Ser Val Leu Ser
1 5 10 15

Asn Ser Val Leu Glu Arg Gly Lys Gln Asp Lys Ser Lys Leu Leu Leu
20 25 30

Val Asp Lys Cys His Tyr Glu Leu Asp Val Glu Glu Arg Lys Glu Asp
35 40 45

Phe Val Gly Gly Phe Gly Phe Gly Val Val Glu Asn Ser His Lys Asp
50 55 60

Val Met Val Leu Pro His His His Tyr Tyr Pro Ser Tyr Ser Ser Pro
65 70 75 80

Ser Ser Ser Ser Leu Cys Tyr Cys Ser Ala Gly Val Ser Asp Pro Met
85 90 95

Phe Ser Val Ser Ser Asn Gln Ala Tyr Thr Ser Ser His Ser Gly Met
100 105 110

[0050]

Phe Thr Pro Ala Gly Ser Gly Ser Ala Ala Val Thr Val Ala Asp Pro
115 120 125

Phe Phe Ser Leu Ser Ser Ser Gly Glu Met Arg Arg Ser Met Asn Glu
130 135 140

Asp Ala Gly Ala Ala Phe Ser Glu Ala Gln Trp His Glu Leu Glu Arg
145 150 155 160

Gln Arg Asn Ile Tyr Lys Tyr Met Met Ala Ser Val Pro Val Pro Pro
165 170 175

Glu Leu Leu Thr Pro Phe Pro Lys Asn His Gln Ser Asn Thr Asn Pro
180 185 190

Asp Val Asp Thr Tyr Arg Ser Gly Met Phe Ser Ile Tyr Ala Asp Tyr
195 200 205

Lys Asn Leu Pro Leu Ser Met Trp Met Thr Val Thr Val Ala Val Ala
210 215 220

Thr Gly Gly Ser Leu Gln Leu Gly Ile Ala Ser Ser Ala Ser Asn Asn
225 230 235 240

Thr Ala Asp Leu Glu Pro Trp Arg Cys Lys Arg Thr Asp Gly Lys Lys

245 250 255
 Trp Arg Cys Ser Arg Asn Val Ile Pro Asp Gln Lys Tyr Cys Glu Arg
 260 265 270
 His Thr His Lys Ser Arg Pro Arg Ser Arg Lys His Val Glu Ser Ser
 275 280 285
 His Gln Ser Ser His His Asn Asp Ile Arg Thr Ala Lys Asn Asp Thr
 290 295 300
 Ser Gln Leu Val Arg Thr Tyr Pro Gln Phe Tyr Gly Gln Pro Ile Ser
 305 310 315 320
 Gln Ile Pro Val Leu Ser Thr Leu Pro Ser Ala Ser Ser Pro Tyr Asp
 325 330 335
 His His Arg Gly Leu Arg Trp Phe Thr Lys Glu Asp Asp Ala Ile Gly
 340 345 350
 Thr Leu Asn Pro Glu Thr Gln Glu Ala Val Gln Leu Lys Val Gly Ser
 355 360 365
 [0051] Ser Arg Glu Leu Lys Arg Gly Phe Asp Tyr Asp Leu Asn Phe Arg Gln
 370 375 380
 Lys Glu Pro Ile Val Asp Gln Ser Phe Gly Ala Leu Gln Gly Leu Leu
 385 390 395 400
 Ser Leu Asn Gln Thr Pro Gln His Asn Gln Glu Thr Arg Gln Phe Val
 405 410 415
 Val Glu Gly Lys Gln Asp Glu Ala Met Gly Ser Ser Leu Thr Leu Ser
 420 425 430
 Met Ala Gly Gly Gly Met Glu Glu Thr Glu Gly Thr Asn Gln His Gln
 435 440 445
 Trp Val Ser His Glu Gly Pro Ser Trp Leu Tyr Ser Thr Thr Pro Gly
 450 455 460
 Gly Pro Leu Ala Glu Ala Leu Cys Leu Gly Val Ser Asn Asn Pro Ser
 465 470 475 480
 Ser Ser Thr Thr Thr Ser Ser Cys Ser Arg Ser Ser Ser
 485 490

<210> 40

<211> 431
 <212> PRT
 <213> 拟南芥

<400> 40

Met Lys Met Gln Ser Pro Lys Met Glu Gln Glu Glu Val Glu Glu Glu
 1 5 10 15

Arg Met Arg Asn Lys Trp Pro Trp Met Lys Ala Ala Gln Leu Met Glu
 20 25 30

Phe Arg Met Gln Ala Leu Val Tyr Arg Tyr Ile Glu Ala Gly Leu Arg
 35 40 45

Val Pro His His Leu Val Val Pro Ile Trp Asn Ser Leu Ala Leu Ser
 50 55 60

Ser Ser Ser Asn Tyr Asn Tyr His Ser Ser Ser Leu Leu Ser Asn Lys
 65 70 75 80

Gly Val Thr His Ile Asp Thr Leu Glu Thr Glu Pro Thr Arg Cys Arg
 85 90 95

Arg Thr Asp Gly Lys Lys Trp Arg Cys Ser Asn Thr Val Leu Leu Phe
 100 105 110

[0052]

Glu Lys Tyr Cys Glu Arg His Met His Arg Gly Arg Lys Arg Ser Arg
 115 120 125

Lys Leu Val Glu Ser Ser Ser Glu Val Ala Ser Ser Ser Thr Lys Tyr
 130 135 140

Asp Asn Thr Tyr Gly Leu Asp Arg Tyr Asn Glu Ser Gln Ser His Leu
 145 150 155 160

His Gly Thr Ile Ser Gly Ser Ser Asn Ala Gln Val Val Thr Ile Ala
 165 170 175

Ser Leu Pro Ser Ala Arg Ser Cys Glu Asn Val Ile Arg Pro Ser Leu
 180 185 190

Val Ile Ser Glu Phe Thr Asn Lys Ser Val Ser His Gly Arg Lys Asn
 195 200 205

Met Glu Met Ser Tyr Asp Asp Phe Ile Asn Glu Lys Glu Ala Ser Met
 210 215 220

Cys Val Gly Val Val Pro Leu Gln Gly Asp Glu Ser Lys Pro Ser Val
 225 230 235 240

Gln Lys Phe Phe Pro Glu Val Ser Asp Lys Cys Leu Glu Ala Ala Lys
 245 250 255

Phe Ser Ser Asn Arg Lys Asn Asp Ile Ile Ala Arg Ser Arg Glu Trp
 260 265 270

Lys Asn Met Asn Val Asn Gly Gly Leu Phe His Gly Ile His Phe Ser
 275 280 285

Pro Asp Thr Val Leu Gln Glu Arg Gly Cys Phe Arg Leu Gln Gly Val
 290 295 300

Glu Thr Asp Asn Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys
 305 310 315 320

Trp Arg Cys Ser Lys Asp Val Leu Ser Gly Gln Lys Tyr Cys Asp Lys
 325 330 335

His Met His Arg Gly Met Lys Lys Lys His Pro Val Asp Thr Thr Asn
 340 345 350

[0053]

Ser His Glu Asn Ala Gly Phe Ser Pro Leu Thr Val Glu Thr Ala Val
 355 360 365

Arg Ser Val Val Pro Cys Lys Asp Gly Asp Asp Gln Lys His Ser Val
 370 375 380

Ser Val Met Gly Ile Thr Leu Pro Arg Val Ser Asp Glu Lys Ser Thr
 385 390 395 400

Ser Ser Cys Ser Thr Asp Thr Thr Ile Thr Asp Thr Ala Leu Arg Gly
 405 410 415

Glu Asp Asp Asp Glu Glu Tyr Leu Ser Leu Phe Ser Pro Gly Val
 420 425 430

<210> 41

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 41

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 42
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 42

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

[0054]

<210> 43
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 43

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 44
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 44

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 45
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 45

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

[0055]

<210> 46
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 46

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 47
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 47

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 48

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 48

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

[0056]

His Arg Gly Arg Asn Arg
 35

<210> 49

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 49

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 50

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 50

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 51

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 51

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

[0057]

His Arg Gly Arg Asn Arg
 35

<210> 52

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 52

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 53

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 53

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 54

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 54

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

[0058]

His Arg Gly Arg Asn Arg
 35

<210> 55

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 55

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 56

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 56

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 57

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 57

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

[0059]

Cys Ser Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 58

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 58

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 59

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 59

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 60

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 60

[0060]

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 61

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 61

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 62
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 62

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 63
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 63

[0061]

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 64
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 64

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 65
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 65

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 66
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

[0062]

<400> 66

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 67
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 67

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
35

<210> 68
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 68

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
35

<210> 69
<211> 38
<212> PRT
<213> 人工序列

[0063]

<220>
<223> 指示物基序变体

<400> 69

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
35

<210> 70
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 70

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
35

<210> 71
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 71

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
20 25 30

His Arg Gly Arg Asn Arg
35

[0064]

<210> 72
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 72

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
20 25 30

His Arg Gly Arg Asn Arg
35

<210> 73
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 73

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 74
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 74

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

[0065]

<210> 75
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 75

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 76
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 76

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 77

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 77

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

[0066]

<210> 78

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 78

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 79

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 79

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 80

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 80

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

[0067]

His Arg Gly Lys Asn Arg
 35

<210> 81

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 81

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 82

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 82

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 83

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 83

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

[0068]

His Arg Gly Lys Asn Arg
 35

<210> 84

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 84

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 85

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 85

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 86

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 86

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

[0069]

His Arg Gly Lys Asn Arg
 35

<210> 87

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 87

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 88

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 88

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 89

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 89

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

[0070]

Cys Ser Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 90

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 90

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 91

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 91

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
20 25 30

His Arg Gly Lys Asn Arg
35

<210> 92

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 92

[0071]

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
20 25 30

His Arg Gly Lys Asn Arg
35

<210> 93

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 93

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
20 25 30

His Arg Gly Lys Asn Arg
35

<210> 94
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 94

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 95
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 95

[0072]

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 96
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 96

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 97
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 97

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 98
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

[0073]

<400> 98

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 99
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 99

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
35

<210> 100
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 100

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
20 25 30

His Arg Gly Lys Asn Arg
35

<210> 101
<211> 38
<212> PRT
<213> 人工序列

[0074]

<220>
<223> 指示物基序变体

<400> 101

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
20 25 30

His Arg Gly Lys Asn Arg
35

<210> 102
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 102

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
20 25 30

His Arg Gly Lys Asn Arg
35

<210> 103
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 103

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
20 25 30

His Arg Gly Lys Asn Arg
35

<210> 104
<211> 38
<212> PRT
<213> 人工序列

[0075]

<220>
<223> 指示物基序变体

<400> 104

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
20 25 30

His Arg Gly Lys Asn Arg
35

<210> 105
<211> 1080
<212> DNA
<213> 人工序列

<220>
<223> GmGRF5的cDNA

<400> 105

atgatgagtg caagtcagg tgcaagaaat aggtctccgt tcacacaaat tcagtggtgca 60

gagcttgagc aacaagtct tgttttaag tacatggta caggaacacc tatcccacca 120

gatctcatct actctattaa aagaagtcta gacactcaa tttctcaag gctcttccca 180

catcatcaa ttgggtgggg atgttttgaa atgggatttg gcagaaaagt agaccagag 240
 ccagggaggt gcagaagaac agatggcaag aatggagat gttcaaagga ggcatatcca 300
 gactcaaagt actgtgaaag acacatgcac agaggcagaa accgttcaag aaagcctgtg 360
 gaagtttctt cagcaacaag caccgccaca aacacctccc aaacaatccc atcatcttat 420
 accagaaacc tttcttgac caataacagt aacccaaca taaccacc accaccacc 480
 tcttcttcc ctttctcta ttgcccctct tctatgccta ttgatcagtc ccaacccttt 540
 tcccaatct accaaaactc ttcttcaat cccttctct actccaatc aacctctct 600
 agacccccag atgtgtgatt tccacccaa gatgccacca cccaccact attcatggac 660
 tctgtggct cttattctca tgatgaaag aattataggc atgttcatgg aataagggaa 720
 gatgtggatg agagagcttt cttccagaa gcatcaggat cagctaggag ctatacagac 780
 tcgtaccaac aactatcaat gagctctac aagtcctatt caaactcaa ctttcagaac 840
 attaataatg atgccaccac caaccaaga cagcaagagc agcaactaca acaacaaca 900
 cactgttttg ttttagggac agacttcaa tcaacaaggc caagcaaga gaaagaagct 960
 gagacaaca caggtcagag acccctcac cgtttcttg gggagtggcc accaaagaac 1020
 acaacaacag attcttgct agatcttct tccaactcca gaatccaac cgatgaatga 1080

[0076]

<210> 106
 <211> 359
 <212> PRT
 <213> 大豆

<400> 106

Met Met Ser Ala Ser Ala Gly Ala Arg Asn Arg Ser Pro Phe Thr Gln
 1 5 10 15

Ile Gln Trp Gln Glu Leu Glu Gln Gln Ala Leu Val Phe Lys Tyr Met
 20 25 30

Val Thr Gly Thr Pro Ile Pro Pro Asp Leu Ile Tyr Ser Ile Lys Arg
 35 40 45

Ser Leu Asp Thr Ser Ile Ser Ser Arg Leu Phe Pro His His Pro Ile
 50 55 60

Gly Trp Gly Cys Phe Glu Met Gly Phe Gly Arg Lys Val Asp Pro Glu
 65 70 75 80

Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg Cys Ser Lys
 85 90 95

Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met His Arg Gly

	100	105	110
	Arg Asn Arg Ser Arg Lys Pro Val Glu Val Ser Ser Ala Thr Ser Thr		
	115	120	125
	Ala Thr Asn Thr Ser Gln Thr Ile Pro Ser Ser Tyr Thr Arg Asn Leu		
	130	135	140
	Ser Leu Thr Asn Asn Ser Asn Pro Asn Ile Thr Pro Pro Pro Pro		
	145	150	155 160
	Ser Ser Phe Pro Phe Ser His Leu Pro Ser Ser Met Pro Ile Asp Gln		
	165	170	175
	Ser Gln Pro Phe Ser Gln Ser Tyr Gln Asn Ser Ser Leu Asn Pro Phe		
	180	185	190
	Phe Tyr Ser Gln Ser Thr Ser Ser Arg Pro Pro Asp Ala Asp Phe Pro		
	195	200	205
	Pro Gln Asp Ala Thr Thr His His Leu Phe Met Asp Ser Ala Gly Ser		
	210	215	220
[0077]	Tyr Ser His Asp Glu Lys Asn Tyr Arg His Val His Gly Ile Arg Glu		
	225	230	235 240
	Asp Val Asp Glu Arg Ala Phe Phe Pro Glu Ala Ser Gly Ser Ala Arg		
	245	250	255
	Ser Tyr Thr Asp Ser Tyr Gln Gln Leu Ser Met Ser Ser Tyr Lys Ser		
	260	265	270
	Tyr Ser Asn Ser Asn Phe Gln Asn Ile Asn Asn Asp Ala Thr Thr Asn		
	275	280	285
	Pro Arg Gln Gln Glu Gln Gln Leu Gln Gln Gln Gln His Cys Phe Val		
	290	295	300
	Leu Gly Thr Asp Phe Lys Ser Thr Arg Pro Ser Lys Glu Lys Glu Ala		
	305	310	315 320
	Glu Thr Thr Thr Gly Gln Arg Pro Leu His Arg Phe Phe Gly Glu Trp		
	325	330	335
	Pro Pro Lys Asn Thr Thr Thr Asp Ser Trp Leu Asp Leu Ala Ser Asn		
	340	345	350
	Ser Arg Ile Gln Thr Asp Glu		

355

<210> 107
 <211> 1173
 <212> DNA
 <213> 人工序列

<220>
 <223> BnGRF5的cDNA

<400> 107
 atgatgagtc taagtggaaa tgggtgggaga acaatagaga ggctccatt tacaccaaca 60
 caatggcaag aactggagaa tcaagccta attacaagt acatggtctc aggagttcct 120
 gtcccacctg agctcatctt ctccattaga agaagcttg actcttctt ggtctctaga 180
 ctctccctc accaatccat tgggtgggga tgctatcaga tggggttgg tagaaaacca 240
 gatccagaac caggaagggtg cagaagaaca gatggaaga aatggagatg ctcaagagaa 300
 gcataccag attcaaagta ctgtgaaaa cacatgcaca gaggaaggaa ccgtgccaga 360
 aaatctattg atcagaatca gacaactgct ctttaacat caccatctct ctcttcccc 420
 aacaacaaca acccaagccc tacctgtct tcttctctct ctactattc agcttctct 480
 tcctctctt ccattgatgc ttacagtaat atcaataggc ttggtgttg taatagtaac 540
 [0078] agtagaggtt actcaacaa ccattccctt gactatcctt atccttctc ctacacataa 600
 cagcaacaac aacaacagac tcttcatcat gcttctgctt tgtctctca ccaaaacgca 660
 tctactgctt ctcaagtcaa tgccttagct tctgcaactg accataaaga cttcagatac 720
 ttcaagga ttggggagag agttggagtt ggagctgggg agaggacttt tttccagaa 780
 gcttctagaa gcttcaaga ttctccatac catcaccaac aaccgtagc tacagtaatg 840
 aatgaccctg tccactctgg tactgatcat aaggttgatc atcagcatca cacatactca 900
 tccgatcat catcatctca gcatgatcaa gatcatcatc gacaacaaca gcagcaatgt 960
 ttgttatgg gcgctgacat gttcaacaaa cccacaagaa ctgtctctgc aaactcatct 1020
 agacaagatc atcaagaaga ggaggagaaa gattcatcag aaacaagaa gtctctacat 1080
 catttcttg gtgaggactg ggcacagAAC aaaaacagtt cagattcttg gcttgacctt 1140
 tcttccatt caagactgga cactgtagt tga 1173

<210> 108
 <211> 390
 <212> PRT
 <213> 欧洲油菜

<400> 108

Met Met Ser Leu Ser Gly Asn Gly Gly Arg Thr Ile Glu Arg Pro Pro
 1 5 10 15

Phe Thr Pro Thr Gln Trp Gln Glu Leu Glu Asn Gln Ala Leu Ile Tyr
 20 25 30

Lys Tyr Met Val Ser Gly Val Pro Val Pro Pro Glu Leu Ile Phe Ser
 35 40 45

Ile Arg Arg Ser Leu Asp Ser Ser Leu Val Ser Arg Leu Leu Pro His
 50 55 60

Gln Ser Ile Gly Trp Gly Cys Tyr Gln Met Gly Phe Gly Arg Lys Pro
 65 70 75 80

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 85 90 95

Cys Ser Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 100 105 110

His Arg Gly Arg Asn Arg Ala Arg Lys Ser Ile Asp Gln Asn Gln Thr
 115 120 125

Thr Ala Pro Leu Thr Ser Pro Ser Leu Ser Phe Pro Asn Asn Asn Asn
 130 135 140

[0079]

Pro Ser Pro Thr Leu Ser Ser Ser Ser Thr Tyr Ser Ala Ser Ser
 145 150 155 160

Ser Ser Pro Ser Ile Asp Ala Tyr Ser Asn Ile Asn Arg Leu Gly Val
 165 170 175

Gly Asn Ser Asn Ser Arg Gly Tyr Phe Asn Asn His Ser Leu Asp Tyr
 180 185 190

Pro Tyr Pro Leu Ser Ser Pro Lys Gln Gln Gln Gln Gln Thr Leu
 195 200 205

His His Ala Ser Ala Leu Ser Leu His Gln Asn Ala Ser Thr Ala Ser
 210 215 220

Gln Phe Asn Ala Leu Ala Ser Ala Thr Asp His Lys Asp Phe Arg Tyr
 225 230 235 240

Phe Gln Gly Ile Gly Glu Arg Val Gly Val Gly Ala Gly Glu Arg Thr
 245 250 255

Phe Phe Pro Glu Ala Ser Arg Ser Phe Gln Asp Ser Pro Tyr His His
 260 265 270

Gln Gln Pro Leu Ala Thr Val Met Asn Asp Pro Phe His Ser Gly Thr
275 280 285

Asp His Lys Val Asp His Gln His His Thr Tyr Ser Ser Val Ser Ser
290 295 300

Ser Ser Gln His Asp Gln Asp His His Arg Gln Gln Gln Gln Cys
305 310 315 320

Phe Val Met Gly Ala Asp Met Phe Asn Lys Pro Thr Arg Thr Val Phe
325 330 335

Ala Asn Ser Ser Arg Gln Asp His Gln Glu Glu Glu Lys Asp Ser
340 345 350

Ser Glu Thr Lys Lys Ser Leu His His Phe Phe Gly Glu Asp Trp Ala
355 360 365

Gln Asn Lys Asn Ser Ser Asp Ser Trp Leu Asp Leu Ser Ser His Ser
370 375 380

Arg Leu Asp Thr Gly Ser
385 390

[0080]

<210> 109

<211> 981

<212> DNA

<213> 人工序列

<220>

<223> HaGRF5的cDNA

<400> 109

atgatgatga tgagtactac aagcagtaac caaaatccaa atgtgttcac agcatcacia 60

tggaagaac tgaacagca agcttaatc tacaagtata tggtttcagg tgttcagtt 120

ccaactgatc tcatctgtc tgtcagaaga agtttgata acacctcagc tcatcacta 180

tctaaccaac acacctcctc cttaggaata tgggaagctg gatcaagctt tccatacaat 240

cagttgatc agattggtgg gtaggtggc agaaagatag atttagaacc aggaagatgc 300

agaagaacag atggaaaaa atggaggtgc ttaaagaag cttacccga ttaaaatac 360

tgcgagagac acatgcacag aggtagaac cgttcaagaa agcctgtgga attctctct 420

tctctctt catctcatc tgctgccaca agtgtaata atgttcttc tcatcagca 480

atctccaaat caatcgatgc ttacctcct ctttctcaa ctttatgga ttctcatct 540

tattctacc aaacccttaa agattacagg cagatgcaag gaatgaagga ttaggagag 600

gatgagagat catcagcagc agcagcagct tacttcaac taaatgatcc ttaccgcc 660

actacacaat ctggtcaaca aaactactct catttcagtt tcaaaacct gaaagatgag 720

cagaagaagg agcaagggca gcaactgttt gtgatgggta ctgatttcat aaagccatca 780
 gaagaacatg aaccaccaa atccactacc acaaaagtta atgaaaccac cagtaaacia 840
 ccattccacc acttctttc accaccaaaa gccacactgc ttaaccctaa ccatgacca 900
 aactggggtg aagttgacca cccaagggcc cctttgtcca cccaagacct tttccaatcc 960
 aaaccaagac ctactggta g 981

<210> 110
 <211> 326
 <212> PRT
 <213> 葵花
 <400> 110

Met Met Met Met Ser Thr Thr Ser Ser Asn Gln Asn Pro Asn Val Phe
 1 5 10 15

Thr Ala Ser Gln Trp Glu Glu Leu Glu Gln Gln Ala Leu Ile Tyr Lys
 20 25 30

Tyr Met Val Ser Gly Val Pro Val Pro Thr Asp Leu Ile Leu Ser Val
 35 40 45

[0081] Arg Arg Ser Leu Tyr Asn Thr Ser Ala Ser Ser Leu Ser Asn Gln His
 50 55 60

Thr Ser Ser Leu Gly Ile Trp Glu Ala Gly Ser Ser Phe Pro Tyr Asn
 65 70 75 80

Gln Leu Tyr Gln Ile Gly Gly Tyr Gly Gly Arg Lys Ile Asp Leu Glu
 85 90 95

Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg Cys Ser Lys
 100 105 110

Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met His Arg Gly
 115 120 125

Arg Asn Arg Ser Arg Lys Pro Val Glu Phe Ser Ser Ser Ser Ser
 130 135 140

Ser Ser Ser Ala Ala Thr Ser Val Asn Asn Val Ser Ser Ser Ala
 145 150 155 160

Ile Ser Lys Ser Ile Asp Ala Tyr Pro Pro Pro Phe Ser Thr Phe Met
 165 170 175

Asp Ser Ser Ser Tyr Ser His Gln Thr Leu Lys Asp Tyr Arg Gln Met

180 185 190

Gln Gly Met Lys Asp Leu Gly Glu Asp Glu Arg Ser Ser Ala Ala Ala
 195 200 205

Ala Ala Tyr Phe Gln Leu Asn Asp Pro Tyr Thr Ala Thr Thr Gln Ser
 210 215 220

Gly Gln Gln Asn Tyr Ser His Phe Ser Phe Gln Asn Leu Lys Asp Glu
 225 230 235 240

Gln Lys Lys Glu Gln Gly Gln His Cys Phe Val Met Gly Thr Asp Phe
 245 250 255

Ile Lys Pro Ser Glu Glu His Glu Pro Thr Lys Ser Thr Thr Thr Lys
 260 265 270

Val Asn Glu Thr Thr Ser Lys Gln Pro Phe His His Phe Phe Ser Pro
 275 280 285

Pro Lys Ala Thr Leu Leu Asn Pro Asn His Asp Pro Asn Trp Gly Glu
 290 295 300

[0082] Val Asp His Pro Lys Ala Pro Leu Ser Thr Gln Asp Leu Phe Gln Ser
 305 310 315 320

Lys Pro Arg Pro Tyr Trp
 325

<210> 111
 <211> 1131
 <212> DNA
 <213> 人工序列

<220>
 <223> ZmGRF5的cDNA

<400> 111
 atgatgatga tgagcggctg agcggccacc gcggggcggg acccggtcac ggcgtcgag 60
 tggcaggagc tggagacca ggcgctcacc tacaagtgcc tggcgtccgg caagccatc 120
 ccgtctacc tcattgcc gctccggcgc atcctcgact ccgccctcgc cacgtcgccg 180
 tcgctcgccg ctttccgcc gcaaccctcg ctgggggtggg ggggctgctt cgggatgggc 240
 ttcagcagga agcccgccga cgaggaccgg gagcccgggc ggtgccggcg cacggacggc 300
 aagaagtggc gctgctccaa ggaggcgtac cgggactcca agtactgcga gaagcacatg 360
 caccggggca agaaccgttc aagaagcct gtggaaatgt ccttgccac gccggcggcg 420
 gcctctctcg ccacaagcgc cgccggcggc gccacctct cgtcccaggc gccgtctctac 480

cacagcccgg cccccccgt gccgtaccac gcgccctacg gcgccgcgta ccatcacacg 540
 cagagcgagg tgacgatgag cccctccac ctctccacc tcgagaccac ccaccgcac 600
 ccgccggccg cgccggccc gccctactac tacgaggacc agagggacta cgctacggc 660
 aaggaggctg gcgagcgcg cttcttccc gacggcgcg gcgagagggga ccggcagcag 720
 caggcccggg ggcagtggca gttcaagcag ctcgggacga tggaggcgac gaagcagccg 780
 tgcaccacgc cgctgctcgt ccccgcccgc gggtagcgcc acggcgcggc gtcgccgtac 840
 ggcgtcggtc agccaagga agacaggag gaggaggaaa cgcgccggca gcagcagcac 900
 tgcttcgttc ttggcgcca cctgcggctg gcggagcggc cgtcgggggc acatgacgcc 960
 gccgcgaga agccgctccg gcatttcac gacgagtggc gcacagagaa ggggagcaat 1020
 aaggcggggg cgtggatggg ggggctcgac ggcgagacga cgagctctc catgtctatc 1080
 ccgatggcgg ccgctgccga cctccccgtc acctcccgt accgtacgtg a 1131

<210> 112
 <211> 376
 <212> PRT
 <213> 玉蜀黍
 <400> 112

[0083]

Met Met Met Met Ser Gly Arg Ala Ala Thr Ala Gly Arg Tyr Pro Phe
 1 5 10 15
 Thr Ala Ser Gln Trp Gln Glu Leu Glu His Gln Ala Leu Ile Tyr Lys
 20 25 30
 Cys Leu Ala Ser Gly Lys Pro Ile Pro Ser Tyr Leu Met Pro Pro Leu
 35 40 45
 Arg Arg Ile Leu Asp Ser Ala Leu Ala Thr Ser Pro Ser Leu Ala Ala
 50 55 60
 Phe Pro Pro Gln Pro Ser Leu Gly Trp Gly Gly Cys Phe Gly Met Gly
 65 70 75 80
 Phe Ser Arg Lys Pro Ala Asp Glu Asp Pro Glu Pro Gly Arg Cys Arg
 85 90 95
 Arg Thr Asp Gly Lys Lys Trp Arg Cys Ser Lys Glu Ala Tyr Pro Asp
 100 105 110
 Ser Lys Tyr Cys Glu Lys His Met His Arg Gly Lys Asn Arg Ser Arg
 115 120 125
 Lys Pro Val Glu Met Ser Leu Ala Thr Pro Ala Pro Ala Ser Ser Ala
 130 135 140

Thr Ser Ala Ala Ala Ala Thr Ser Ser Ser Gln Ala Pro Ser Tyr
145 150 155 160

His Ser Pro Ala Pro Ala Val Pro Tyr His Ala Pro Tyr Gly Ala Ala
165 170 175

Tyr His His Thr Gln Thr Gln Val Thr Met Ser Pro Phe His Leu Leu
180 185 190

His Leu Glu Thr Thr His Pro His Pro Pro Pro Pro Pro Pro Pro
195 200 205

Tyr Tyr Tyr Ala Asp Gln Arg Asp Tyr Ala Tyr Gly Lys Glu Val Gly
210 215 220

Glu Arg Ala Phe Phe Ser Asp Gly Ala Gly Glu Arg Asp Arg Gln Gln
225 230 235 240

Gln Ala Ala Gly Gln Trp Gln Phe Lys Gln Leu Gly Thr Met Glu Ala
245 250 255

Thr Lys Gln Pro Cys Thr Thr Pro Leu Leu Val Pro Ala Ala Gly Tyr
260 265 270

[0084]

Gly His Gly Ala Ala Ser Pro Tyr Gly Val Gly Gln Ala Lys Glu Asp
275 280 285

Glu Glu Glu Glu Glu Thr Arg Arg Gln Gln Gln His Cys Phe Val Leu
290 295 300

Gly Ala Asp Leu Arg Leu Ala Glu Arg Pro Ser Gly Ala His Asp Ala
305 310 315 320

Ala Ala Gln Lys Pro Leu Arg His Phe Ile Asp Glu Trp Pro His Glu
325 330 335

Lys Gly Ser Asn Lys Ala Gly Ser Trp Met Gly Gly Leu Asp Gly Glu
340 345 350

Thr Thr Gln Leu Ser Met Ser Ile Pro Met Ala Ala Ala Ala Asp Leu
355 360 365

Pro Val Thr Ser Arg Tyr Arg Thr
370 375

<210> 113

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 113

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 114

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 114

[0085]

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 115

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 115

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 116
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 116

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 117
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 117

[0086]

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 118
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 118

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 119
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 119

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 120
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

[0087]

<400> 120

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 121
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 121

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
35

<210> 122
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 122

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
35

<210> 123
<211> 38
<212> PRT
<213> 人工序列

[0088]

<220>
<223> 指示物基序变体

<400> 123

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
35

<210> 124
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 124

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
35

<210> 125
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 125

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
20 25 30

His Arg Gly Arg Asn Arg
35

[0089]

<210> 126
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 126

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
20 25 30

His Arg Gly Arg Asn Arg
35

<210> 127
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 127

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 128
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 128

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

[0090]

<210> 129
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 129

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 130
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 130

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 131
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 131

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

[0091]

<210> 132
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 132

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 133
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 133

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 134

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 134

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

[0092]

His Arg Gly Arg Asn Arg
 35

<210> 135

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 135

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 136

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 136

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 137

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 137

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

[0093]

His Arg Gly Arg Asn Arg
 35

<210> 138

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 138

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 139

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 139

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 140

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 140

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

[0094]

His Arg Gly Arg Asn Arg
 35

<210> 141

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 141

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 142

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 142

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 143

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 143

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

[0095]

Cys Ser Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 144

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 144

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 145

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 145

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 146

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 146

[0096]

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 147

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 147

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 148
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 148

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 149
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 149

[0097]

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 150
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 150

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 151
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 151

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 152
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

[0098]

<400> 152

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 153
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 153

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
35

<210> 154
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 154

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
35

<210> 155
<211> 38
<212> PRT
<213> 人工序列

[0099]

<220>
<223> 指示物基序变体

<400> 155

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
35

<210> 156
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 156

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
35

<210> 157
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 157

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
20 25 30

His Arg Gly Lys Asn Arg
35

<210> 158
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 158

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
20 25 30

His Arg Gly Lys Asn Arg
35

<210> 159
<211> 38
<212> PRT
<213> 人工序列

<220>
<223> 指示物基序变体

<400> 159

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

[0100]

Cys Ser Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 160
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 160

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

[0101]

<210> 161
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 161

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 162
 <211> 38
 <212> PRT
 <213> 人工序列

<220>
 <223> 指示物基序变体

<400> 162

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 163

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 163

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

[0102]

<210> 164

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 164

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 165

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 165

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 166

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 166

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

[0103] His Arg Gly Lys Asn Arg
 35

<210> 167

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 167

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 168

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 168

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Asp Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 169

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 169

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

[0104]

His Arg Gly Lys Asn Arg
 35

<210> 170

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 170

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Arg Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 171

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 171

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 172

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 172

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Glu Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

[0105]

His Arg Gly Lys Asn Arg
 35

<210> 173

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 173

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 174

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 174

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Arg Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 175

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 175

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

[0106]

Cys Ser Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 176

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> 指示物基序变体

<400> 176

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ala Lys Asp Ala His Pro Asp Ser Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 177

<211> 38

<212> PRT
 <213> 人工序列

<220>
 <223> GRF5特异性指示物基序

<220>
 <221> VARIANT
 <222> (2)..(2)
 <223> X是脯氨酸(P)或亮氨酸(L)

<220>
 <221> VARIANT
 <222> (18)..(18)
 <223> X是丝氨酸(S)或丙氨酸(A)

<220>
 <221> VARIANT
 <222> (19)..(19)
 <223> X是精氨酸(R)或赖氨酸(K)

<220>
 <221> VARIANT
 <222> (20)..(20)
 <223> X是谷氨酸(E)或天冬氨酸(D)

<220>
 <221> VARIANT
 <222> (22)..(22)
 <223> X是酪氨酸(Y)或组氨酸(H)

[0107]

<220>
 <221> VARIANT
 <222> (30)..(30)
 <223> X是赖氨酸(K)或精氨酸(R)

<220>
 <221> VARIANT
 <222> (36)..(36)
 <223> X是精氨酸(R)或赖氨酸(K)

<400> 177

Asp Xaa Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Xaa Xaa Xaa Ala Xaa Pro Asp Ser Lys Tyr Cys Glu Xaa His Met
 20 25 30

His Arg Gly Xaa Asn Arg
 35

<210> 178
 <211> 38
 <212> PRT
 <213> 拟南芥

<400> 178

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg

1 5 10 15
 Cys Ser Arg Asp Ala Val Pro Asp Gln Lys Tyr Cys Glu Arg His Ile
 20 25 30

Asn Arg Gly Arg His Arg
 35

<210> 179
 <211> 38
 <212> PRT
 <213> 拟南芥

<400> 179

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Asp Ala Val Pro Asp Gln Lys Tyr Cys Glu Arg His Ile
 20 25 30

Asn Arg Gly Arg His Arg
 35

[0108]

<210> 180
 <211> 38
 <212> PRT
 <213> 拟南芥

<400> 180

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Asp Val Phe Ala Gly His Lys Tyr Cys Glu Arg His Met
 20 25 30

His Arg Gly Arg Asn Arg
 35

<210> 181
 <211> 38
 <212> PRT
 <213> 拟南芥

<400> 181

Asp Pro Glu Pro Gly Arg Cys Lys Arg Thr Asp Gly Lys Lys Trp Arg
 1 5 10 15

Cys Ser Arg Asp Val Val Ala Gly His Lys Tyr Cys Asp Arg His Ile
 20 25 30

His Arg Gly Arg Asn Arg
35

<210> 182
<211> 38
<212> PRT
<213> 拟南芥

<400> 182

Asp Ala Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
20 25 30

His Arg Gly Lys Asn Arg
35

<210> 183
<211> 38
<212> PRT
<213> 拟南芥

<400> 183

[0109]

Asp Leu Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ala Lys Glu Val Val Ser Asn His Lys Tyr Cys Glu Lys His Leu
20 25 30

His Arg Gly Arg Pro Arg
35

<210> 184
<211> 38
<212> PRT
<213> 拟南芥

<400> 184

Asp Leu Glu Pro Trp Arg Cys Lys Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Asn Val Ile Pro Asp Gln Lys Tyr Cys Glu Arg His Thr
20 25 30

His Lys Ser Arg Pro Arg
35

<210> 185
<211> 38
<212> PRT

<213> 拟南芥

<400> 185

Glu Thr Glu Pro Thr Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Asn Thr Val Leu Leu Phe Glu Lys Tyr Cys Glu Arg His Met
20 25 30

His Arg Gly Arg Lys Arg
35

<210> 186

<211> 38

<212> PRT

<213> 拟南芥

<400> 186

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Arg Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
20 25 30

[0110] His Arg Gly Arg Asn Arg
35

<210> 187

<211> 38

<212> PRT

<213> 甜菜

<400> 187

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
20 25 30

His Arg Gly Lys Asn Arg
35

<210> 188

<211> 38

<212> PRT

<213> 玉蜀黍

<400> 188

Asp Pro Glu Pro Gly Arg Cys Arg Arg Thr Asp Gly Lys Lys Trp Arg
1 5 10 15

Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
 20 25 30

His Arg Gly Lys Asn Arg
 35

<210> 189

<211> 38

<212> PRT

<213> 普通小麦

<400> 189

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His Arg Gly Lys Asn Arg
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<211> 38

<212> PRT

<213> 欧洲油菜

[0111]

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His Arg Gly Arg Asn Arg
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<213> 芜菁

<400> 191

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His Arg Gly Arg Asn Arg
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His Arg Gly Arg Asn Arg
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His Arg Gly Arg Asn Arg
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 <213> 两色蜀黍

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His Arg Gly Lys Asn Arg
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Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
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His Arg Gly Arg Asn Arg
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<213> 马铃薯

<400> 196

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His Arg Gly Arg Asn Arg
 35

[0113]

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His Arg Gly Lys Asn Arg
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His Arg Gly Lys Asn Arg
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<210> 199
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<400> 199

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Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
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His Arg Gly Arg Asn Arg
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[0114]

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His Arg Gly Arg Asn Arg
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His Arg Gly Lys Asn Arg
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Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Arg His Met
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His Arg Gly Arg Asn Arg
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<210> 203
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<400> 203

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His Arg Gly Arg Asn Arg
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<210> 204
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 <213> 葵花

<400> 204

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His Arg Gly Arg Asn Arg
 35

<210> 205
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 <213> 玉蜀黍

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Cys Ser Lys Glu Ala Tyr Pro Asp Ser Lys Tyr Cys Glu Lys His Met
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His Arg Gly Lys Asn Arg
 35

<210> 206
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 <213> 玉蜀黍

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His Arg Gly Lys Asn Arg
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[0116]

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	gggttcagcg ggtcatctat ggatgagtct tggcaaatcg gcggagggtc taacatcgat	780
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	cagctccagt ctaacagcac cacctctaac aacggaacca gcgctaagca agagaagcag	900
	tgttacatct ggggccgtga ttcaactgc gacctctcta tgaaggctga agaggaacgt	960
	gagaactcc acgaaaagac gaccaccac ttcttcgatg agtggcctat caaatctggt	1020
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	tga	1143
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<213> 玉蜀黍

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Arg Tyr Pro Phe Thr Ala Ser Gln Trp Gln Glu Leu Glu His Gln Ala
20 25 30

Leu Ile Tyr Lys Cys Leu Ala Ser Gly Lys Pro Ile Pro Ser Tyr Leu
35 40 45

Met Pro Pro Leu Arg Arg Ile Leu Asp Ser Ala Leu Ala Thr Ser Pro
50 55 60

Ser Leu Ala Tyr Pro Pro Gln Pro Ser Leu Gly Trp Gly Cys Phe Gly
65 70 75 80

[0118]

Met Gly Phe Thr Arg Lys Ala Asp Glu Asp Pro Glu Pro Gly Arg Cys
85 90 95

Arg Arg Thr Asp Gly Lys Lys Trp Arg Cys Ser Lys Glu Ala Tyr Pro
100 105 110

Asp Ser Lys Tyr Cys Glu Lys His Met His Arg Gly Lys Asn Arg Ser
115 120 125

Arg Lys Pro Val Glu Met Ser Leu Ala Thr Pro Ala Pro Ala Pro Ala
130 135 140

Pro Ala Ala Ala Thr Thr Ala Thr Ala Thr Ser Ser Pro Ala Pro Ser
145 150 155 160

Tyr His Arg Pro Ala His Asp Ala Thr Pro Ser Pro Tyr His Ala Leu
165 170 175

Tyr Gly Gly Gly Gly Gly Gly Gly Gly Ser Pro Tyr Ser Ala Ser Ala
180 185 190

Arg Pro Gly Ala Thr Gly Gly Gly Gly Ala Tyr His His Ala Gln His
195 200 205

Val Ser Pro Phe His Leu His Leu Glu Thr Thr His Pro His Pro Pro
 210 215 220

Pro Pro Tyr Asn Tyr Ser Ala Asp Gln Arg Asp Tyr Ala Tyr Gly His
 225 230 235 240

Ala Ala Ala Lys Glu Val Gly Glu His Ala Phe Phe Ser Asp Gly Ala
 245 250 255

Gly Glu Arg Val Asp Arg Gln Ala Ala Ala Gly Gln Trp Gln Phe Arg
 260 265 270

Gln Leu Gly Val Glu Thr Lys Pro Gly Pro Thr Pro Leu Phe Pro Val
 275 280 285

Ala Gly Tyr Gly His Gly Ala Ala Ser Pro Tyr Gly Val Glu Met Gly
 290 295 300

Lys Asp Asp Asp Glu Gln Glu Glu Arg Arg Arg Gln His Cys Phe Val
 305 310 315 320

Leu Gly Ala Asp Leu Arg Leu Glu Arg Pro Ser Ser Gly His Gly His
 325 330 335

[0119]

Gly His Asp His Asp Asp Ala Ala Ala Ala Gln Lys Pro Leu Arg Pro
 340 345 350

Phe Phe Asp Glu Trp Pro His Gln Lys Gly Asp Lys Ala Gly Ser Trp
 355 360 365

Met Gly Leu Asp Gly Glu Thr Gln Leu Ser Met Ser Ile Pro Met Ala
 370 375 380

Ala Thr Asp Leu Pro Val Thr Ser Arg Phe Arg Asn Gly Gly His Tyr
 385 390 395 400

Glu

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	aagaagtggc gctgcagcaa agaggcttac ccggacagca agtactgcga gaagcacatg	360
	cacaggggca agaacaggtc ccgcaagcca gttgagatga gcttggttac tccagctcca	420
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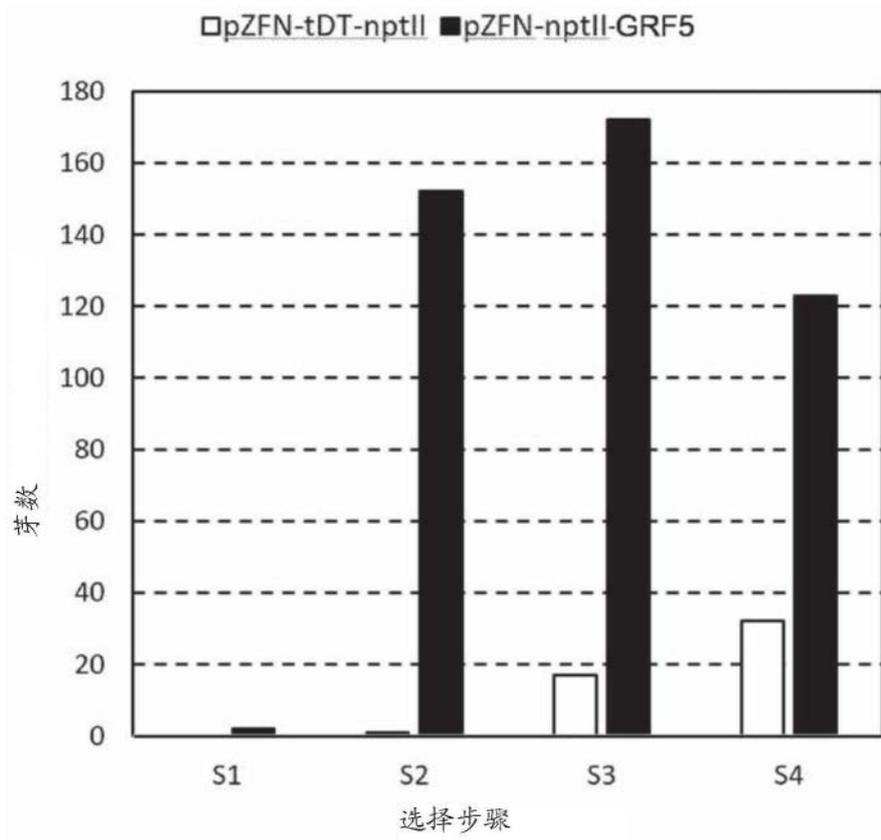


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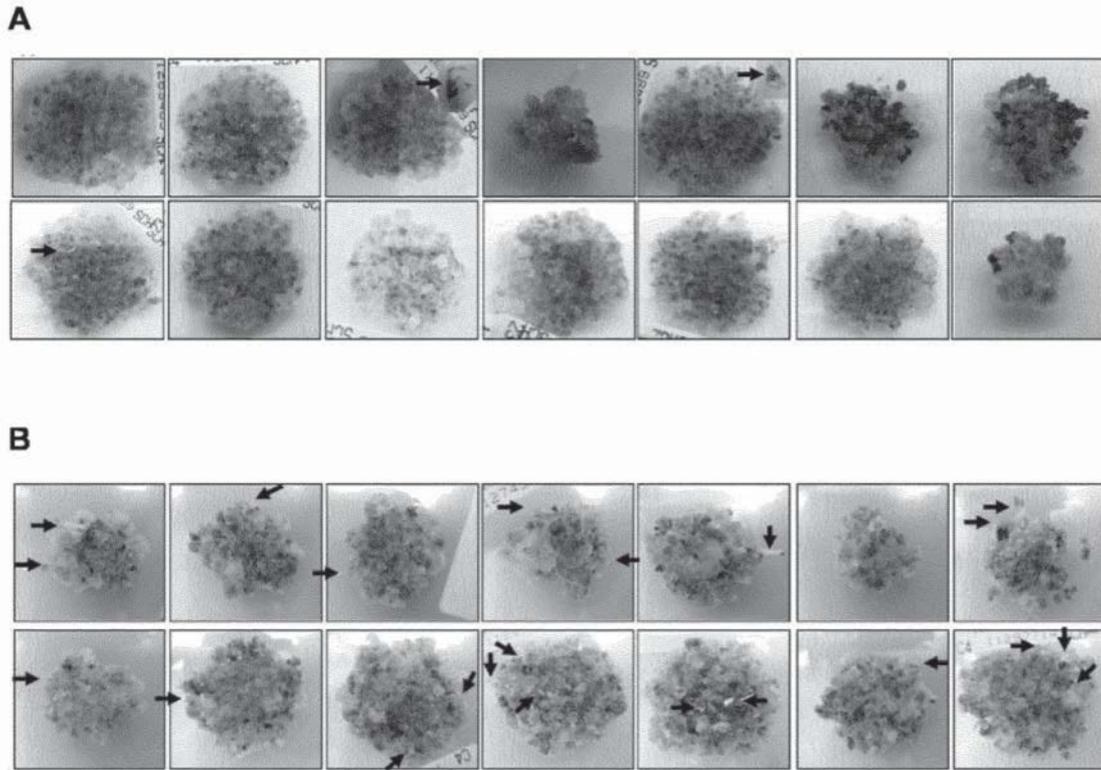
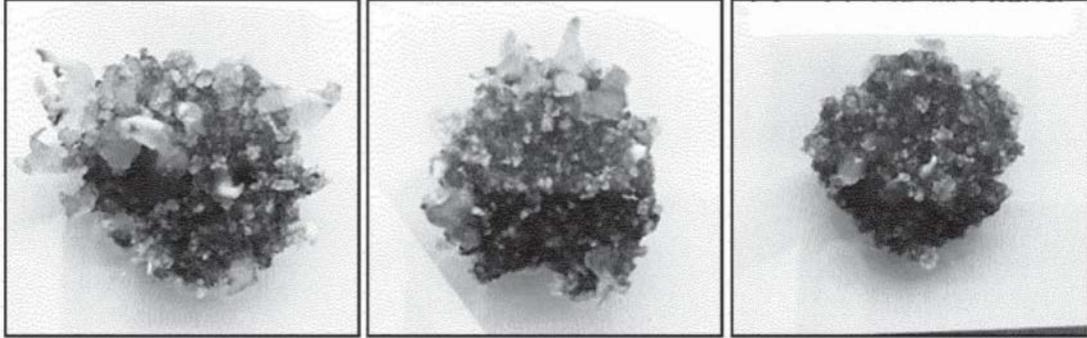


图2

A

pABM70S.1-GRF5-2014 + pUbi-tDT-nptII (1:1)



B

pABM70S-TurboYFP-2014 + pUbi-tDT-nptII (1:1)

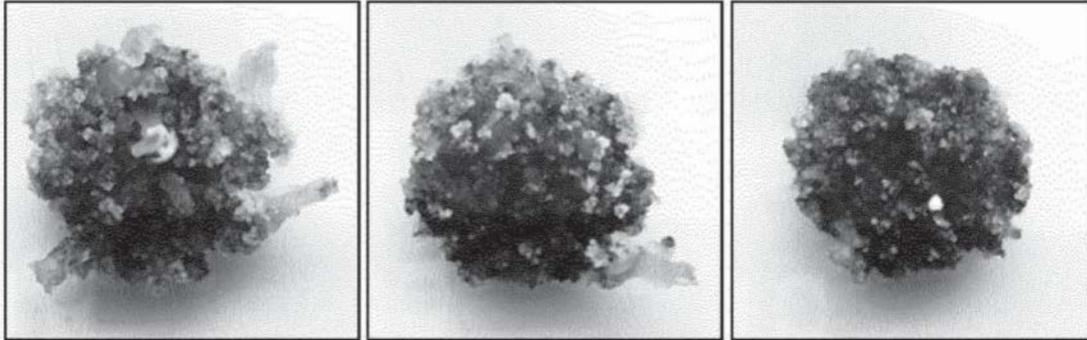


图3

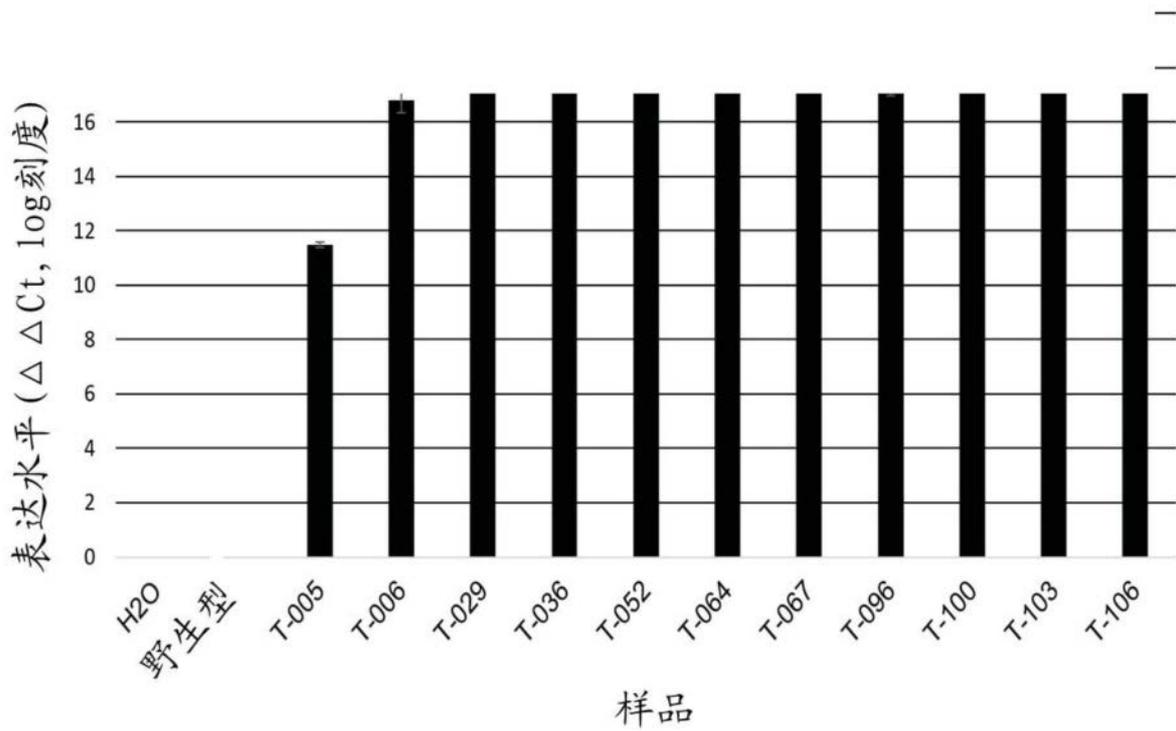


图4

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AtGRF3_AT2G36400.1	: DDEPGRCRRTDGKKWRC	SRD	V	FAGHKYCE RHMHRGRNR	
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SEQ ID NO: 8	: DDEPGRCRRTDGKKWRC	SKE	A	Y PDSKYCE KHMHRCKNR	
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SEQ ID NO: 112	: DDEPGRCRRTDGKKWRC	SKE	A	Y PDSKYCE KHMHRCKNR	
		DDEPGRCRRTDGKKWRC	SKE	A	Y PDSKYCE KHMHRCKNR

基序

: DDEPGRCRRTDGKKWRC SREAYPDSKYCEKHMHRGRNR
L AKD H R K

B

命中-ID	命中计数	起始	终止	错配	命中-序列-区域
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AtGRF6 AT2G06200.1	1	80	117	1	DAEPGRCRRTDGKKWRCSEAYPDSKYCERHMRGKNNR
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SEQ ID NO: 8	1	93	130	0	DPEPGRCRRTDGKKWRCSEAYPDSKYCEKHMHRGKNNR
SEQ ID NO: 10	1	81	118	0	DPEPGRCRRTDGKKWRCSEAYPDSKYCEKHMHRGKNNR
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图5

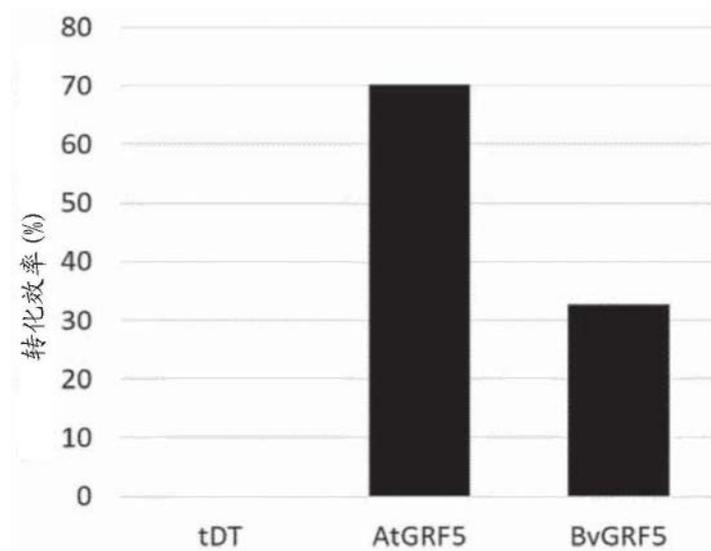


图6

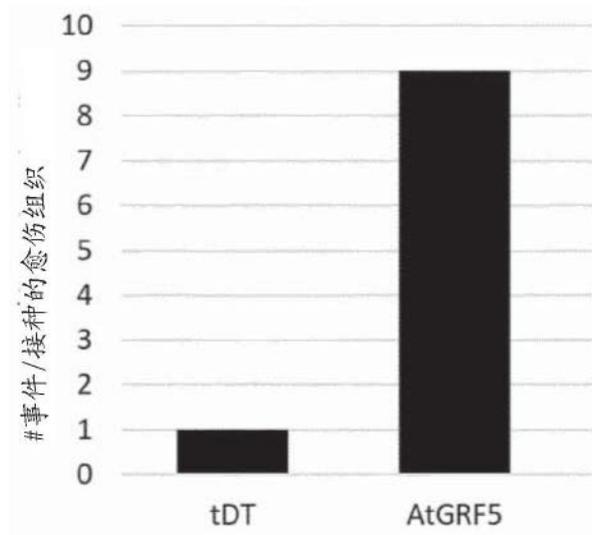


图7

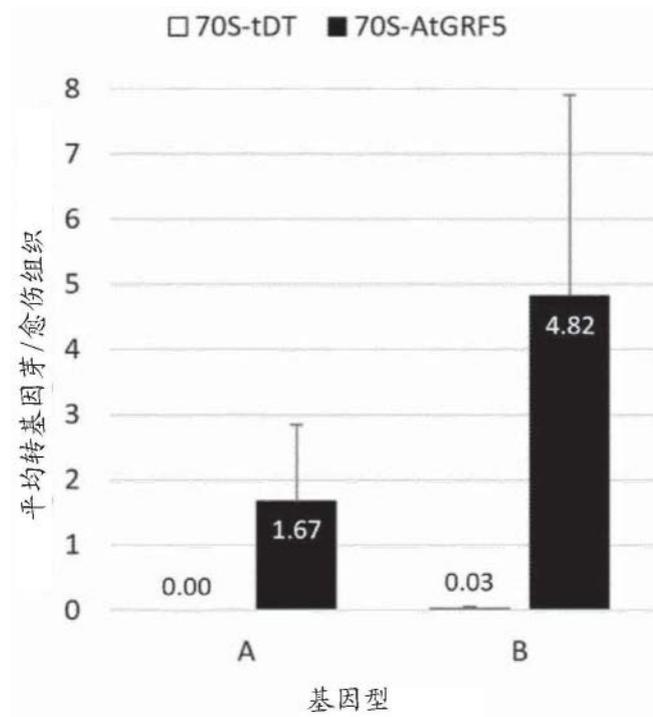


图8

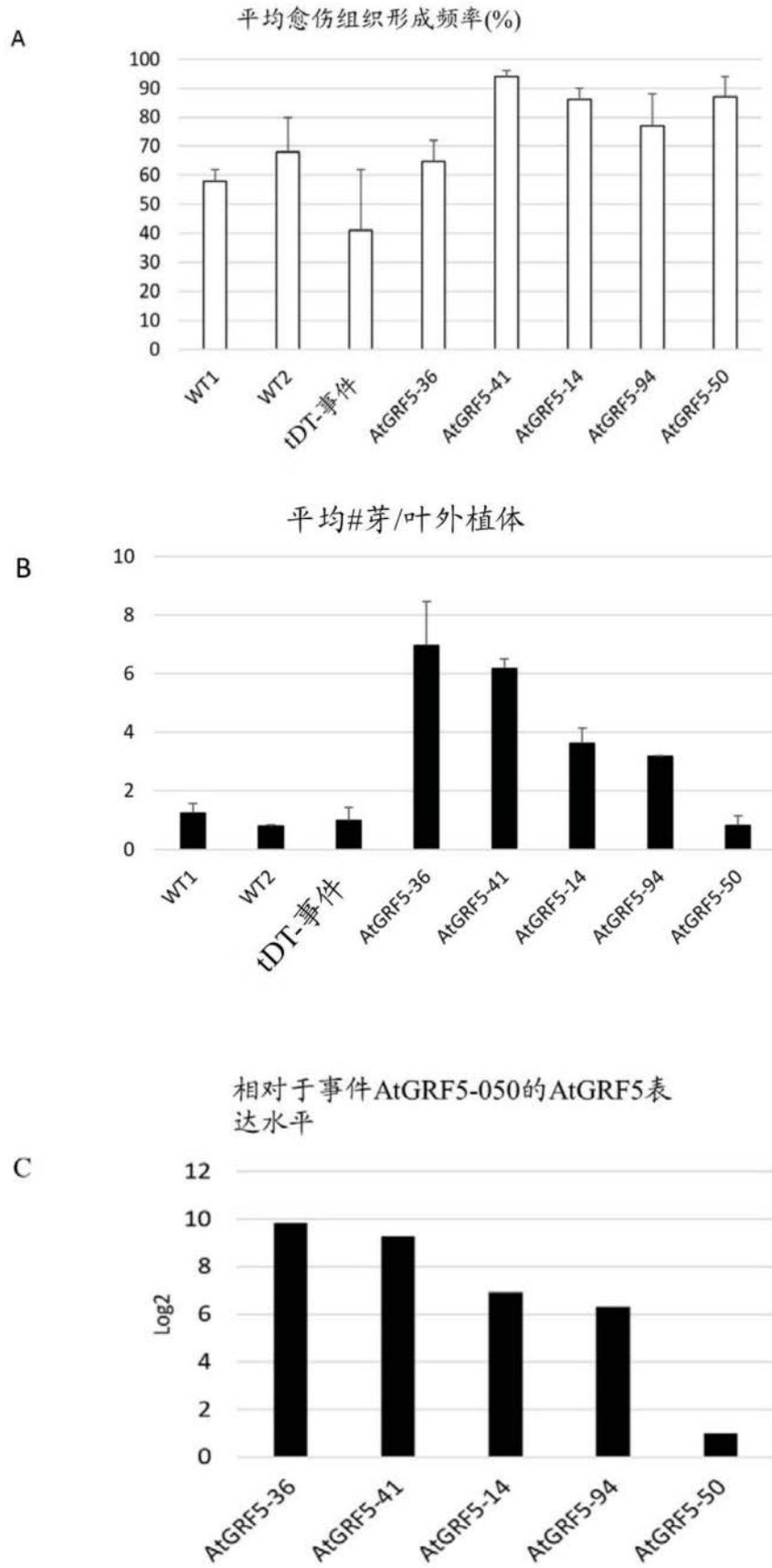


图9

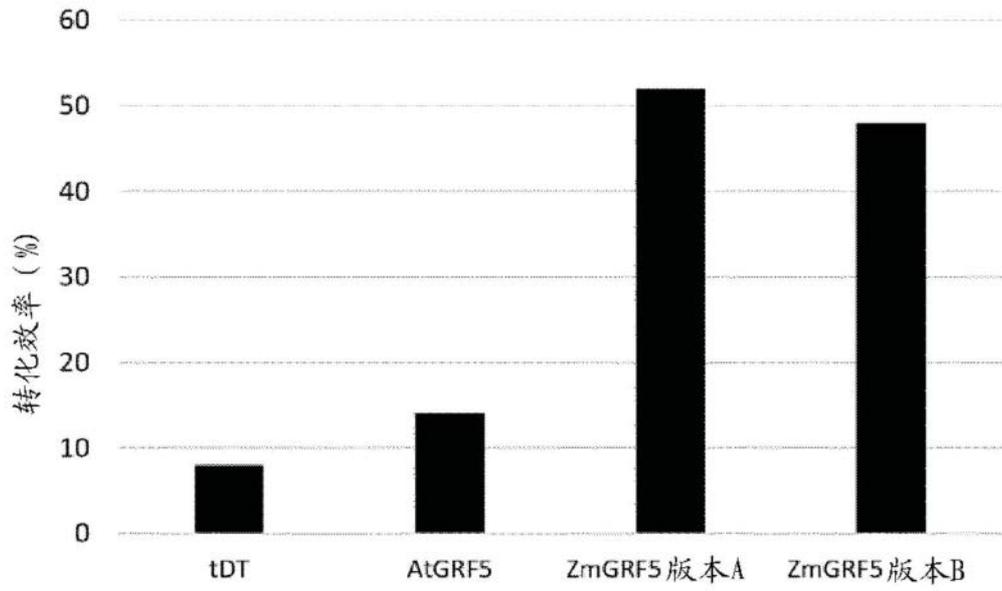


图10

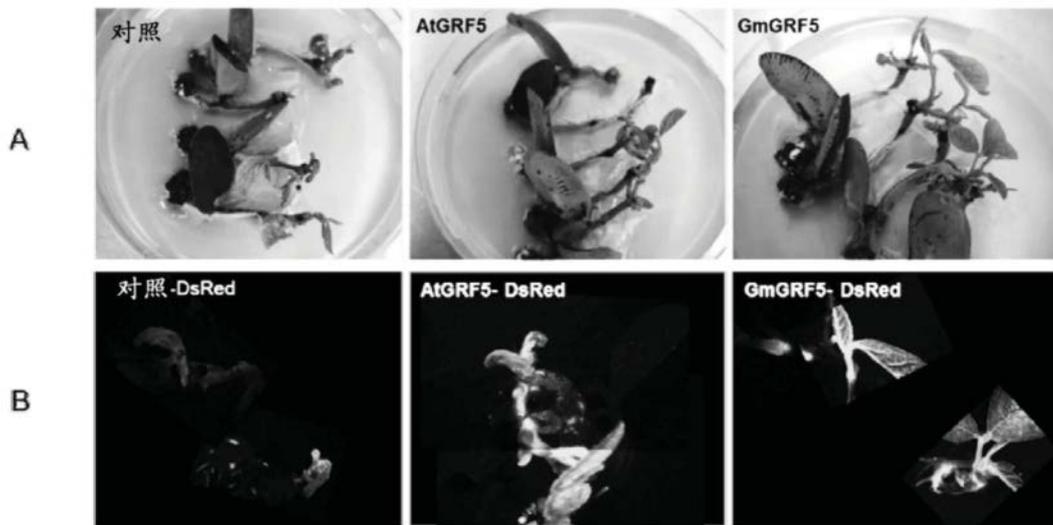


图11

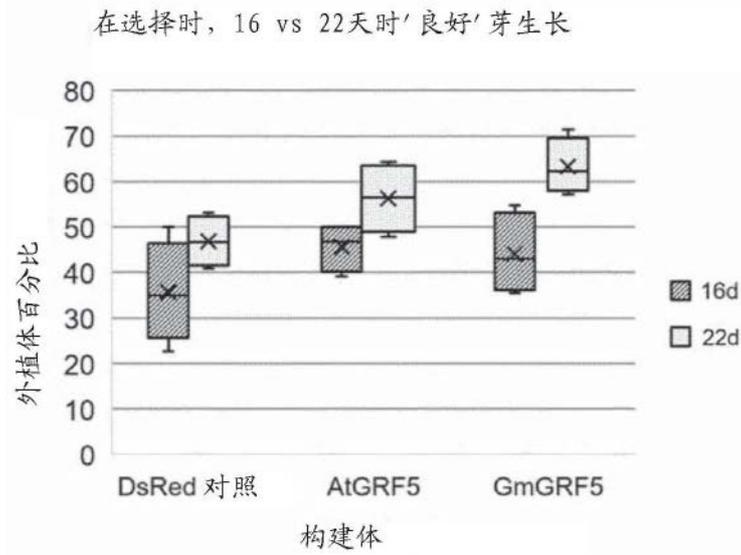


图12

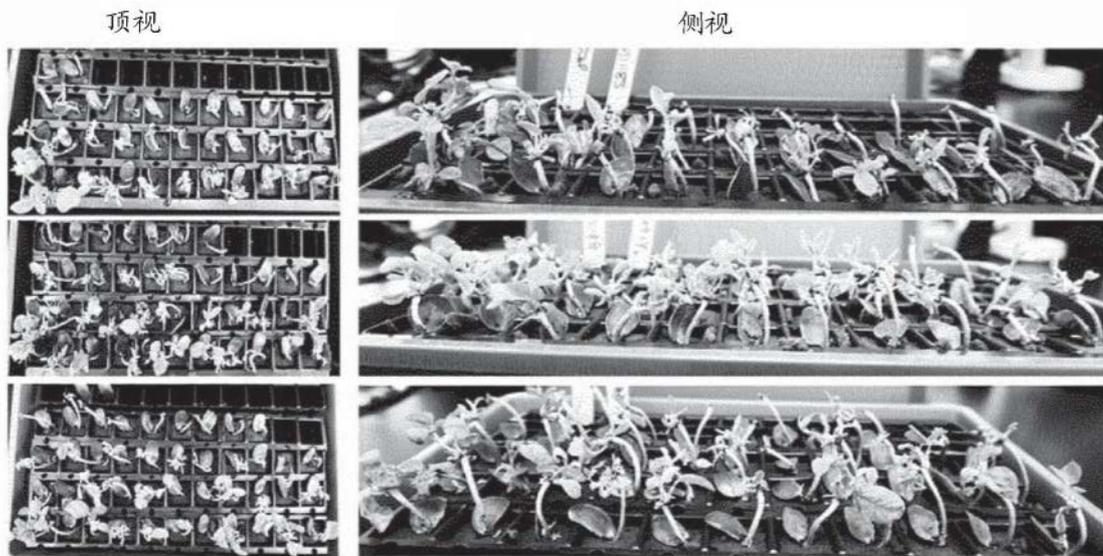


图13

在选择时，16 vs 22天时具有表达DsRed的芽的外植体的百分比

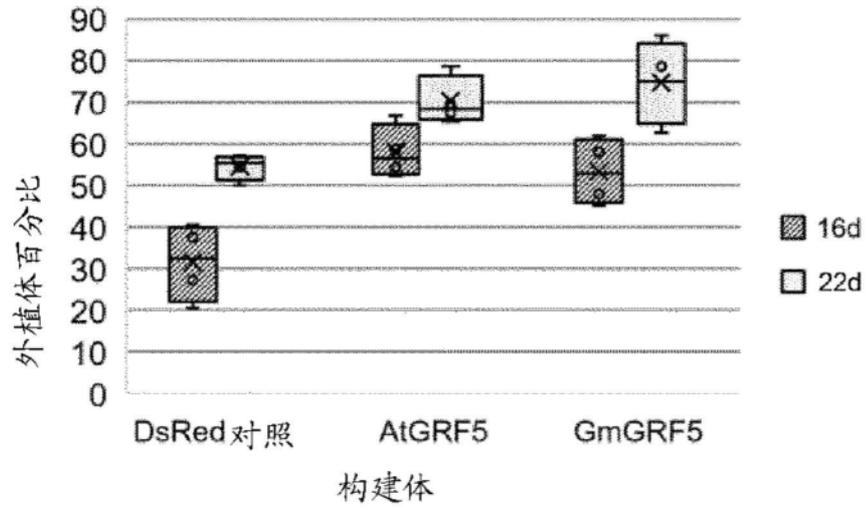


图14

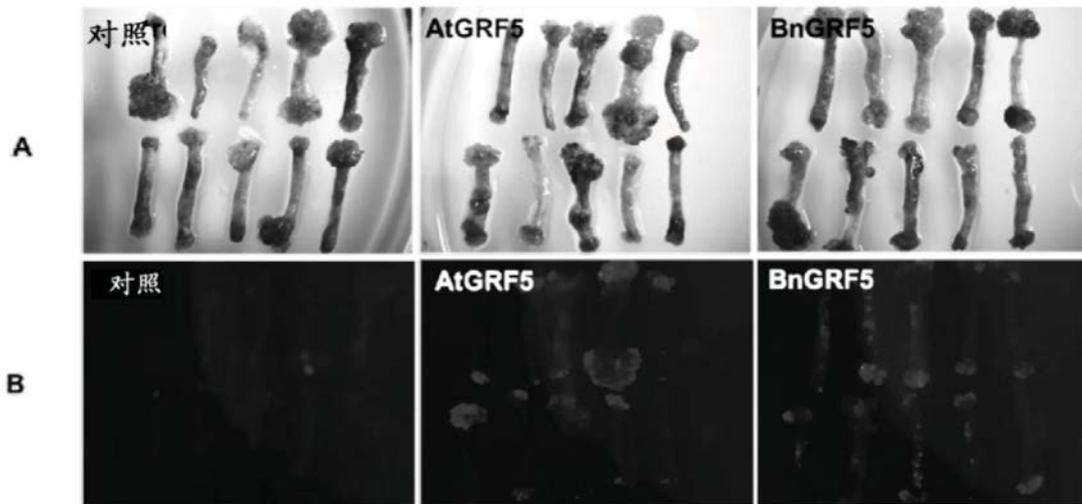


图15

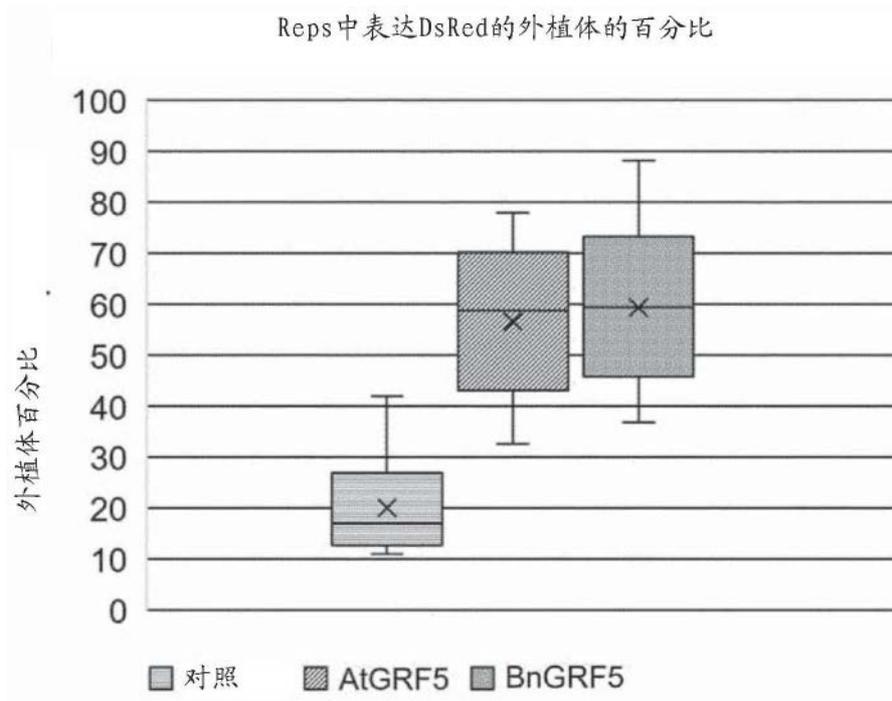


图16

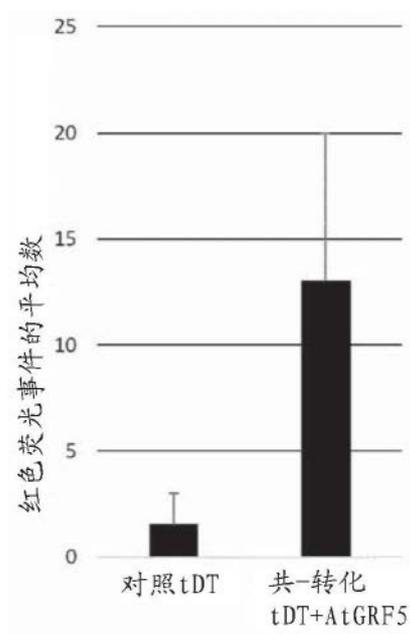


图17