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(54) 发明名称

用于植物改良的方法

(57) 摘要

本发明提供了通过增加植物中至少一种PEAPOD蛋白或其片段的表达以增加所述植物中的根生物量的方法和材料。本发明还提供了用于产生具有增加的根生物量的植物的方法和材料,所述方法包括增加所述植物中至少一种PEAPOD蛋白或其片段的表达的步骤。

1. 一种增加植物中的根生物量的方法,该方法包括增加植物中至少一种PEAPOD蛋白的表达的步骤,其中,所述PEAPOD蛋白以PEAPOD蛋白共享的共有序列为特征,其中,所述共享序列选自SEQ ID NO: 28、29、31和32所组成的组中。

2. 根据权利要求1所述的方法,其中,至少一种PEAPOD蛋白的表达增加是用编码所述PEAPOD蛋白的多核苷酸转化植物、或其祖先植物或植物细胞的结果。

3. 根据权利要求1或2所述的方法,其中,所述植物为用编码和表达所述PEAPOD蛋白的至少一种多核苷酸进行转基因的。

4. 一种产生具有增加的根生物量的植物的方法,该方法包括增加植物中至少一种PEAPOD蛋白的表达的步骤;

其中,所述PEAPOD蛋白以PEAPOD蛋白共享的共有序列为特征,其中,所述共享序列选自SEQ ID NO: 28、29、31和32所组成的组中。

5. 根据权利要求4所述的方法,其中,用编码PEAPOD蛋白的至少一种多核苷酸或其片段转化植物。

6. 根据权利要求4或5所述的方法,该方法包括用编码所述PEAPOD蛋白的多核苷酸转化植物,或转化再生为植物的植物细胞的步骤。

7. 根据权利要求6所述的方法,该方法包括测试或评估所述植物的增加的根生物量的额外步骤。

8. 根据权利要求1、2、4、5和7中任意一项的方法,其中,所述共有序列为TIFY基序,所述TIYF基序选自SEQ ID NO: 34和35所组成的组中。

9. 根据权利要求1、2、4、5和7中任意一项的方法,其中,所述PEAPOD蛋白的序列为SEQ ID NO: 1-26中任意一项的序列。

10. 根据权利要求1、2、4、5和7中任意一项的方法,其中,通过将编码PEAPOD蛋白的多核苷酸引入所述植物细胞或植物中以增加表达。

11. 根据权利要求10所述的方法,其中,所述多核苷酸的序列选自SEQ ID NO: 83-107所组成的组中。

12. 根据权利要求10所述的方法,其中,将所述多核苷酸作为表达构建体的一部分被引入植物。

13. 根据权利要求12所述的方法,其中,所述表达构建体包含与所述多核苷酸可操作地连接的启动子。

14. 根据权利要求13所述的方法,其中,所述启动子能够驱动或驱动可操作地连接的多核苷酸在植物的所有组织中组成型表达。

15. 根据权利要求13所述的方法,其中,所述启动子是组织用启动子。

16. 根据权利要求13所述的方法,其中,所述启动子能够驱动或驱动可操作地连接的多核苷酸在植物的地下组织中表达。

17. 根据权利要求13所述的方法,其中,所述启动子是地下组织用启动子。

18. 根据权利要求13所述的方法,其中,所述启动子是光阻遏型启动子。

19. 根据权利要求13所述的方法,其中,所述启动子能够驱动或驱动可操作地连接的多核苷酸在植物的根中表达。

20. 根据权利要求13所述的方法,其中,所述启动子是根用启动子。

21. 根据权利要求13所述的方法,其中,所述启动子是根特异性启动子。

用于植物改良的方法

技术领域

[0001] 本发明涉及产生具有增加的根生物量的植物的方法。

背景技术

[0002] 维管植物的根具有多种功能。这些功能包括：1) 吸收水和无机养分，2) 将植物固着至地面并支持植物，3) 储存食物和养分，4) 无性繁殖。在对养分的浓度的反应中，根也合成细胞分裂素，其充当影响芽生长快慢的信号。

[0003] 具有增加的根生物量的植物也潜在地具有多种优势，包括更好的固着、更有效的水摄取、更有效的养分摄取和提高的耐旱性。这些特征的组合也可以导致产量提高，包括谷物或果实生物量增加和/或叶生物量增加。

[0004] 目前，对控制植物中的根生物量的遗传机制的理解有限。

[0005] 因此，获得控制植物中的根生物量的可用替代方法是有利的。

[0006] 因此，本发明的目的是提供增加植物中的根生物量的产生的方法和材料，和/或至少为公众提供有用的选择。

发明内容

[0007] 之前，White (2006) 在拟南芥属 (*Arabidopsis*) 中发现了两个邻近的同源基因 (命名为PEAPOD, PPD1和PPD2)，其在叶和种荚 (seed pod) 发育的后期阶段调控拟分生组织的细胞增殖。在拟南芥属中这些基因的缺失导致叶肥大和种荚变宽，而PPD1的过表达导致叶和长角果的大小减小 (White, 2006)。

[0008] 本申请人现已令人惊讶地证明PEAPOD基因在植物中的过表达导致根生物量产生的增加。

[0009] 因此，本申请人的发明涉及通过异位表达PEAPOD以增加植物中的根生物量的方法。具体地，本发明涉及异位表达PEAPOD蛋白，其特征在于存在对于由广泛的植物物种表现出的所有PEAPOD蛋白均常见的共有氨基酸基序。

[0010] 方法

[0011] 第一方面，本发明提供了增加植物中根生物量的方法，该方法包括增加植物中至少一种PEAPOD蛋白的表达的步骤。

[0012] 在一种实施方式中，根生物量相对于同一物种或品种的对照植物中的根生物量增加。

[0013] 在一种实施方式中，至少一种PEAPOD蛋白的表达增加是用编码PEAPOD蛋白的多核苷酸转化植物、或其祖先植物或植物细胞的结果。

[0014] 在其他实施方式中，所述植物为用编码和表达PEAPOD蛋白的至少一种多核苷酸进行转基因的。

[0015] 在其他方面，本发明提供了产生具有增加的根生物量的植物的方法，所述方法包括增加所述植物中至少一种PEAPOD蛋白的表达的步骤。

[0016] 在一种实施方式中,使用编码PEAPOD蛋白的至少一种多核苷酸转化所述植物。

[0017] 在其他实施方式中,所述方法包括用编码PEAPOD蛋白的多核苷酸转化植物,或转化再生为植物的植物细胞的步骤。

[0018] 在一种实施方式中,所述方法包括测试或评估所述植物的增加的根生物量的额外步骤。

[0019] 在其他实施方式中,所述方法包括通过无性或有性繁殖被测试其增加的生物量的植物以进一步产生具有增加的根生物量的植物的步骤。

[0020] PEAPOD蛋白

[0021] 在一种实施方式中,所述PEAPOD蛋白是包含SEQ ID NO:28、29、31、32、34和35中的至少一种序列的多肽。

[0022] 在其他实施方式中,所述PEAPOD蛋白包含SEQ ID NO:28的序列。在其他实施方式中,所述PEAPOD蛋白包含SEQ ID NO:29的序列。在其他实施方式中,所述PEAPOD蛋白包含SEQ ID NO:31的序列。在其他实施方式中,所述PEAPOD蛋白包含SEQ ID NO:32的序列。在其他实施方式中,所述PEAPOD蛋白包含SEQ ID NO:34的序列。在其他实施方式中,所述PEAPOD蛋白包含SEQ ID NO:35的序列。

[0023] 在其他实施方式中,所述PEAPOD蛋白是包含与SEQ ID NO:1-26中任意一项具有至少70%同一性的序列的多肽。

[0024] 在其他实施方式中,所述PEAPOD蛋白是包含选自SEQ ID NO:1-26中任意一项序列的多肽。

[0025] 在其他实施方式中,所述PEAPOD蛋白是包含与SEQ ID NO:1具有至少70%同一性的序列的多肽。

[0026] 在其他实施方式中,所述PEAPOD蛋白是包含SEQ ID NO:1的序列的多肽。

[0027] 表达PEAPOD

[0028] 在植物中表达蛋白的方法是本领域技术人员公知的,并在本文中进行描述。所有这些方法都包括在本发明的范围内。

[0029] 通过引入多核苷酸增加PEAPOD的表达

[0030] 在一种实施方式中,通过将至少一种多核苷酸引入植物细胞或植物中以增加表达。

[0031] 在一个优选的实施方式中,所述多核苷酸编码如本文所定义的PEAPOD蛋白。

[0032] 在其他实施方式中,所述多核苷酸包含与SEQ ID NO:83-107中任意一项的编码序列具有至少70%同一性的序列。

[0033] 在其他实施方式中,所述多核苷酸包含与SEQ ID NO:83-107中任意一项的序列具有至少70%同一性的序列。

[0034] 在其他实施方式中,所述多核苷酸包含SEQ ID NO:83-107中任意一项的编码序列。

[0035] 在其他实施方式中,所述多核苷酸包含SEQ ID NO:83-107中任意一项的序列。

[0036] 在其他实施方式中,所述多核苷酸包含以上所述的序列的片段,其能够编码与PEAPOD蛋白具有相同功能的多肽。在一种实施方式中,所述片段编码能够增加根生物量的多肽。

- [0037] 通过表达构建体表达PEAPOD
- [0038] 在一个优选的实施方式中,将所述多核苷酸作为表达构建体的一部分被引入植物。
- [0039] 在一个优选的实施方式中,所述表达构建体包含与所述多核苷酸可操作地连接的启动子。
- [0040] 用于增加PEAPOD表达的启动子
- [0041] 在一种实施方式中,所述启动子能够驱动或驱动可操作地连接的多核苷酸在植物的所有组织中组成型表达。
- [0042] 在其他实施方式中,所述启动子是组织优选的启动子。
- [0043] 在其他实施方式中,所述启动子能够驱动或驱动可操作地连接的多核苷酸在植物的地下组织中表达。
- [0044] 在一种实施方式中,所述启动子是地下组织优选的启动子。
- [0045] 在其他实施方式中,所述启动子是地下组织特异性启动子。
- [0046] 在一种实施方式中,所述启动子是光阻遏型启动子(light-repressed promoter)。
- [0047] 在其他实施方式中,所述启动子能够驱动或驱动可操作地连接的多核苷酸在植物的根中表达。
- [0048] 在一种实施方式中,所述启动子是根优选的启动子。
- [0049] 在其他实施方式中,所述启动子是根特异性启动子。
- [0050] 多核苷酸和多肽的来源
- [0051] 本发明的或本发明方法中使用多核苷酸和多核苷酸的变体可以衍生自任何物种。所述多核苷酸和变体也可以是合成产生的或重组产生的,并且也可以是“基因改组(gene shuffling)”方法的产物。
- [0052] 本发明的或本发明方法中使用多肽和多肽的变体可衍生自任何物种。所述多肽和变体也可以是重组产生的,并且还可以由“基因改组”方法的产物表达。
- [0053] 在一种实施方式中,所述多核苷酸、多肽或变体衍生自植物物种。
- [0054] 在其他实施方式中,所述多核苷酸、多肽或变体衍生自裸子植物(gymnosperm plant)物种。
- [0055] 在其他实施方式中,所述多核苷酸、多肽或变体衍生自被子植物(angiosperm plant)物种。
- [0056] 在其他实施方式中,所述多核苷酸、多肽或变体衍生自双子叶植物(dicotyledonous)物种。
- [0057] 在一个优选的实施方式中,所述多核苷酸、多肽或变体衍生自真双子叶植物(eudicot)物种。
- [0058] 在其他实施方式中,所述多核苷酸、多肽或变体衍生自真双子叶植物物种。
- [0059] 在其他实施方式中,所述多核苷酸、多肽或变体衍生自单子叶植物物种。优选的单子叶植物包括:棕榈、香蕉、浮萍和兰花物种。
- [0060] 待转化的植物细胞和植物
- [0061] 本发明的,或在本发明的方法中使用的植物细胞和植物来自于任何植物物种。

[0062] 在一种实施方式中,所述植物细胞或植物来自于裸子植物物种。

[0063] 在其他实施方式中,所述植物细胞或植物来自于被子植物物种。

[0064] 在其他实施方式中,所述植物细胞或植物来自于双子叶植物物种。

[0065] 优选的单子叶植物属包括:冰草属(*Agropyron*)、葱属(*Allium*)、看麦娘属(*Alopecurus*)、须芒草属(*Andropogon*)、燕麦草属(*Arrhenatherum*)、天门冬属(*Asparagus*)、燕麦属(*Avena*)、刺竹属(*Bambusa*)、孔颖草属(*Bothrichloa*)、格兰马草属(*Bouteloua*)、雀麦属(*Bromus*)、沙茅属(*Calamovilfa*)、蒺藜草属(*Cenchrus*)、虎尾草属(*Chloris*)、香茅属(*Cymbopogon*)、狗牙根属(*Cynodon*)、鸭茅属(*Dactylis*)、双花草属(*Dichanthium*)、马唐属(*Digitaria*)、稊属(*Eleusine*)、画眉草属(*Eragrostis*)、荞麦属(*Fagopyrum*)、羊茅属(*Festuca*)、向日葵属(*Helianthus*)、大麦属(*Hordeum*)、黑麦草属(*Lolium*)、芒属(*Miscanthis*)、芒属(*Miscanthus x giganteus*)、稻属(*Oryza*)、黍属(*Panicum*)、雀稗属(*Paspalum*)、狼尾草属(*Pennisetum*)、藨草属(*Phalaris*)、梯牧草属(*Phleum*)、早熟禾属(*Poa*)、甘蔗属(*Saccharum*)、黑麦属(*Secale*)、狗尾草属(*Setaria*)、印第安草属(*Sorghastum*)、高粱属(*Sorghum*)、小麦属(*Triticum*)、香荚兰属(*Vanilla*)、小黑麦属(*X Triticosecale Triticale*)和玉蜀黍属(*Zea*)。

[0066] 优选的单子叶植物物种包括:冰草(*Agropyron cristatum*)、冰生冰草(*Agropyron desertorum*)、长穗偃麦草(*Agropyron elongatum*)、中间偃麦草(*Agropyron intermedium*)、蓝莲冰草(*Agropyron smithii*)、丛生小麦草(*Agropyron spicatum*)、细莲冰草(*Agropyron trachycaulum*)、毛冰草(*Agropyron trichophorum*)、古月葱(*Allium ascalonicum*)、洋葱(*Allium cepa*)、蒜头(*Allium chinense*)、韭葱(*Allium porrum*)、北葱(*Allium schoenoprasum*)、大葱(*Allium fistulosum*)、大蒜(*Allium sativum*)、大看麦娘(*Alopecurus pratensis*)、须芒草(*Andropogon gerardi*)、大须芒草(*Andropogon Gerardii*)、小须芒草(*Andropogon scoparius*)、燕麦草(*Arrhenatherum elatius*)、石刁柏(*Asparagus officinalis*)、裸燕麦(*Avena nuda*)、燕麦(*Avena sativa*)、大佛肚竹(*Bambusa vulgaris*)、藤茎须芒草(*Bothrichloa barbinodis*)、白羊草(*Bothrichloa ischaemum*)、针草(*Bothrichloa saccharoides*)、垂穗草(*Bouteloua curipendula*)、黑格兰马草(*Bouteloua eriopoda*)、格兰马草(*Bouteloua gracilis*)、直立雀麦(*Bromus erectus*)、无芒雀麦(*Bromus inermis*)、沙拂子茅(*Calamovilfa longifolia*)、纤毛蒺藜草(*Cenchrus ciliaris*)、非洲虎尾草(*Chloris gayana*)、香茅(*Cymbopogon nardus*)、狗牙根(*Cynodon dactylon*)、鸭茅(*Dactylis glomerata*)、双花草(*Dichanthium annulatum*)、毛梗双花草(*Dichanthium aristatum*)、昆士兰蓝草(*Dichanthium sericeum*)、俯仰马唐(*Digitaria decumbens*)、南非马唐(*Digitaria smutsii*)、圆形薯蓣(*Dioscorea rotundata*)、参薯(*Dioscorea alata*)、麻山药(*Dioscorea opposita*)、黄药(*Dioscorea bulbifera*)、甘薯(*Dioscorea esculenta*)、三浅裂薯蓣(*Dioscorea trifida*)、稊子(*Eleusine coracana*)、窄颖新麦草(*Elymus angustus*)、俄罗斯新麦草(*Elymus junceus*)、弯叶画眉草(*Eragrostis curvula*)、埃塞俄比亚画眉草(*Eragrostis tef*)、荞麦(*Fagopyrum esculentum*)、苦荞麦(*Fagopyrum tataricum*)、苇状羊茅(*Festuca arundinacea*)、羊茅(*Festuca ovina*)、草甸羊茅(*Festuca pratensis*)、紫羊茅(*Festuca rubra*)、向日葵(*Helianthus annuus sunflower*)、二列大麦(*Hordeum distichum*)、大麦

(*Hordeum vulgare*)、多花黑麦草(*Lolium multiflorum*)、多年生黑麦草(*Lolium perenne*)、中国银色草(*Miscanthus sinensis*)、奇岗(*Miscanthus x giganteus*)、水稻(*Oryza sativa*)、秣米(*Panicum italicum*)、大黍(*Panicum maximum*)、稷(*Panicum miliaceum*)、紫黍草(*Panicum purpurascens*)、柳枝稷(*Panicum virgatum*)、柳枝稷(*Panicum virgatum*)、毛花雀稗(*Paspalum dilatatum*)、百喜草(*Paspalum notatum*)、狼尾草(*Pennisetum clandestinum*)、珍珠狼尾草(*Pennisetum glaucum*)、象草(*Pennisetum purpureum*)、御谷(*Pennisetum spicatum*)、藨草(*Phalaris arundinacea*)、梯牧草(*Phleum bertolinii*)、猫尾草(*Phleum pratense*)、羊肉早熟禾(*Poa fendleriana*)、草地早熟禾(*Poa pratensis*)、林地早熟禾(*Poa nemoralis*)、甘蔗(*Saccharum officinarum*)、大茎野生蔗(*Saccharum robustum*)、竹蔗(*Saccharum sinense*)、割手密(*Saccharum spontaneum*)、黑麦(*Secale cereale*)、非洲狗尾草(*Setaria sphacelata*)、印第安草(*Sorghastrum nutans*)、印第安草(*Sorghastrum nutans*)、甜高粱(*Sorghum dochna*)、假高粱(*Sorghum halepense*)、苏丹草(*Sorghum sudanense*)、高粱(*Sorghum vulgare*)、高粱(*Sorghum vulgare*)、小麦(*Triticum aestivum*)、二粒小麦(*Triticum dicoccum*)、硬粒小麦(*Triticum durum*)、一粒小麦(*Triticum monococcum*)、香荚兰(*Vanilla fragrans*)、小黑麦(*X Triticosecale*)和玉米(*Zea mays*)。

[0067] 单子叶植物的优选科系是禾本科。

[0068] 优选的禾本科亚科包括：柊叶竺亚科(*Anomochlooideae*)、服叶竺亚科(*Pharoideae*)、姜叶竺亚科(*Puelioideae*)、竹亚科(*Bambusoideae*)、早熟禾亚科(*Pooideae*)、稻亚科(*Ehrhartoideae*)、三芒草亚科(*Aristidoideae*)、芦竹亚科(*Arundinoideae*)、虎尾草亚科(*Chloridoideae*)、黍亚科(*Panicoideae*)、扁芒草亚科(*Danthonioideae*)和百生草亚科(*Micrairoideae*)。

[0069] 优选的禾本科亚科为早熟禾亚科。优选的早熟禾亚科植物包括小麦、大麦、燕麦、雀麦草和芦苇草。

[0070] 另一个优选的禾本科亚科为稻亚科。优选的稻亚科植物包括水稻。

[0071] 另一个优选的禾本科亚科为黍亚科。优选的黍亚科植物包括稗草、玉米、高粱、甘蔗(sugar cane)、能源甘蔗(energy cane)、小米、福尼奥米(fonio)和须芒草。

[0072] 另一个优选的禾本科亚科为芦竹亚科。优选的芦竹亚科植物包括芦竹(*Arundo donax*)。

[0073] 另一个优选的禾本科亚科为竹亚科。优选的竹亚科植物包括竹子。

[0074] 优选的禾本科物种包括来自黑麦草属(*Lolium*)的那些物种。

[0075] 优选的黑麦草属物种包括长花黑麦草(*Lolium longiflorum*)、多花黑麦草(*Lolium multiflorum*)、多年生黑麦草、西方黑麦草(*Lolium westerwoldicum*)、毒麦(*Lolium temulentum*)和杂种黑麦草(*Lolium hybridum*)。

[0076] 其他优选的禾本科物种包括来自羊茅属(*Festuca*)的那些物种。优选的羊茅属物种包括高羊茅(*Festuca arundinacea*)、硬羊茅(*Festuca ovina*)、草甸羊茅(*Festuca pratensis*)和紫羊茅(*Festuca rubra*)。

[0077] 优选地，所述植物细胞或植物来自双子叶植物物种。

[0078] 优选的双子叶植物属包括：桃属(*Amygdalus*)、檳如树属(*Anacardium*)、花生属

(*Arachis*)、芸苔属(*Brassica*)、木豆属(*Cajanus*)、大麻属(*Cannabis*)、红花属(*Carthamus*)、山核桃属(*Carya*)、吉贝属(*Ceiba*)、鹰嘴豆属(*Cicer*)、椰子属(*Cocos*)、芫荽属(*Coriandrum*)、小冠花属(*Coronilla*)、棉属(*Cossypium*)、猪屎豆属(*Crotalaria*)、扁豆属(*Dolichos*)、油棕属(*Elaeis*)、大豆属(*Glycine*)、棉花属(*Gossypium*)、向日葵属(*Helianthus*)、香豌豆属(*Lathyrus*)、兵豆属(*Lens*)、胡枝子属(*Lespedeza*)、亚麻属(*Linum*)、莲属(*Lotus*)、羽扇豆属(*Lupinus*)、澳洲坚果属(*Macadamia*)、苜蓿属(*Medicago*)、草木犀属(*Melilotus*)、黎豆属(*Mucuna*)、木犀榄属(*Olea*)、驴食草属(*Onobrychis*)、料豆属(*Ornithopus*)、罂粟属(*Papaver*)、菜豆属(*Phaseolus*)、刺葵属(*Phoenix*)、黄连木属(*Pistacia*)、豌豆属(*Pisum*)、樱桃属(*Prunus*)、葛属(*Pueraria*)、茶藨子属(*Ribes*)、蓖麻属(*Ricinus*)、胡麻属(*Sesamum*)、可可(*Theobroma*)、三叶草属(*Trifolium*)、胡芦巴属(*Trigonella*)、蚕豆属(*Vicia*)和豇豆属(*Vigna*)。

[0079] 优选的双子叶植物物种包括：扁桃(*Amygdalus communis*)、腰果(*Anacardium occidentale*)、花生(*Arachis hypogaea*)、花生(*Arachis hypogaea*)、油菜(*Brassica napus* Rape)、黑芥(*Brassica nigra*)、白菜(*Brassica campestris*)、木豆(*Cajanus cajan*)、木豆(*Cajanus indicus*)、大麻(*Cannabis sativa*)、红花(*Carthamus tinctorius*)、美国山核桃(*Carya illinoensis*)、吉贝(*Ceiba pentandra*)、鹰嘴豆(*Cicer arietinum*)、可可椰子(*Cocos nucifera*)、芫荽(*Coriandrum sativum*)、绣球小冠花(*Coronilla varia*)、陆地棉(*Cossypium hirsutum*)、菽麻(*Crotalaria juncea*)、扁豆(*Dolichos lablab*)、油棕(*Elaeis guineensis*)、亚洲棉(*Gossypium arboreum*)、中棉(*Gossypium nanking*)、海岛棉(*Gossypium barbadense*)、草棉(*Gossypium herbaceum*)、陆地棉(*Gossypium hirsutum*)、大豆(*Glycine max*)、野大豆(*Glycine ussuriensis*)、宽叶蔓豆(*Glycine gracilis*)、向日葵(*Helianthus annus*)、*Jatropha cuneata*、麻疯树(*Jatropha curcas*)、窄叶羽扇豆(*Lupinus angustifolius*)、黄羽扇豆(*Lupinus luteus*)、珍珠羽扇豆(*Lupinus mutabilis*)、铁扫帚(*Lespedeza sericea*)、鸡眼草(*Lespedeza striata*)、大百脉根(*Lotus uliginosus*)、山黧豆(*Lathyrus sativus*)、兵豆(*Lens culinaris*)、长萼灼眼草(*Lespedeza stipulacea*)、亚麻(*Linum usitatissimum*)、百脉根(*Lotus corniculatus*)、白羽扇豆(*Lupinus albus*)、窄叶羽扇豆(*Lupinus angustifolius*)、黄羽扇豆(*Lupinus luteus*)、木本苜蓿(*Medicago arborea*)、黄花苜蓿(*Medicago falcate*)、金花菜(*Medicago hispida*)、黄香草木犀(*Medicago officinalis*)、紫花苜蓿(*Medicago sativa* (alfalfa))、硬果苜蓿(*Medicago tribuloides*)、澳洲坚果(*Macadamia integrifolia*)、褐斑苜蓿(*Medicago arabica*)、白花草木樨(*Melilotus albus*)、刺毛黎豆(*Mucuna pruriens*)、油橄榄(*Olea europaea*)、红豆草(*Onobrychis viciifolia*)、锯齿草(*Ornithopus sativus*)、绿豆(*Phaseolus aureus*)、红叶李(*Prunus cerasifera*)、欧洲酸樱桃(*Prunus cerasus*)、荷包豆(*Phaseolus coccineus*)、欧洲李(*Prunus domestica*)、棉豆(*Phaseolus lunatus*)、马哈利樱桃(*Prunu. mahaleb*)、绿豆(*Phaseolus mungo*)、桃(*Prunus. persica*)、樱桃(*Prunus. pseudocerasus*)、菜豆(*Phaseolus vulgaris*)、罂粟(*Papaver somniferum*)、宽叶菜豆(*Phaseolus acutifolius*)、海枣(*Phoenix dactylifera*)、阿月浑子(*Pistacia vera*)、豌豆(*Pisum sativum*)、甜扁桃(*Prunus amygdalus*)、杏(*Prunus armeniaca*)、野葛(*Pueraria thunbergiana*)、黑茶藨子(*Ribes nigrum*)、红茶藨子(*Ribes rubrum*)、鹅莓

(*Ribes grossularia*)、蓖麻(*Ricinus communis*)、芝麻(*Sesamum indicum*)、马铃薯(*Solanum tuberosum*)、狭叶三叶草(*Trifolium augustifolium*)、分散三叶草(*Trifolium diffusum*)、杂种车轴草(*Trifolium hybridum*)、降车轴草(*Trifolium incarnatum*)、球花车轴草(*Trifolium ingrescens*)、红车轴草(*Trifolium pratense*)、白三叶草(*Trifolium repens*)、波斯三叶草(*Trifolium resupinatum*)、地三叶草(*Trifolium subterraneum*)、可可(*Theobroma cacao*)、埃及车轴草(*Trifolium alexandrinum*)、胡芦巴(*Trigonella foenumgraecum*)、油桐(*Vernicia fordii*)、窄叶野豌豆(*Vicia angustifolia*)、深紫花野豌豆(*Vicia atropurpurea*)、距花豌豆(*Vicia calcarata*)、广布野豌豆(*Vicia dasycarpa*)、苦野豌豆(*Vicia ervilia*)、红莓苔子(*Vaccinium oxycoccos*)、褐毛野豌豆(*Vicia pannonica*)、长豇豆(*Vigna sesquipedalis*)、豇豆(*Vigna sinensis*)、长柔毛野豌豆(*Vicia villosa*)、蚕豆(*Vicia faba*)、野豌豆(*Vicia sativa*)和赤豆(*Vigna angularis*)。

[0080] 植物和植物部分

[0081] 在其他方面,本发明提供了由于具有增加的PEAPOD蛋白或其片段的表达而具有增加的根生物量的植物。

[0082] 在一种实施方式中,所述PEAPOD蛋白或其片段的表达增加是用编码PEAPOD蛋白或其片段的多核苷酸转化植物、或其祖先植物或植物细胞的结果。

[0083] 在其他实施方式中,所述植物为用表达PEAPOD蛋白或其片段的多核苷酸进行转基因的。

[0084] 在其他实施方式中,所述多核苷酸或其片段是与组织优选的启动子可操作地连接的多核苷酸。

[0085] 在其他实施方式中,所述启动子是根优选的启动子。

[0086] 在其他实施方式中,所述启动子是根特异性启动子。

[0087] 在其他实施方式中,所述PEAPOD蛋白如本文所定义。

[0088] 在其他实施方式中,所述编码PEAPOD蛋白的多核苷酸如本文所定义。

[0089] 在其他方面,本发明提供了植物的细胞、部分、繁殖体或子代,其为用以下中的至少一种进行转基因的:

[0090] a) 多核苷酸,和

[0091] b) 多核苷酸和可操作地连接的启动子。

具体实施方式

[0092] 在本说明书中,提及专利说明书、其他外部文献或其他信息来源时,通常是为了提供用于讨论本发明特征的上下文。除非另有具体说明,对这些外部文献的参考并不被解释为承认这些文献或这些信息来源以任何权限作为现有技术,或形成本领域的公知常识的一部分。

[0093] 本说明书中使用的术语“包含(comprising)”意指“至少部分由…组成”。当解释本说明书中的每个包括术语“包含”的语句时,除了以该术语开始的特征以外的特征也可能存在。相关的术语如“包含(comprise)”将以相同的方式解释。

[0094] 增加的根生物量

[0095] 具有“增加的根生物量”的植物比同一类型和年龄的对照植物产生更多的根生物量。因此，“增加的”意指相对于同一类型和年龄的对照植物的增加。

[0096] 优选地，具有“增加的根生物量”的植物比同一类型和年龄的对照植物产生的根生物量多至少10%、优选至少20%、更优选至少30%、更优选至少40%、更优选至少50%、更优选至少60%、更优选至少70%、更优选至少80%、更优选至少90%、更优选至少100%、更优选至少150%、更优选至少200%、更优选至少300%、更优选至少400%。

[0097] 在一种实施方式中，具有“增加的根生物量”的植物相比于对照植物具有以下中的至少一种：更大的根、更多的根或更延伸的根系。

[0098] 根生物量

[0099] 术语根生物量是指由植物产生的根组织的总质量。这可以由干重或湿重来评估。

[0100] 根

[0101] 本文使用的术语根意指与该术语的标准用法相同。术语根包含初生根、次生根、不定根、根分枝和根毛。根通常在地下，但是该术语也包含气生根。在一种实施方式中，术语根包含植物的非含叶、非含节部分。

[0102] 增加的耐旱性

[0103] 在一种实施方式中，具有“增加的根生物量”的植物也具有增加的耐旱性。同样地，“增加的”意指相对于同一类型和年龄的对照植物的增加。

[0104] 术语“增加的耐旱性”旨在描述在次优的水化条件下其在生长和发育的任一方面都比在相同条件下的适宜的对照植物表现更好的植物。

[0105] 对照植物

[0106] 在一种实施方式中，所述对照植物是野生型植物。在其他实施方式中，所述对照植物是未转化的植物。在其他实施方式中，所述对照植物是尚未用PEAPOD多核苷酸转化的植物。在其他实施方式中，所述对照植物是尚未用构建体转化的植物。在其他实施方式中，所述对照植物是用对照构建体转化的植物。在一种实施方式中，所述构建体是空载体构建体。

[0107] 异位表达

[0108] 术语“异位表达”旨在被广义地解释。该术语是指多核苷酸或多肽在以下的任意一项中的表达：

[0109] • 在其中所述多核苷酸或多肽未被正常表达的细胞、器官、组织或植物，

[0110] • 在所述多核苷酸或多肽未被正常表达时的时间或发育阶段的细胞、器官、组织或植物，和

[0111] • 在其中所述多核苷酸或多肽以比其正常表达时更高的水平表达的细胞、器官、组织或植物。

[0112] 组织优选的启动子

[0113] 在某些实施方式中，所述编码多核苷酸的PEAPOD蛋白在组织优选的启动子的控制下表达。关于组织优选的启动子的术语“优选的”意指启动子首先在该组织中驱动表达。因此，例如，根优选的启动子在根组织中比在植物的其他组织或器官中驱动更高水平的可操作地连接的多核苷酸的表达。

[0114] 根优选的启动子

[0115] 根优选的启动子在根组织中比在植物的其他组织或器官中驱动更高水平的可操

作地连接的多核苷酸的表达。

[0116] 根优选的启动子可以包括非光合组织优选的启动子和光阻遏调节型启动子。

[0117] 非光合组织优选的启动子

[0118] 非光合组织优选的启动子包括优选在植物的非光合组织/器官中表达的启动子。

[0119] 非光合组织优选的启动子也可包括光阻遏型启动子。

[0120] 光阻遏型启动子

[0121] 光阻遏型启动子的实例可见于US 5,639,952和US 5,656,496。

[0122] 根特异性启动子

[0123] 根特异性启动子的实例可见于US 5,837,848和US 2004/0067506以及US 2001/0047525。

[0124] 关于优选在某一组织中表达的启动子的术语“优选表达”意指在该组织中比在植物的其他组织中以更高水平表达的启动子。

[0125] 关于启动子的术语“组织特异性的”意指所述启动子基本上仅在该组织而非植物的其他组织中表达。

[0126] 在一种实施方式中,所述根优选的启动子是根特异性启动子。

[0127] 本文使用的术语“基因”意指包括编码多肽或蛋白的编码序列的内源基因组序列。所述编码序列被一个或多个内含子间隔。基因通常还包括启动子序列、5' 非翻译序列、3' 非翻译序列和终止子序列。调节蛋白表达的基因组序列也可被认为是基因的一部分。

[0128] 多核苷酸和片段

[0129] 本文使用的术语“多核苷酸”意指任意长度但优选至少15个核苷酸的单链或双链脱氧核糖核苷酸或核糖核苷酸聚合物,并且包括以下非限制性实例:基因的编码和非编码序列、正义和反义序列互补物、外显子、内含子、基因组DNA、cDNA、前mRNA、mRNA、rRNA、siRNA、miRNA、tRNA、核酶、重组多肽、分离的和纯化的天然存在的DNA或RNA序列、合成的RNA和DNA序列、核酸探针、引物和片段。

[0130] 多核苷酸的“片段”是指较长的多核苷酸序列的连续子序列。优选地,所述片段为所公开的多核苷酸的至少15个核苷酸、优选至少16个核苷酸、更优选至少17个核苷酸、更优选至少18个核苷酸、更优选至少19个核苷酸、更优选至少20个核苷酸、更优选至少21个核苷酸、更优选至少22个核苷酸、更优选至少23个核苷酸、更优选至少24个核苷酸、更优选至少25个核苷酸、更优选至少26个核苷酸、更优选至少27个核苷酸、更优选至少28个核苷酸、更优选至少29个核苷酸、更优选至少30个核苷酸、更优选至少31个核苷酸、更优选至少32个核苷酸、更优选至少33个核苷酸、更优选至少34个核苷酸、更优选至少35个核苷酸、更优选至少36个核苷酸、更优选至少37个核苷酸、更优选至少38个核苷酸、更优选至少39个核苷酸、更优选至少40个核苷酸、更优选至少41个核苷酸、更优选至少42个核苷酸、更优选至少43个核苷酸、更优选至少44个核苷酸、更优选至少45个核苷酸、更优选至少46个核苷酸、更优选至少47个核苷酸、更优选至少48个核苷酸、更优选至少49个核苷酸、更优选至少50个核苷酸、更优选至少51个核苷酸、更优选至少52个核苷酸、更优选至少53个核苷酸、更优选至少54个核苷酸、更优选至少55个核苷酸、更优选至少56个核苷酸、更优选至少57个核苷酸、更优选至少58个核苷酸、更优选至少59个核苷酸、更优选至少60个核苷酸、更优选至少61个核苷酸、更优选至少62个核苷酸、更优选至少63个核苷酸、更优选至少64个核苷酸、更优选至

少65个核苷酸、更优选至少66个核苷酸、更优选至少67个核苷酸、更优选至少68个核苷酸、更优选至少69个核苷酸、更优选至少70个核苷酸、更优选至少71个核苷酸、更优选至少72个核苷酸、更优选至少73个核苷酸、更优选至少74个核苷酸、更优选至少75个核苷酸、更优选至少76个核苷酸、更优选至少77个核苷酸、更优选至少78个核苷酸、更优选至少79个核苷酸、更优选至少80个核苷酸、更优选至少81个核苷酸、更优选至少82个核苷酸、更优选至少83个核苷酸、更优选至少84个核苷酸、更优选至少85个核苷酸、更优选至少86个核苷酸、更优选至少87个核苷酸、更优选至少88个核苷酸、更优选至少89个核苷酸、更优选至少90个核苷酸、更优选至少91个核苷酸、更优选至少92个核苷酸、更优选至少93个核苷酸、更优选至少94个核苷酸、更优选至少95个核苷酸、更优选至少96个核苷酸、更优选至少97个核苷酸、更优选至少98个核苷酸、更优选至少99个核苷酸、更优选至少100个核苷酸、更优选至少150个核苷酸、更优选至少200个核苷酸、更优选至少250个核苷酸、更优选至少300个核苷酸、更优选至少350个核苷酸、更优选至少400个核苷酸和最优选至少500个核苷酸的连续核苷酸。多核苷酸序列的片段可用于反义RNA干扰(RNAi)、基因沉默、三螺旋或核酶技术中,或作为包括在微阵列中的引物、探针,或用于本发明的基于多核苷酸的可选方法中。

[0131] 在一种实施方式中,所述片段编码执行或能够执行与由该片段是其一部分的更长的多核苷酸编码的多肽相同的功能的多肽。

[0132] 术语“引物”是指通常具有游离的3' OH基团的短多核苷酸,其与或能够与模板杂交,并用于引发与靶标互补的多核苷酸的聚合。

[0133] 术语“探针”是指在基于杂交的测定中,用于或可用于检测与探针互补的多核苷酸序列的短多核苷酸。所述探针可由如本文所定义的多核苷酸的“片段”组成。

[0134] 多肽和片段

[0135] 本文使用的术语“多肽”包含任意长度但优选至少5个氨基酸的氨基酸链,包括其中氨基酸残基通过共价肽键连接的全长蛋白。本发明的或用于本发明方法中的多肽可以是纯化的天然产物,或者可以部分或全部使用重组或合成技术产生。该术语可是指多肽、多肽的聚合体,例如,二聚体或其他多聚体、融合多肽、多肽片段、多肽变体或其衍生物。

[0136] 多核苷酸的“片段”是指较长的多核苷酸序列的连续子序列。优选所述片段

[0137] 多肽的“片段”是指较长的多肽的连续子序列。优选所述片段为至少5个、更优选至少10个、更优选至少20个、更优选至少30个、更优选至少40个、更优选至少50个、更优选至少100个、更优选至少120个、更优选至少150个、更优选至少200个、更优选至少250个、更优选至少300个、更优选至少350个、更优选至少400个。

[0138] 在一种实施方式中,所述片段执行或能够执行与该片段是其一部分的多肽相同的功能。

[0139] 优选地,所述片段执行生物活性所必需的功能和/或提供多肽的三维结构。

[0140] 应用于本文公开的多核苷酸或多肽序列的术语“分离的”用于指从其天然细胞环境中去除的序列。在一种实施方式中,所述序列与其天然存在的侧翼序列相分离。分离的分子可以通过包括生化、重组和合成技术的任意方法或方法的组合来获得。

[0141] 术语“重组体(recombinant)”是指经合成产生的或从在其天然环境中围绕它的序列中去除的多核苷酸序列。所述重组序列可以与其天然环境中不存在的序列重新组合。

[0142] “重组体”多肽序列通过从“重组体”多核苷酸序列的翻译而产生。

[0143] 关于衍生自特定属或物种的本发明的多核苷酸或多肽的术语“衍生自”意指所述多核苷酸或多肽与天然存在于该属或物种中的多核苷酸或多肽具有相同的序列。衍生自特定属或物种的多核苷酸或多肽因此可以合成产生或重组产生。

[0144] 变体

[0145] 本文使用的术语“变体”是指不同于特定鉴定的序列的多核苷酸或多肽序列，其中一个或多个核苷酸或氨基酸残基被缺失、置换或添加。变体可以是天然存在的等位基因变体或非天然存在的变体。变体可以来自同一物种或来自其他物种并且可以包含同源物(homologue)、旁系同源物(paralogue)和直系同源物(orthologue)。在某些实施方式中，本文公开的多肽和多核苷酸的变体具有与所公开的多肽或多核苷酸的生物活性相同或相似的生物活性。关于多肽和多核苷酸的术语“变体”包含如本文所定义的多肽和多核苷酸的所有形式。

[0146] 多核苷酸变体

[0147] 变体核苷酸序列优选显示出与本发明的序列至少50%、更优选至少51%、更优选至少52%、更优选至少53%、更优选至少54%、更优选至少55%、更优选至少56%、更优选至少57%、更优选至少58%、更优选至少59%、更优选至少60%、更优选至少61%、更优选至少62%、更优选至少63%、更优选至少64%、更优选至少65%、更优选至少66%、更优选至少67%、更优选至少68%、更优选至少69%、更优选至少70%、更优选至少71%、更优选至少72%、更优选至少73%、更优选至少74%、更优选至少75%、更优选至少76%、更优选至少77%、更优选至少78%、更优选至少79%、更优选至少80%、更优选至少81%、更优选至少82%、更优选至少83%、更优选至少84%、更优选至少85%、更优选至少86%、更优选至少87%、更优选至少88%、更优选至少89%、更优选至少90%、更优选至少91%、更优选至少92%、更优选至少93%、更优选至少94%、更优选至少95%、更优选至少96%、更优选至少97%、更优选至少98%和最优选至少99%的同一性。同一性通过至少20个核苷酸位置、优选至少50个核苷酸位置、更优选至少100个核苷酸位置以及最优选本发明的全长多核苷酸的比较窗口而发现。

[0148] 多核苷酸序列同一性可以通过以下方式测定。使用bl2seq中的BLASTN(来自BLAST程序套件,2.2.5版本[2002年11月])将所述目标多核苷酸序列与候选多核苷酸序列进行比较(Tatiana A.Tatusova,Thomas L.Madden(1999),“Blast 2 sequences-a new tool for comparing protein and nucleotide sequences”,FEMS Microbiol Lett.174:247-250),该程序可从NCBI公开获得(ftp:jjftp.ncbi.nih.govjblastj)。在一种实施方式中,使用bl2seq的默认参数。在其他实施方式中,使用bl2seq的默认参数,除了应该关闭对低复杂度部分的过滤以外。

[0149] 也可以使用全局序列比对程序(例如,Needleman,S.B.and Wunsch,C.D.(1970) J.Mol.Biol.48,443-453)在候选多核苷酸序列和目标多核苷酸序列之间的整个重叠长度上计算多核苷酸序列同一性。Needleman-Wunsch全局比对算法的完整实施方式可见于EMBOSS程序包中的needle程序(Rice,P.Longden,l.and Bleasby,A.EMBOSS:The European Molecular Biology Open Software Suite,Trends in Genetics June 2000,第16卷,第6期,第276-277页),其可从<http://www.hgmp.mrc.ac.uk/Software/EMBOSS/>获得。欧洲生物信息学研究所服务器也提供了在线上(在<http://www.ebi.ac.uk/emboss/align/>上)进行两

条序列之间的EMBOSS-needle全局比对的工具。

[0150] 或者,可以使用GAP程序,其计算两条序列的最佳全局比对,而不会对末端空位进行罚分。GAP记载于以下文章中:Huang,X.(1994) On Global Sequence Alignment.Computer Applications in the Biosciences 10,227-235。

[0151] 计算多核苷酸的序列同一性%的优选方法基于使用Clustal X比对待比较的序列(Jeanmougin et al.,1998,Trends Biochem.Sci.23,403-5)。

[0152] 本发明的多核苷酸变体还包含表现出与一个或多个特定鉴定的序列具有相似性的那些,其可能保留这些序列的功能等效性并且无法被合理预期已随机发生。所述关于多肽的序列相似性可以使用可公开获得的NCBI的BLAST程序套件的bl2seq程序(2.2.5版本[2002,11月])(ftp://ftp.ncbi.nih.gov/blast/)来确定。

[0153] 或者,本发明的变体多核苷酸在严格条件下与特定的多核苷酸序列或其互补物杂交。

[0154] 术语“在严格条件下杂交”及其语法上的同义语是指多核苷酸分子与靶多核苷酸分子(如固定在DNA或RNA印迹(如Southern印迹或Northern印迹)上的靶多核苷酸分子)在确定的温度和盐浓度的条件下杂交的能力。在严格杂交条件下杂交的能力可以通过在较不严格条件下初始杂交然后增加严格性至预期严格性的条件下杂交来测定。

[0155] 对于长度大于约100个碱基的多核苷酸分子,典型的严格杂交条件是不超过25至30℃(例如,10℃),低于天然双链的解链温度(T_m) (通常参见,Sambrook et al.,Eds,1987,Molecular Cloning,A Laboratory Manual,第2版,Cold Spring Harbor Press;Ausubel et al.,1987,Current Protocols in Molecular Biology,Green Publishing)。大于约100个碱基的多核苷酸分子的 T_m 可以通过公式 $T_m = 81.5 + 0.41\% (G+C - \log(Na^+))$ 来计算(Sambrook et al.,Eds,1987,Molecular Cloning,A Laboratory Manual,第2版,Cold Spring Harbor Press;Bolton and McCarthy,1962,PNAS 84:1390)。长度大于约100个碱基的多核苷酸的典型严格条件为杂交条件,如在6X SSC、0.2%SDS的溶液中预洗涤;在65℃、6X SSC、0.2%SDS中过夜杂交;然后在65℃下在1X SSC、0.1%SDS中洗涤两次,每次30分钟,以及在65℃下在0.2X SSC、0.1%SDS中洗涤两次,每次30分钟。

[0156] 对于长度小于100个碱基的多核苷酸分子,示例性的严格杂交条件为低于 T_m 的5至10℃。平均而言,长度小于100bp的多核苷酸分子的 T_m 降低约(500/寡核苷酸长度)℃。

[0157] 对于已知作为肽核酸(PNA)的DNA模拟物(Nielsen et al.,Science.1991 Dec 6; 254(5037):1497-500), T_m 值比DNA-DNA杂交体或DNA-RNA杂交体的 T_m 值高,并且可以使用Giesen et al.,Nucleic Acids Res.1998 Nov 1;26(21):5004-6中记载的公式计算。具有长度小于100个碱基的DNA-PNA杂交体的示例性严格杂交条件为低于 T_m 的5至10℃。

[0158] 本发明的变体多核苷酸还包含这样的多核苷酸,其不同于本发明的序列,但是因遗传密码的简并性而编码与由本发明的多核苷酸编码的多肽具有相似活性的多肽。不改变多肽的氨基酸序列的序列改变是“沉默变异”。除了ATG(甲硫氨酸)和TGG(色氨酸),相同氨基酸的其他密码子可以通过本领域公知的技术改变,例如,在特定的宿主生物体中以优化密码子的表达。

[0159] 本发明也包括这样的多核苷酸序列改变,其导致所编码的多肽序列的一个或几个氨基酸的保守置换,而不明显改变其生物活性。本领域技术人员将会知晓制备表型沉默氨

氨基酸置换的方法(参见,例如,Bowie et al.,1990,Science 247,1306)。

[0160] 由于所编码的多肽序列中的沉默变异和保守置换而导致的变体多核苷酸可以通过如前所述的tblastx算法使用可公开获得的NCBI的BLAST程序套件的bl2seq程序(2.2.5版本[2002,11月])(<ftp://ftp.ncbi.nih.gov/blast/>)来测定。

[0161] 多肽变体

[0162] 关于多肽的术语“变体”包含天然存在的、重组产生的和合成产生的多肽。变体多肽序列优选表现出与本发明的序列至少50%、更优选至少51%、更优选至少52%、更优选至少53%、更优选至少54%、更优选至少55%、更优选至少56%、更优选至少57%、更优选至少58%、更优选至少59%、更优选至少60%、更优选至少61%、更优选至少62%、更优选至少63%、更优选至少64%、更优选至少65%、更优选至少66%、更优选至少67%、更优选至少68%、更优选至少69%、更优选至少70%、更优选至少71%、更优选至少72%、更优选至少73%、更优选至少74%、更优选至少75%、更优选至少76%、更优选至少77%、更优选至少78%、更优选至少79%、更优选至少80%、更优选至少81%、更优选至少82%、更优选至少83%、更优选至少84%、更优选至少85%、更优选至少86%、更优选至少87%、更优选至少88%、更优选至少89%、更优选至少90%、更优选至少91%、更优选至少92%、更优选至少93%、更优选至少94%、更优选至少95%、更优选至少96%、更优选至少97%、更优选至少98%和最优选至少99%的同一性。同一性通过至少20个氨基酸位置、优选至少50个氨基酸位置、更优选至少100个氨基酸位置以及最优选本发明的全长多肽的比较窗口而发现。

[0163] 多肽序列同一性可以通过以下方式确定。使用bl2seq中的BLASTN(来自BLAST程序套件,2.2.5版本[2002年11月],其可从NCBI公开获得(<ftp://ftp.ncbi.nih.gov/blast/>))将所述目标多肽序列与候选多肽序列进行比较。在一种实施方式中,使用bl2seq的默认参数。在其他实施方式中,使用bl2seq的默认参数,除了应关闭对低复杂度部分的过滤。

[0164] 也可以使用全局序列比对程序在候选多肽序列和目标多肽序列之间的整个重叠长度上计算多肽序列同一性。如上所讨论的EMBOSS-needle(可从<http://www.ebi.ac.uk/emboss/align/>上获得)和GAP(Huang,X.(1994)On Global Sequence Alignment.Computer Applications in the Biosciences 10,227-235)也是用于计算多肽序列同一性的合适的全局序列比对程序。

[0165] 计算多肽的序列同一性%的优选方法基于使用Clustal X比对待比较的序列(Jeanmougin et al.,1998,Trends Biochem.Sci.23,403-5)。

[0166] 变体多肽包括这样的多肽,其中氨基酸序列与本文的多肽的区别在于一个或多个不影响肽的生物活性的保守氨基酸置换、缺失、添加或插入。保守置换通常包括一个氨基酸置换为具有相似特征的另一个,例如,以下组中的置换:缬氨酸、甘氨酸;甘氨酸、丙氨酸;缬氨酸、异亮氨酸、亮氨酸;天冬氨酸、谷氨酸;天冬酰胺、谷氨酰胺;丝氨酸、苏氨酸;赖氨酸、精氨酸;和苯丙氨酸、酪氨酸。

[0167] 非保守置换需要将这些类别之一的一个成员换成另一类别的成员。

[0168] 对进化的生物序列的分析已证明并不是所有的序列改变都具有同样可能性,反映了保守置换和非保守置换在生物水平上的至少部分差异。例如,某些氨基酸置换可以频繁发生,而其他则非常少见。氨基酸残基的进化性改变或置换可以通过评分矩阵(也称为置换矩阵)来模拟。将所述矩阵用于生物信息学分析中以鉴定序列之间的关系,一个实例为以下

所示的BLOSUM62矩阵(表1)。

[0169] 表1:含有所有可能的置换分数的BLOSUM62矩阵[Henikoff and Henikoff,1992]

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-4	1	0	-3	-2	0
R	-1	4	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	6	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	6	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-4	0	-1	-4	-3	-3
C	0	-3	-3	-3	9	-3	-4	-3	-3	-1	-4	-3	-1	-3	-3	-1	-1	-3	-2	-1
Q	-1	1	0	0	-3	5	2	-2	0	-3	-2	1	0	-3	-4	0	-1	-3	-1	-2
E	-1	0	0	2	-4	2	5	-2	0	-3	-3	1	-2	-3	-4	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	6	-2	-4	-4	-2	-3	-3	-2	0	-2	-3	-3	-3
H	-2	0	1	-1	-3	0	0	-2	6	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-1	-3	-3	-1	-3	-3	-4	-3	4	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5	-1	-3	-2	0	-1	-3	-2	-2
M	-1	-1	-3	-3	-1	0	-3	-3	-2	1	2	-1	5	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6	-4	-2	-2	1	3	-1
P	-4	-2	-2	-4	-3	-4	-4	-2	-2	-3	-2	-2	-3	-4	7	-1	-1	-4	-3	-2
S	1	-1	0	0	-1	0	0	-1	-1	-2	0	-1	-2	-2	-1	4	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5	-2	-2	0
W	-3	-3	-4	-4	-3	-3	-3	-2	-2	-3	-2	-3	-1	-3	-4	-3	-2	11	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	-2	-1	-4	-2	-1	3	-3	-2	-2	2	7	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	0	-3	-1	-1	4

[0171] 使用所示的BLOSUM62矩阵生成相应的行和列的交汇点处存在的各比对的氨基酸对的分数。例如,从谷氨酸残基(E)至天冬氨酸残基(D)的置换分数为2。对角线示出了还未改变的氨基酸的分数。大部分置换改变具有负分数。所述矩阵只包含整数。

[0172] 确定合适的评分矩阵以产生对给定序列组最佳的比对被认为是在本领域技术的范围内。表1中的BLOSUM62矩阵也可以用作BLAST检索中的缺省矩阵,尽管并不局限于此。

[0173] 其他变体包括具有影响肽稳定性的修饰的肽。这种类似物可以包含,例如,肽序列中的一个或多个非-肽键(其替换肽键)。也包括这样的类似物,其包括非天然存在的L-氨基酸的残基,例如,D-氨基酸或非天然存在的合成氨基酸,例如, β 或 γ 氨基酸和环状类似物。

[0174] 构建体、载体和其组件

[0175] 术语“遗传构建体”是指多核苷酸分子,通常为双链DNA,其可能已经被插入至另一个多核苷酸分子(插入多核苷酸分子)(例如,但不限于cDNA分子)中。遗传构建体可以包含必要的元件,其允许转录插入多核苷酸分子,并且,任选地,将转录物翻译为多肽。所述插入多核苷酸分子可衍生自宿主细胞,或可衍生自不同的细胞或生物体和/或可以是重组多核苷酸。一旦在宿主细胞内,所述遗传构建体可以整合在宿主染色体DNA中。所述遗传构建体可与载体连接。

[0176] 术语“载体”是指多核苷酸分子,通常为双链DNA,其被用于将所述遗传构建体转运到宿主细胞中。所述载体能够在至少一种其他宿主系统(如大肠杆菌)中复制。

[0177] 术语“表达构建体”是指包括必要元件的遗传构建体,所述元件允许转录所述插入多核苷酸分子,并且,任选地,将转录物翻译为多肽的必要元件。表达构建体通常在5'至3'方向上包含:

[0178] a) 在所述构建体将转化至其中的宿主细胞中具有功能的启动子;

[0179] b) 待表达的多核苷酸;

[0180] c) 在所述构建体将转化至其中的宿主细胞中具有功能的终止子。

[0181] 术语“编码区域”或“开放阅读框”(ORF)是指能够在适当的调控序列的控制下产生

转录产物和/或多肽的基因组DNA序列的正义链或cDNA序列。所述编码序列通过5' 翻译起始密码子和3' 翻译终止密码子的存在而鉴定。当插入至遗传构建体中时,“编码序列”在其与启动子序列和终止子序列可操作地连接时能够被表达。

[0182] “可操作地连接”意指待表达的序列被置于调控元件的控制下,所述调控元件包括启动子、组织特异性调控元件、时间调控元件、增强子、抑制子和终止子。

[0183] 术语“非编码区域”是指非翻译序列,其为翻译起始位点的上游和翻译终止密码子的下游。这些序列也分别称为5' UTR和3' UTR。这些区域包括转录起始和终止以及调控翻译效率所必需的元素。

[0184] 终止子是终止转录的序列,并存在于所翻译序列下游的基因的3' 非翻译末端。终止子是mRNA稳定性的重要决定因素,并且在一些情况下被发现具有空间调控功能。

[0185] 术语“启动子”是指编码区域上游的调控基因转录的非转录顺式调控元件。启动子包含指定转录起始位点和保守框(如TATA框)以及被转录因子结合的基序的顺式起始元件。

[0186] 启动子可以与待表达的多核苷酸是同源的。这意指所述启动子和多核苷酸天然以可操作地连接的形式存在。

[0187] 或者,所述启动子可以与待表达的多核苷酸是异源的。这意指所述启动子和多核苷酸天然不以可操作地连接的形式存在。

[0188] “转基因”是通过转化被引入生物体中的多聚核苷酸。所述转基因可以衍生自相同物种或与所述转基因被引入至其中的生物体的物种不同的物种。所述转基因也可以是合成的并且天然不存在于任何物种中。

[0189] “转基因植物”是指含有作为遗传操作或转化结果的新遗传物质的植物。所述新遗传物质可衍生自与所得转基因植物的物种相同或不同的植物,或可是合成的。

[0190] 优选地,所述“转基因植物”因转基因的存在而与天然存在的任何植物不同。

[0191] “反向重复”是重复的序列,其中,重复序列的后半段是互补链,例如:

[0192] (5') GATCTA.....TAGATC (3')

[0193] (3') CTAGAT.....ATCTAG (5')

[0194] 只要在重复区域间具有3-5个碱基对的间隔区,通读转录将产生经过互补碱基配对来形成发卡结构的转录物。所述间隔区可以是通常长度至少为3个碱基对的任意多核苷酸序列。

[0195] 宿主细胞

[0196] 宿主细胞可以衍生自,例如,细菌、真菌、昆虫、哺乳动物或植物生物体。

[0197] 分离或产生多核苷酸的方法

[0198] 本发明的多核苷酸分子可以使用本领域普通技术人员已知的多种技术分离。例如,可以通过使用Mullis et al.,Eds.1994The Polymerase Chain Reaction,Birkhauser (以引用的方式纳入本文)中记载的聚合酶链式反应(PCR)分离所述多肽。本发明的多肽可以使用如本文所定义的衍生自本发明的多核苷酸序列的引物来扩增。

[0199] 分离本发明的多核苷酸的其他方法包括使用具有如本文所示的杂交探针的序列的多肽的全部或部分。将标记的多核苷酸探针与固定在固体支持物(如硝化纤维素滤膜或尼龙膜)上的多核苷酸杂交的技术可以被用于筛选基因组文库或cDNA文库。示例性的杂交和洗涤条件为:在65℃下在5.0X SSC、0.5%十二烷基硫酸钠、1X Denhardt溶液中杂交20小

时;在1.0X SSC,1% (w/v) 十二烷基硫酸钠中洗涤(在55℃下洗涤3次,每次20分钟),以及任选地,在60℃下在0.5X SSC、1% (w/v) 十二烷基硫酸钠中洗涤1次(20分钟)。在60℃、0.1X SSC、1% (w/v) 十二烷基硫酸钠的条件下进行任选的进一步洗涤(20分钟)。

[0200] 本发明的多核苷酸片段可以通过本领域公知的技术(例如,限制性内切酶消化、寡核苷酸合成和PCR扩增)产生。

[0201] 部分多核苷酸序列可以在本领域公知的方法中使用,以鉴定相应的全长多核苷酸序列。这种方法包括基于PCR的方法,5' RACE (Frohman MA,1993,Methods Enzymol.218:340-56)和基于杂交的方法、基于计算机/数据库的方法。此外,例如,使用以基于已知区域的引物为起始的反向PCR允许捕获位于本文公开的多核苷酸序列侧翼的未知序列(Triglia et al.,1998,Nucleic Acids Res 16,8186,以引用的方式纳入本文)。该方法使用若干个限制性酶来以在基因的已知区域中产生合适的片段。所述片段然后通过分子内连接进行环化并用作PCR模板。发散引物(divergent primer)从已知区域设计而来。为了完全组装全长的克隆,可使用标准分子生物学方法(Sambrook et al.,Molecular Cloning:A Laboratory Manual,第2版Cold Spring Harbor Press,1987)。

[0202] 当从特定物种产生转基因植物时,使用衍生自该物种的序列转化这样的植物可能是有益的。益处可能是改善公众对在产生转基因生物中的物种间转化的关注。此外,当基因的下调是期望的结果时,利用与所述植物中的序列相同(或至少是高度相似的)的序列可能是必需的,对该基因而言,降低的表达是期望的。因为这些以及其他原因,能够鉴定和分离在几个不同植物物种中鉴定和分离出特定基因的直系同源物是可期望的。

[0203] 变体(包括直系同源物)可以通过所描述的方法进行鉴定。

[0204] 用于鉴定变体的方法

[0205] 物理方法

[0206] 变体多肽可以通过使用基于PCR的方法进行鉴定(Mullis et al.,Eds.1994 The Polymerase Chain Reaction,Birkhauser)。通常,可用于通过PCR扩增本发明的多聚核苷酸分子变体的引物的多聚核苷酸序列可以基于编码相应氨基酸序列的保守区域的序列。

[0207] 或者,可以使用本领域技术人员所公知的文库筛选方法(Sambrook et al.,Molecular Cloning:A Laboratory Manual,第2版,Cold Spring Harbor Press,1987)。当鉴定所述探针序列的变体时,相对于当寻找精确的序列匹配时,其杂交和/或洗涤的严格性通常将被降低。

[0208] 多肽变体也可以通过物理方法进行鉴定,例如,通过使用抗本发明的多肽的抗体筛选表达文库(Sambrook et al.,Molecular Cloning:A Laboratory Manual,第2版,Cold Spring Harbor Press,1987)或在这样的抗体的帮助下将多肽从天然来源中鉴定出。

[0209] 基于计算机的方法

[0210] 包括多核苷酸和多肽变体的本发明的变体序列也可以通过本领域技术人员所公知的基于计算机的方法使用公开的域序列比对算法和序列相似性搜索工具来搜索序列数据库(公开的域数据库包括Genbank、EMBL、Swiss-Prot、PIR等)而进行鉴定。参见,例如,Nucleic Acids Res.29:1-10and 11-16,2001中在线资源的实例。相似性搜索检索和比对用于与待分析序列相比较的靶序列(即查询序列)。序列比较算法使用评分矩阵来为每一条比对序列赋予总分数。

[0211] 用于鉴定序列数据库中变体的示例性程序族是包括BLASTN、BLASTP、BLASTX、tBLASTN和tBLASTX的BLAST程序套件(2.2.5版本[2002年11月]),它们可以从(<ftp://ftp.ncbi.nih.gov/blast/>)或从美国马里兰州贝塞斯达市(邮编20894)38A栋8N805房间的国家医学图书馆国家生物技术信息中心(NCBI)公开获得。NCBI服务器也提供了使用这些程序来筛选大量可公开获得的序列数据库的工具。BLASTN比较核苷酸查询序列和核苷酸序列数据库。BLASTP比较氨基酸查询序列和蛋白序列数据库。BLASTX比较在所有阅读框中被翻译的核苷酸查询序列和蛋白序列数据库。tBLASTN比较蛋白查询序列和在所有阅读框中动态翻译的核苷酸序列数据库。tBLASTX比较核苷酸查询序列的六读码框翻译和核苷酸序列数据库的六读码框翻译。BLAST程序可以以默认参数使用或者可以根据需要改变参数以改进筛选。

[0212] 包括BLASTN、BLASTP和BLASTX的BLAST算法族的使用在出版物Altschul et al., *Nucleic Acids Res.* 25:3389-3402, 1997中进行了描述。

[0213] 由BLASTN、BLASTP、BLASTX、tBLASTN、tBLASTX或相似算法产生的被查询序列产生的对于一个或多个数据库序列的“击中序列数”(hit)比对和鉴定了序列的相似部分。击中序列数以相似性的程度和序列重叠的长度的顺序排列。对数据库序列的击中序列数通常代表了仅是被查询序列的序列长度的部分的重叠。

[0214] BLASTN、BLASTP、BLASTX、tBLASTN和tBLASTX算法也针对比对产生“期望”值。期望值(E)表明当搜索含有随机连续序列的相同大小的数据库时,人们能“期望”随机看到的击中的数目。期望值用作决定对于数据库的击中是否表明真的相似性的显著性阈值。例如,指定到多核苷酸击中数的E值为0.1被解释为意味着,在被筛选的数据库大小的数据库中,人们可能期望看到具有简单随机地相似分数的序列的被比对部分的0.1匹配。对于被比对和匹配部分具有0.01或更低的E值的序列,通过使用所述BLASTN、BLASTP、BLASTX、tBLASTN或tBLASTX算法在那个数据库中发现随机匹配的可能性是1%或更低。

[0215] 一组相关序列的多序列比对可以使用CLUSTALW (Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22:4673-4680, <http://www-igbmc.u-strasbg.fr/BioInfo/ClustalW/Top.html>) 或T-COFFEE (Cedric Notredame, Desmond G. Higgins, Jaap Heringa, T-Coffee: A novel method for fast and accurate multiple sequence alignment, *J. Mol. Biol.* (2000) 302:205-217) 或PILEUP进行,这些算法使用渐进配对比对。(Feng and Doolittle, 1987, *J. Mol. Evol.* 25, 351)。

[0216] 模式识别软件应用可用于发现基序或标记序列。例如, MEME (用于基序挖掘的多重Em) 在一组序列中发现基序标记序列, 和MAST (基序比对和搜索工具) 使用这些基序来鉴定查询序列中的相似或相同基序。MAST结果以具有适当统计数据和发现的基序的可视化概况的系列比对的形式提供。MEME和MAST在圣地亚哥的加利福尼亚大学开发。

[0217] PROSITE (Bairoch and Bucher, 1994, *Nucleic Acids Res.* 22, 3583; Hofmann et al., 1999, *Nucleic Acids Res.* 27, 215) 是鉴定从基因组或cDNA序列翻译而来的未表征的蛋白的功能的方法。PROSITE数据库 (www.expasy.org/prosite) 含有生物学上重要的模式和配置文件并被设计以使其能与适当的计算机工具一起使用,以指定新的序列至已知的蛋

白家族或测定哪些已知的结构域存在于所述序列中 (Falquet et al., 2002, *Nucleic Acids Res.* 30, 235)。Prosearch 是能够使用给定序列模式或标记搜索 SWISS-PROT 和 EMBL 数据库的工具。

[0218] 分离多肽的方法

[0219] 包括变体多肽的本发明的, 或用于本发明的方法中使用的多肽可以通过使用本领域公知的肽合成方法进行制备, 例如使用固相合成技术的定向肽合成 (例如 Stewart et al., 1969, in *Solid-Phase Peptide Synthesis*, WH Freeman Co, 加利福尼亚圣弗朗西斯科) 或自动化合成, 例如使用应用 Applied Biosystems 431A 肽合成仪 (加利福尼亚, 福斯特市)。所述多肽的突变形式也可以在该合成过程中产生。

[0220] 本发明的或在本发明方法中使用的多肽和变体多肽也可以通过使用本领域公知的各种技术从天然来源中纯化而来 (例如, Deutscher, 1990, Ed, *Methods in Enzymology*, Vol. 182, *Guide to Protein Purification*)。

[0221] 或者, 本发明的或在本发明的方法中使用的多肽和变体多肽可能在合适的宿主细胞中重组表达并按照如下所述从细胞中分离而来。

[0222] 产生构建体和载体的方法

[0223] 本发明的遗传构建体包含一条或多条本发明的多核苷酸序列和/或编码本发明的多肽的多核苷酸, 并可用于转化例如细菌、真菌、昆虫、哺乳动物或植物生物体。本发明的遗传构建体旨在包括如本文所定义的表达构建体。

[0224] 产生和使用遗传构建体和载体的方法在本领域中是公知的并通常记载于 Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 第2版, Cold Spring Harbor Press, 1987; Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing, 1987) 中。

[0225] 产生包含多核苷酸、构建体或载体的宿主细胞的方法

[0226] 本发明提供了包含本发明的遗传构建体或载体的宿主细胞。

[0227] 包含本发明的遗传构建体 (如表达构建体) 的宿主细胞可以用于本领域公知的方法中 (例如, Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 第2版, Cold Spring Harbor Press, 1987; Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing, 1987) 以用于重组产生本发明的多肽。该方法可以涉及在适当的培养基中在适于或有助于本发明的多肽的表达的条件下培养宿主细胞。可任选地分泌至培养物中的经表达的重组多肽然后可以被从培养基、宿主细胞或培养物培养基中通过本领域公知的方法分离 (例如, Deutscher, Ed, 1990, *Methods in Enzymology*, 第182卷, *Guide to Protein Purification*)。

[0228] 产生包含构建体和载体的植物细胞和植物的方法

[0229] 本发明还提供了包含本发明的遗传构建体的植物细胞, 以及被修饰以改变本发明的多核苷酸或多肽的表达的植物细胞。包含该细胞的植物也形成了本发明的一个方面。

[0230] 用多肽转化植物细胞、植物和其部分的方法在下述文献中进行了描述: Draper et al., 1988, *Plant Genetic Transformation and Gene Expression. A Laboratory Manual* Blackwell Sci. Pub. Oxford, p. 365; Potrykus and Spangenburg, 1995, *Gene Transfer to Plants*. Springer-Verlag, Berlin.; 和 Gelvin et al., 1993, *Plant Molecular*

Biol.Manual.Kluwer Acad.Pub.Dordrecht。包括转化技术的转基因植物的综述在Galun and Breiman,1997,Transgenic Plants.Imperial College Press,London中提供。

[0231] 植物遗传操纵的方法

[0232] 大量植物转化策略是可获得的(例如,Birch,1997,Ann Rev Plant Phys Plant Mol Biol,48,297,Hellens RP,et al (2000) Plant Mol Biol 42:819-32,Hellens R et al Plant Meth 1:13)。例如,在多核苷酸/多肽被正常表达时,可以设计策略以增加多核苷酸/多肽在植物细胞、器官中和/或在特定发育阶段的表达,或在多核苷酸/多肽不能正常被表达时,设计策略以在细胞、组织、器官中和/或在特定发育阶段异位表达多核苷酸/多肽。所表达的多核苷酸/多肽可以衍生自待转化的植物物种或可衍生自不同的植物物种。

[0233] 在转基因植物中用于表达基因的遗传构建体通常包括驱动一个或多个克隆多核苷酸表达的启动子、终止子和用于检测遗传构建体在被转化的植物中存在的选择性标记序列。

[0234] 适用于遗传构建体的启动子在单子叶植物或双子叶植物的细胞、组织或器官中具有功能并包括细胞、组织和器官特异性启动子,细胞循环特异性启动子,时间启动子,诱导型启动子,在大多数植物组织中有活性的组成型启动子和重组启动子。启动子的选择将依赖于所期望的克隆的多核苷酸在时间和空间上的表达。所述启动子可能是与目的转基因正常相关的启动子,或衍生自其他植物、病毒和植物病原性细菌和真菌的基因的启动子。无需过多的实验,本领域技术人员将能够通过使用包含本发明的多核苷酸序列的遗传构建体选择适用于修饰和调控植物特性的启动子。组成型植物启动子的实例包括CaMV 35S启动子、胆脂碱合成酶启动子和章鱼碱合成酶启动子和来自于玉米的Ubi 1启动子。在特定组织中有活性并对内部发育信号或外部非生物胁迫或生物胁迫产生应答的植物启动子记载于科学文献中。示例性的启动子记载于例如WO 02/00894中,其以引用的方式纳入本文。

[0235] 在植物转化遗传构建体中普遍使用的示例性的终止子包括,例如,花椰菜花叶病毒(CaMV) 35S终止子、根癌农杆菌(*Agrobacterium tumefaciens*) 胭脂碱合成酶或章鱼肉碱合成酶终止子、玉米(*Zea mays*) 醇溶蛋白基因终止子、水稻(*Oryza sativa*) ADP葡萄糖焦磷酸化酶终止子和马铃薯PI-II终止子。

[0236] 在植物转化中普遍使用的选择性标志物包括赋予卡那霉素抗性的新霉素磷酸转移酶II基因(NPT II)、赋予壮观霉素和链霉素抗性的aadA基因、赋予Ignite (AgrEvo) 和Basta (Hoechst) 抗性的膦丝菌素乙酰基转移酶(bar基因) 和赋予潮霉素抗性的潮霉素磷酸转移酶基因(hpt)。

[0237] 包含可能用于植物和植物组织中启动子表达分析的报告基因(表达对于宿主而言是外来的、通常是酶活性和/或可视信号(例如荧光素酶、GUS、GFP)的活性的编码序列)的遗传构建体的使用也是被考虑的。报告基因的文献在Herrera-Estrella et al.,1993, Nature 303,209,和Schrott,1995,In:Gene Transfer to Plants (Potrykus,T., Spangenberg.Eds) Springer Verlag.Berline,pp.325-336中进行了综述。

[0238] 基因沉默

[0239] 在多核苷酸/多肽被正常表达时,可以设计转化策略以降低多核苷酸/多肽在植物细胞、组织、器官中和/或在特定发育阶段的表达。

[0240] 基因沉默策略可能会关注基因本身或影响所编码的多肽的表达的调控元件。“调

控元件”在本文中以其最广泛可能的意义使用并包括与目的基因相互作用的其他基因。

[0241] 被设计用于降低或沉默本发明的多核苷酸/多肽的表达的遗传构建体可以包括本发明多核苷酸的反义拷贝。在这样的构建体中,所述多核苷酸被以相对于启动子和终止子的反义方向进行放置。

[0242] “反义”多核苷酸通过将多核苷酸或多核苷酸或片段反向获得,以使得所产生的转录物将与该基因的mRNA转录物互补,例如,

[0243] 5' GATCTA 3' (编码链) 3' CTAGAT 5' (反义链)

[0244] 3' CUAGAU 5' mRNA 5' GAUCUCG 3' 反义RNA

[0245] 被设计用于基因沉默的遗传构建体也可以包括反向重复序列。“反向重复序列”是重复的序列,其中,重复序列的后半段是互补链,例如,

[0246] 5' -GATCTA·····TAGATC-3'

[0247] 3' -CTAGAT·····ATCTAG-5'

[0248] 所形成的转录物可能经历互补碱基配对以形成发卡结构。通常在重复区域之间的至少3-5个碱基对的间隔区是允许发卡形成所必需的。包括这种反向重复序列的构建体可用于RNA干扰(RNAi)中,并因此可称为RNAi构建体。

[0249] 另一种沉默方法涉及靶向于miRNA的转录等价物的小反义RNA的使用(Llave et al.,2002,Science 297,2053)。这样的对应于本发明的多核苷酸的小反义RNA的使用是特别考虑的。

[0250] 本文所用的术语遗传构建体也包括小反义RNA和其他影响基因沉默的这样的多肽。

[0251] 如本文所定义的,用表达构建体进行的转化也可以通过被称为有义抑制的过程产生基因沉默(例如,Napoli et al.,1990,Plant Cell 2,279;de Carvalho Niebel et al.,1995,Plant Cell,7,347)。在一些情况下,有义抑制可涉及全部或部分编码序列的过表达但也可涉及所述基因的非编码区(如内含子或5' 或3' 非翻译区(UTR))的表达。嵌合的部分正义构建体能用于协调沉默多个基因(Abbott et al.,2002,Plant Physiol.128(3):844-53;Jones et al.,1998,Planta 204:499-505)。这样的用于沉默本发明的多核苷酸的表达的有义抑制的使用也是被考虑的。

[0252] 被设计用于基因沉默的遗传构建体中多核苷酸插入物可能对应于相应基因的编码序列和/或非编码序列(如启动子和/或内含子和/或5' 或3' UTR序列)。

[0253] 其他基因沉默策略包括显性负向方法和核酶构建体的使用(McIntyre,1996,Transgenic Res,5,257)。

[0254] 转录前沉默可能通过基因本身或其调控元件的突变而实现。这样的突变可以包括点突变、移码、插入、缺失和置换。

[0255] 转化方案

[0256] 以下是公开的能够被用于遗传转化下述植物物种的遗传转化方案的代表性出版物:水稻(Alam et al.,1999,Plant Cell Rep.18,572)、苹果(Yao et al.,1995,Plant Cell Reports 14,407-412)、玉米(美国专利序列No.5,177,010和5,981,840)、小麦(Ortiz et al.,1996,Plant Cell Rep.15,1996,877)、番茄(美国专利序列No.5,159,135)、马铃薯(Kumar et al.,1996 Plant J.9,:821)、木薯(Li et al.,1996 Nat.Biotechnology 14,

736)、莴苣(Micheltmore et al.,1987,Plant Cell Rep.6,439)、烟草(Horsch et al.,1985,Science 227,1229)、棉花(美国专利序列No.5,846,797和5,004,863)、草(美国专利No.5,187,073和6,020,539)、薄荷(Niu et al.,1998,Plant Cell Rep.17,165)、柑橘植物(Pena et al.,1995,Plant Sci.104,183);葛缕子(Kren et al.,1997,Plant Cell Rep,17,39)、香蕉(美国专利序列No.5,792,935)、大豆(美国专利No.5,416,011、5,569,834、5,824,877、5,563,04455和5,968,830)、菠萝(美国专利序列No.5,952,543)、白杨(美国专利No.4,795,855)、普通单子叶植物(美国专利No.5,591,616和6,037,522)、芸苔(美国专利No.5,188,958、5,463,174和5,750,871)、谷物(美国专利No.6,074,877)、梨(Matsuda et al.,2005,Plant Cell Rep.24(1):45-51)、李子(Ramesh et al.,2006、Song和Sink 2005、Gonzalez Padilla et al.,2003)、草莓(Oosumi et al.,2006、Oosumi et al.,2006Planta.223(6):1219-30;Folta et al.,2006Planta Apr 14;PMID:16614818Folta et al.,2006)、玫瑰(Li et al.,2003)、悬钩子(Graham et al.,1995Methods Mol Biol.1995;44:129-33)、番茄(Dan et al.,2006,Plant Cell Reports V25:432-441)、苹果(Yao et al.,1995,Plant Cell Rep.14,407-412)和毛花猕猴桃(*Actinidia eriantha*)(Wang et al.,2006,Plant Cell Rep.25,5:425-31)、欧洲桦(silver birch)(Keinonen-Mettala et al.,1998,Plant Cell Rep.17:356-361.)和山杨(Nilsson O,et al.,1992,Transgenic Research.1:209-220)。其他物种的转化也被本发明所考虑。合适的方法和方案在科学文献中可以获得。

[0257] 本领域已知的多个其他方法可能被用于改变本发明的核苷酸和/或多肽的活性的表达。这样的方法包括但不限于Tilling(Till et al.,2003,Methods Mol Biol,2%,205)、所谓的“Deletagene”技术(Li et al.,2001,Plant Journal 27(3),235)和人工转录因子(如合成的锌指转录因子)的使用(例如,Jouvenot et al.,2003,Gene Therapy 10,513)。此外,靶向于特定多肽的抗体或其片段也可以在植物中表达以调控该多肽的活性(Jobling et al.,2003,Nat.Biotechnol,21(1),35)。转座子标签方法也可以被使用。此外,与本发明的多肽相互作用的肽可以通过如相位展示的技术(Dyax Corporation)进行鉴定。这样的相互作用的多肽可以在植物中表达或应用到植物以影响本发明的多肽的活性。上述改变本发明的核苷酸和/或多肽的表达的每一种方法的使用都是被特别考虑的。

[0258] 术语“改变本发明的或在本发明的方法中使用的多核苷酸或多肽的表达”或“改变的本发明的或在本发明的方法中使用的多核苷酸或多肽的表达”旨在包括对应于本发明的多核苷酸的基因组DNA被修饰从而导致本发明的多核苷酸或多肽的改变的表达的情况。所述基因组DNA的修饰可能是通过遗传转化或其他的本领域已知的诱导突变的方法而进行。所述“改变的表达”可能与产生的信使RNA和/或多肽的量的增加或减少有关,并可能由于产生的多核苷酸和多肽的序列的改变而导致多肽的活性改变。

[0259] 选择植物的方法

[0260] 还提供了选择具有增加的根生物量的植物的方法。该方法包括测试植物中是否存在至少一种PEAPOD多核苷酸或多肽的表达改变,包括本文所定义的或所公开的那些。当增加的根生物量特征不必然易于测量时,该方法可以在幼年或早期发育阶段使用。

[0261] 多核苷酸(如信使RNA)的表达经常被用作相应多肽表达的指标。测量多核苷酸的表达的示例性的方法包括但不限于Northern分析、RT-PCR和斑点印记(dot-blot)分析

(Sambrook et al., Molecular Cloning: A Laboratory Manual, 第2版, Cold Spring Harbor Press, 1987)。因此, 如本文所定义的, 本发明的多核苷酸或其部分在鉴定具有增加的根生物量的植物的方法中可用作探针或引物。本发明或本文公开的多肽可以在被设计以鉴定这样的植物的杂交实验中用作探针或基于PCR的实验中用作引物。

[0262] 或者, 可以获得抗本文所述或所公开的PEAPOD多肽的抗体。获得和使用抗体的方法是本领域中的标准方法(参见例如: Antibodies, A Laboratory Manual, Harlow A Lane, Eds, Cold Spring Harbour Laboratory, 1998)。这样的抗体可以被用在检测所述多肽的表达改变的方法中。这样的方法可以包括ELISA (Kemeny, 1991, A Practical Guide to ELISA, NY Pergamon Press) 和Western分析 (Towbin & Gordon, 1994, J Immunol Methods, 72, 313)。

[0263] 这些分析多核苷酸或多肽表达和选择具有增加的根生物量的植物的方法在被设计以产生具有所述改变的特征的品种的传统育种程序中是有用的。

[0264] 植物

[0265] 术语“植物”旨在包括完整的植物或植物的任意部分、植物的繁殖体和子代。

[0266] 术语“繁殖体”意指可用在有性或无性生殖或繁殖中的植物的任意部分, 包括种子和插穗。

[0267] 本发明的植物可能被种植, 并且进行自交或与不同的植物植株进行杂交且所产生的具有期望的表型特征的杂交体可以被鉴定。两代或更多代植物可以被种植以确保目标表型特征被稳定维持和遗传。从这样的标准育种方法产生的植物也形成本发明的一个方面。

[0268] 通过赤霉素(GA)、油菜素内酯(BR)和其他植物激素控制植物生长和发育

[0269] 赤霉素(GA)和油菜素内酯(BR)是两类植物激素, 它们参与植物形态发生和生长的很多方面, 包括: 种子萌发、细胞伸长、维管组织发育、种子大小、叶直立、开花、叶和果实衰老(Mathew et al 2009, NZJAR 52, 213-225; Hou et al 2010, Developmental Cell 19, 884-894; Jiang and Lin 2013, Plant Signaling and Behaviour 8:10, e25928)。

[0270] 考虑到GA和BR在植物发育中的作用, 调控GA和BR的水平或它们下游靶标的能力在提高很多植物物种的产量和质量方面是高度期望的。实际上, 有一些这两种激素中的一种的外源施用被用于提高农艺价值的商业实例。

[0271] GA可被应用于黑麦草以刺激过季生长以及促进开花(Mathew et al 2009, NZJAR 52, 213-225), 其也可以被用于抵消较低温度对甘蔗(热带C4草)的副作用。GA也可以用于增大无籽葡萄和樱桃的果实大小, 以促进苹果和梨的结实并延迟特别是柑橘属作物的果皮老化(Sun 2011, Current Biology 21, R338-R345)。类似地, 建议将BR制品用于提高马铃薯、番茄、黄瓜、辣椒和大麦、水稻、玉米、小麦、棉花和烟草的作物产量和质量(Prusakova et al 1999, Agrarian Russia, 41-44; Khripach et al 2000 Annals of Botany 86, 441-447; Anjum et al 2011 J. Agronomy Crop Sci. 197, 177-185; Vardhini 2012 J. Phytology 4, 1-3)。但是, 商业上应用的油菜素内酯的低采用率可以反映出成本和在外源施用植物不能对其进行有效吸收的事实。此外, 需要严格控制时机和外源施用的GA和BR的浓度限制了它们的应用。

[0272] 在大多数情况下, 被子植物中的GA和BR生物合成和分解代谢途径已经被表征并包括负调控因子和下游转录因子靶标。GA或BR一旦结合至其各自的受体, 复杂的信号通路便

接续发生,在这两种情况中,调控的中心点均涉及改变负调控因子DELLA(在GA的情况下)和转录调控因子(在BR的情况下)的泛素-蛋白酶体通路。

[0273] DELLA蛋白的去除导致对生长抑制的去除和对GA应答的生长和发育的促进。相反地,对BR的检测导致未被磷酸化的BZR1蛋白在细胞核中的累积。BZR1的去磷酸化阻止了其被蛋白酶体降解并反而使得BZR1能与其他DNA结合转录因子结合并与转录辅因子相互作用。这导致对参与生长和其他细胞过程的成千上万个基因的调控,包括抑制BR生物合成基因的表达(He et al 2005,Science 307,1634-1638;Guo et al 2013,Current Opinion Plant Biol.16,545-553)。

[0274] 存在很多外源信号和环境提示,它们影响GA-GID1-DELLA调控模块,在该模块中DELLA通过与多种来自其他通路的关键调控蛋白的直接蛋白-蛋白相互作用整合了不同的信号传导活性。因此,DELLA蛋白是主要的生长抑制子,其通过整合来自其他激素通路的内部信号(植物生长素、脱落酸、茉莉酸和乙烯)和外部生物刺激(病原体)和非生物刺激(光照条件、冷和盐胁迫)来控制植物生长和发育(Sun 2011,Current Biology 21,R338-R345)。干旱是限制植物生长和农业生产力的最重要的环境限制因素之一。不出所料地,在提高的耐旱性和更延伸的根系之间存在正相关性,所述更延伸的根系包括更深的根和更多的侧根,它们都能够进行土壤勘探和地下资源获取(Yu et al 2008,Plant Cell 20,1134-1151;Werner et al 2010,Plant Cell 22,3905-3920)。因此,由此可见,共同的农业目标是对根系结构的优化以有助于克服由水或养分缺乏造成的作物植物的产量限制。但是,在所有缩减作物产量的非生物胁迫中,干旱是最具破坏性的一种并且是对抗育种者工作的最顽固胁迫。因为性状受很多基因控制,传统的育种方法是困难的并且难以评估(Werner et al 2010,Plant Cell 22,3905-3920)。当标记辅助选择(MAS)、数量性状基因座(QTL)和其他基因组学方法被广泛用于辅助育种工作以产生抵抗干旱的栽培种时(Tuberosa and Salvi,2006,Trends in Plant Science,11:405-412),所述系统受限于筛选群体中存在的变异。

[0275] 有趣地是,水稻仅具有一个DELLA蛋白(SLR1),玉米具有两个(d8和d9)(Lawit et al 2010,Plant Cell Physiol 51,1854-1868),而拟南芥属具有5个(GA1、RGA、RGL1、RGL2和RGL3)(Achard and Genschik 2009,J.Exp.Bot.60,1085-1092)。此外,在最近的系统进化分析中,Chen et al 2013发现了它们所分析的六个禾本科物种有5个仅具有单个DELLA,而18个双子叶植物物种中有14个具有两个或更多个DELLA蛋白。相比之下,水稻中存在BZR家族中的6个成员,玉米中存在10个成员(www.Grassius.org)以及拟南芥属中存在6个成员(Wang et al 2002,Developmental Cell 2,505-513)。

[0276] 植物的生长和发育依赖于提供其固着生活习性所需的高发育可塑性的信号传导通路之间的多种连接(Gallego-Bartolome et al 2012,PNAS 109,13446-13451)。因此,目前的证据,而不是以绝缘形式存在的各激素信号传导通路表明不同的通路之间存在高程度的相互作用,以及给定的激素通常调控由其余激素引起的输出。例如,近来已证明GA和BR信号传导通路之间的相互干预包括DELLA和BZR1/BES1之间的直接相互作用,由此DELLA蛋白不仅影响蛋白稳定性而且抑制BZR1的转录活性(Li and He 2013,Plant Signaling and Behaviour 8:7,e24686及其中的文献)。因此,由GA促进细胞伸长部分是通过去除DELLA介导的对BZR1的抑制。

[0277] 近来已证明植物生长和发育可以通过直接操控主生长调节子DELLA (Lawit, Kundu, Rao and Tomes, 2007, ISOLATED POLYNUCLEOTIDE MOLECULES CORRESPONDING TO MUTANT AND WILD-TYPE ALLELES OF THE MAIZE D9 GENE AND METHODS OF USE, WO 2007124312 A2) 和BZR1 (Chory and Wang, 2005, GENES INVOLVED IN BRASSINOSTEROID HORMONE ACTION IN PLANTS, US 6,921,848B2) 来改变。

[0278] 类固醇激素在协调植物和动物的广泛的发育和生理过程中起着重要作用 (Thummel and Chory 2002, Genes Dev. 16, 3113-3129)。在植物中,类固醇激素油菜素内酯 (BR) 在生长、发育和对生物和非生物胁迫的应答中起着广泛的作用 (Zhu et al 2013, Development 140, 1615-1620; Clouse 2011, Plant Cell 23, 1219-1230)。相比于通过核受体起作用的动物类固醇激素信号传导,在植物中,BR结合于细胞表面受体激酶 BRASSINOSTEROID INSENSITIVE 1 (BRI1) 的胞外结构域并激活调控基因表达的细胞内信号转导级联 (Clouse 2011, Plant Cell 23, 1219-1230; Kinoshita et al 2005, Nature 433, 167-171)。有包括中间体的激活和失活的多个步骤,所述中间体导致两个转录因子 BRASSINAZOLE RESISTANT 1 (BZR1) 和BZR2 (也称为BES1) 的磷酸化。因此,信号转导BZR转录因子是将信号传导转换为BR应答基因表达的靶组分。

[0279] 在植物激素信号传导中存在一种新兴模式,其中,由激素激活的靶转录因子也被特定的阻遏因子复合物负调控。例如,在茉莉酸 (JA)、植物生长素、脱落酸 (ABA) 和独角金内酯 (SL) 信号传导通路中,所述靶转录因子被利用作为由激素通路特异性抑制因子招募的常用协同阻遏物的TOPLESS (TPL) 的抑制因子复合物负调控 (Pauwels et al 2010, Nature 464, 788-791)。在JA转导通路中,JASMONATE ZIM DOMAIN (JAZ) 家族的转录阻遏因子直接地或通过衔接蛋白NOVEL INTERACTOR OF JAZ (NINJA) 与靶JA-应答转录激活子MYC2相互作用并招募TPL (Pauwels et al 2010, Nature 464, 788-791)。

[0280] 因此,调控影响很多不同的目的农业性状的GA和BR通路的能力对于商业性农业具有相当大的价值。

[0281] 本申请人的发明

[0282] 如上所述,本发明涉及通过异位表达PEAPOD以增加植物中的根生物量的方法。

[0283] 不希望被理论所束缚,本申请人已经证明PEAPOD (PPD) 似乎通过与主生长调节子DELLA和BZR的直接或间接相互作用来调控GA和BR通路。

[0284] 对PPD蛋白的一级氨基酸结构的分析表明高度保守的新的植物特异性结构域仅存在于这些蛋白中。在广泛的双子叶植物、松柏类植物和一些单子叶植物 (棕榈、香蕉、兰花和浮萍) 而非禾本科植物中发现了这些基因的同源物。

[0285] 拟南芥属的PPD基因编码植物特异性TIFY家族 (其以称为ZIM的结构域内发现的核心TIF[F/Y]XG基序命名) 成员的蛋白 (Vanholme et al 2007, Trends Plant Sci. 12, 239-244)。这两个拟南芥属PPD蛋白PPD1和PPD2包括在与12个已经充分表征的充当茉莉酮酸酯应答的阻遏因子的JAZ蛋白相同的II类TIFY组中。但是,PPD蛋白和该组中的另一个非-JAZ蛋白似乎并不参与对茉莉酮酸酯激素信号传导的应答 (Pauwels et al 2010, Nature 464, 788-791)。

[0286] 同样地,不希望被理论所束缚,本申请人提出根据本发明,根生物量的增加由新的机制介导,该机制使用PPD基因调控GA和BR通路。以下实施例3和4支持该提议。

[0287] 本发明也可以广义地说是由本申请的说明书中单独或共同提及或指出的部分、元素和特征,以及所述部分、元素或特征中的任意两个或更多个的任意或全部组合组成,并且其中在本文中提到的特定整体在本发明涉及的领域中具有已知的等同性,这种已知的等同性被认为如同单独提出一样被纳入本文。

附图说明

[0288] 参照以下附图可以更好地理解本发明,其中:

[0289] 图1示出了包含来自一些植物物种的PEAPOD区域的46个氨基酸残基,相同的残基用星号表示。

[0290] 图2示出了来自一些植物物种的PEAPOD区域内的内部27个氨基酸残基,相同的残基用星号表示。

[0291] 图3示出了来自一些植物物种的PEAPOD蛋白的TIFY结构域的6个氨基酸残基,相同的残基用星号表示。

[0292] 图4示出了PPD蛋白和保守的PPD、TIFY和Jas*区域的大致位置的示意图。

[0293] 图5示出了在Y2H测定中PPD蛋白的二聚化以及TPL和NINJA之间的相互作用。

[0294] 图6示出了在Y2H测定中PPD和NINJA之间的相互作用以及TPL和BZR1之间的相互作用。

[0295] 图7示出了使用BiFC测定证明的在嫩叶(A和B)和老叶(C)中的PPD、NINJA、TPL和BZR1之间的相互作用。

[0296] 图8示出了PPD-NINJA-TPL-BZR1复合物的示意图。

[0297] 图9示出了在Y1H测定中PPD和BZR1之间的相互作用。

[0298] 图10示出了野生型、 Δ ppd突变体和PEAPOD过表达子(PPD-0X)下胚轴长度对外源GA和PAC施用的应答。

[0299] 图11示出了与野生型和载体对照相比过表达来自拟南芥(*Arabidopsis thaliana*)的PPD(PPD-0X)或来自无油樟(*Ambroella trichopoda*)的PEAPOD的黑麦草植物的根生长的增加。

[0300] 图12示出了与对照植物相比过表达PPD(PPD-0X)的苜蓿植物的根长度的增加。

[0301] 图13示出了来自拟南芥、北美云杉(*Picea sitchensis*)、无油樟、小果野蕉(*Musa acuminata*)、白三叶草(*Trifolium repens*)和江南卷柏(*Selaginella moellendorffii*)的PEAPOD蛋白是功能等效的。来自每一个的优化的PEAPOD编码序列被用于互补PEAPOD缺失突变体 Δ ppd拟南芥(生态型兰兹贝格(*Landsberg erecta*))。在等同的发育阶段拍摄幼苗图片。

[0302] 实施例

[0303] 现在参照以下非限制性实施例对本发明进行说明。

[0304] 实施例1:对多个植物物种的PEAPOD基因的表征

[0305] 为了鉴定其他植物物种中的PPD基因直系同源物,将来自拟南芥属PPD1基因(SEQ ID NO:27)的保守的PPD区域(46个氨基酸)用于使用搜索程序TBLASTN和BLASTP进行公共植物基因序列数据库搜索(Altschul et al 1990)。从多种植物物种(包括苔藓植物、松柏类植物、所检查的双子叶植物的所有目和一些单子叶植物目,包括棕榈、香蕉、兰花和浮萍)中

鉴定PEAPOD序列。PEAPOD序列不存在于禾本科植物中。代表性的PEAPOD蛋白和核酸序列分别示于SEQ ID NO:1-26和SEQ ID NO:83-107中。

[0306] 来自拟南芥PPD1的46个氨基酸PEAPOD区域示于SEQ ID NO:27中。如图1所示,用vector NTI (VNTI) 比对来自多肽SEQ ID NO:1-26的该区域。

[0307] SEQ ID NO:28示出了这46个氨基酸PPD区域的共有序列。SEQ ID NO:29示出了相同的共有区域,但是示出了哪些氨基酸可存在于各可变位置处。

[0308] 来自拟南芥PPD1的46个氨基酸PEAPOD区域内的27个氨基酸子序列示于SEQ ID NO:30中。

[0309] 对图1中的每个相同序列的这27个氨基酸子序列的比对示于图2中。

[0310] SEQ ID NO:30示出了这27个氨基酸PPD区域的共有序列。SEQ ID NO:32示出了相同的共有区域,但是示出了哪些氨基酸可存在于各可变位置处。

[0311] 在每一个SEQ ID NO:1-26的PPD肽序列中,也有保守的TIFY基序,其位于所述46个氨基酸PPD区域之后。分隔开PPD区域的C-末端和TIFY基序的N-末端的氨基酸残基的数目取决于PPD的来源;例如,对于SEQ ID NO:1-26,所述数目在46至140个氨基酸之间变化。

[0312] SEQ ID NO:33示出了拟南芥属PPD1序列的TIFY基序。对来自SEQ ID NO:1-26的TIFY基序的比对(如Vanholme et al 2007, Trends Plant Sci.12,239-244中所述)示于图3中。

[0313] SEQ ID NO:34示出了这6个氨基酸TIFY基序的共有序列。SEQ ID NO:35示出了相同的共有区域,但是示出了哪些氨基酸可存在于各可变位置。PPD和TIFY结构域中完全保守的残基在图1-3中用星号突出显示。

[0314] 本申请人认为以上所述的这些区域和基序存在于所有鉴定的PEAPOD蛋白中并且可用于诊断这些PEAPOD蛋白。

[0315] 实施例2:证明来自多个植物物种的PEAPOD序列的PEAPOD功能

[0316] 可以通过拟南芥属 Δ ppd 突变体叶表型的互补作用来确认任意PEAPOD序列的功能。首先将拟南芥属 Δ ppd 突变体叶表型的互补作用用于鉴定拟南芥属PPD基因(White 2006)。这可以通过将与圆顶叶和玫瑰花结的扭曲相反的野生型扁平叶表型和正常玫瑰花结形恢复为“螺旋桨”表型而看到。

[0317] PEAPOD序列,如SEQ IN NO:1-26中的那些(包括棕榈、松柏类植物、苔藓、兰花和其他双子叶植物物种),或任意其他待测试的PEAPOD序列可以通过本领域技术人员公知的方法被转化到拟南芥属 Δ ppd 突变体中。这类方法中的一个实例在下文描述。

[0318] 克隆和基因构建体

[0319] 用于在拟南芥属 Δ ppd 突变体中过表达拟南芥属PPD1的CaMV35s::拟南芥PPD1构建体的产生合成表达构建体以使得在拟南芥属 Δ ppd 突变体中拟南芥PPD1在CaMV35s启动子(SEQ ID NO:129)下过表达。优化PPD ORF以用于在拟南芥属中表达,这包括修饰的Joshi序列(Joshi 1997, Nucleic Acid Research 15,6643-6653)、密码子的优化、mRNA不稳定性序列的去除、多聚A信号序列的去除、隐性剪接位点的去除、BamHI可去除的C-末端V5表位和His标签尾部(编码SEQ ID NO:37)的添加和双终止密码子的添加。然后将该构建体(有或没有所述尾部)通过GATEWAY®LR反应置于CaMV35s启动子和ocs终止子之间,其分别编码为SEQ ID NO:105和SEQ ID NO:111。

[0320] 用于在拟南芥属 Δ ppd 突变体中过表达白三叶草PPD1的CaMV35s::白三叶草PPD构建体的产生

[0321] 合成表达构建体以使得在拟南芥属 Δ ppd 突变体中白三叶草PPD在CaMV35s启动子 (SEQ ID NO:129) 下过表达。优化PPD ORF以用于在拟南芥属中表达,这包括修饰的Joshi序列 (Joshi 1997,Nucleic Acid Research 15,6643-6653)、密码子的优化、mRNA不稳定性序列的去除、多聚A信号序列的去除、隐性剪接位点的去除、BamHI可去除的C-末端V5表位和His标签尾部 (编码SEQ ID NO:37) 的添加和双终止密码子的添加。然后将该构建体 (有或没有所述尾部) 通过 **GATEWAY®** LR反应置于CaMV35s启动子和ocs终止子之间,其分别编码为SEQ ID NO:106和SEQ ID NO:112。

[0322] 用于在拟南芥属 Δ ppd 突变体中过表达无油樟PPD的CaMV35s::无油樟PPD构建体的产生

[0323] 合成表达构建体以使得在拟南芥属 Δ ppd 突变体中无油樟PPD在CaMV35s启动子 (SEQ ID NO:129) 下过表达。优化PPD ORF以用于在拟南芥属中表达,这包括修饰的Joshi序列 (Joshi 1997,Nucleic Acid Research 15,6643-6653)、密码子的优化、mRNA不稳定性序列的去除、多聚A信号序列的去除、隐性剪接位点的去除、BamHI可去除的C-末端V5表位和His标签尾部 (编码SEQ ID NO:37) 的添加和双终止密码子的添加。然后将该构建体 (有或没有所述尾部) 通过 **GATEWAY®** LR反应置于CaMV35s启动子和ocs终止子之间,其分别编码为SEQ ID NO:107和SEQ ID NO:113。

[0324] 用于在拟南芥属 Δ ppd 突变体中过表达小果野蕉PPD的CaMV35s::小果野蕉PPD构建体的产生

[0325] 合成表达构建体以使得在拟南芥属 Δ ppd 突变体中小果野蕉PPD在CaMV35s启动子 (SEQ ID NO:129) 下过表达。优化PPD ORF以用于在拟南芥属中表达,这包括修饰的Joshi序列 (Joshi 1997,Nucleic Acid Research 15,6643-6653)、密码子的优化、mRNA不稳定性序列的去除、多聚A信号序列的去除、隐性剪接位点的去除、BamHI可去除的C-末端V5表位和His标签尾部 (编码SEQ ID NO:37) 的添加和双终止密码子的添加。然后将该构建体 (有或没有所述尾部) 通过 **GATEWAY®** LR反应置于CaMV35s启动子和ocs终止子之间,其分别编码为SEQ ID NO:108和SEQ ID NO:114。

[0326] 用于在拟南芥属 Δ ppd 突变体中过表达北美云杉PPD的CaMV35s::北美云杉PPD1构建体的产生

[0327] 合成表达构建体以使得在拟南芥属 Δ ppd 突变体中北美云杉PPD在CaMV35s启动子 (SEQ ID NO:129) 下过表达。优化PPD ORF以用于在拟南芥属中表达,这包括修饰的Joshi序列 (Joshi 1997,Nucleic Acid Research 15,6643-6653)、密码子的优化、mRNA不稳定性序列的去除、多聚A信号序列的去除、隐性剪接位点的去除、BamHI可去除的C-末端V5表位和His标签尾部 (编码SEQ ID NO:37) 的添加和双终止密码子的添加。然后将该构建体 (有或没有所述尾部) 通过 **GATEWAY®** LR反应置于CaMV35s启动子和ocs终止子之间,其分别编码为SEQ ID NO:109和SEQ ID NO:115。

[0328] 用于在拟南芥属 Δ ppd 突变体中过表达江南卷柏PPD1的CaMV35s::江南卷柏PPD构建体的产生

[0329] 合成表达构建体以使得在拟南芥属 Δ ppd 突变体中江南卷柏PPD在CaMV35s启动子 (SEQ ID NO:129) 下过表达。优化PPD ORF以用于在拟南芥属中表达,这包括修饰的Joshi序列 (Joshi 1997, Nucleic Acid Research 15, 6643-6653)、密码子的优化、mRNA不稳定性序列的去除、多聚A信号序列的去除、隐性剪接位点的去除、BamHI可去除的C-末端V5表位和His标签尾部 (编码SEQ ID NO:37) 的添加和双终止密码子的添加。然后将该构建体 (有或没有所述尾部) 通过 **GATEWAY®** LR反应置于CaMV35s启动子和ocs终止子之间,其分别编码为SEQ ID NO:110和SEQ ID NO:116。

[0330] 植物材料和生长条件

[0331] 拟南芥 (L.) Heynh生态型Ler可以被用作野生型 (WT)。功能缺失突变体 (缺失PPD1和PPD2) 的 Δ ppd缺失如先前White 2006, PNAS 103, 13238-13243中所述。

[0332] 使植物生长于持续为21℃的温度可控的温室中或在23℃下在16h光照_8h黑暗循环下的可控环境室中。

[0333] 拟南芥属转化

[0334] 可以通过蘸花 (floral dip) 浸润法将上述构建体转化到拟南芥属中 (Clough and Bent, 1998, Plant J 16, 735-43)。转化 Δ ppd株系以通过标准技术表达PPD多肽。通过标准PCR分析技术并结合转基因特异性和T-DNA引物确认转基因植物。

[0335] 在T1幼苗 (经浸润的植物的子代) 中产生野生型叶和玫瑰花结表型的 Δ ppd株系的互补作用确认了所引入的基因的PEAPOD功能, 如照片所示。

[0336] 该方法可用于确认本申请人指定的任意基因的PEAPOD功能, 表明其适用于本发明。

[0337] 通过PEAPOD缺失突变体 Δ ppd拟南芥生态型Landsberg erecta的互补作用证明来自拟南芥、北美云杉、无油樟、小果野蕉和江南卷柏的PEAPOD蛋白是功能等效的 (图13)。

[0338] 实施例3:PEAPOD可能参与调控油菜素类固醇信号传导通路

[0339] 本申请人使用酵母双杂交 (Y2H) 测定、双分子荧光 (BiFC) 来研究PPD、NINJA、TPL和BZR1之间的相互作用。

[0340] 克隆和构建体

[0341] 如下产生用于Y2H和BiFC测定的构建体。合成编码以下的开放阅读框的拟南芥属DNA序列: At4g14713 (PPD1) 和PPD1的截短和缺失衍生物、PPD1、PPD1 Δ ppd (编码氨基酸1-61的序列的N-末端截短)、PPD1 Δ tify (编码氨基酸154-186的序列的内部缺失)、PPD1 Δ jas* (编码氨基酸229-313的序列的C-末端截短) (图4)、At4g28910 (NINJA)、At1g15750 (TPL)、At1g75080 (BZR1)、合成的PUAS-35S启动子和编码GAL4DBD和c-myc融合蛋白的序列, 并通过GeneArt验证序列。提供大部分序列作为备用于Gateway克隆到酵母和植物表达载体中的pENTR221中的克隆。例外的是, 整合了5' XhoI和3' NcoI限制性酶切位点的用于植物内转录激活测定的启动子序列被提供在pMA-RQ中进行克隆。用于瞬时LUC报告测定的质粒: 将具有-105bp CaMV35S启动子上游的5X UAS GAL4 DNA结合位点的合成启动子克隆至包含内含子的萤火虫荧光素酶基因 (LUC) 和35Spro::海肾荧光素酶 (REN) 作为内标的双荧光素酶构建体pNWA62内的XhoI-NcoI位点, 以构建pAML7。对于GAL4DBD融合蛋白的过表达, 使用Gateway技术将编码GAL4 DNA结合结构域 (GAL4DBD氨基酸1-147) 以及N-末端GAL4DBD与2X VP16激活子结构域 (GAL4DBD-VP16) 或PPD1 (GAL4DBD-PPD1) 的融合体 (使用编码GGGS的接

头)的DNA序列克隆至pRSh1 (Winichayakul et al 2008) 中以构建载体pRSh1-GAL4DBD、pRSh1-GAL4DBD-VP16和pRSh1-GAL4DBD-PPD1,用于在植物内表达该融合蛋白。

[0342] 用于酵母双杂交分析的质粒

[0343] 将BZR1、NINJA、TPL和PPD1,以及PPD1的截短或缺失衍生物 (PPD1 Δ ppd、PPD1 Δ tify和PPD1 Δ jas*) 的全长编码序列Gateway亚克隆至pDEST32 (N-末端GAL4DBD) 或pDEST22 (N-末端GAL4AD),以构建作为诱饵载体的pDEST32-PPD1、pDEST32-PPD1 Δ ppd、pDEST32-PPD1 Δ tify、pDEST32-PPD1 Δ jas*、pDEST32-TPL以及作为猎物载体的pDEST22-PPD1、pDEST22-BZR1和pDEST22-NINJA。当被表达时,这些构建体产生序列56-67和70-72中所列的蛋白,包括DNA结合结构域 (DBD)、激活结构域 (AD)、与DBD融合的PPD1 (PPD1-DBD)、与AD融合的PPD1 (PPD1-AD)、未与AD融合的TIFY结构域的PPD1 (PPD1-tify-AD)、未与AD融合的jas结构域的PPD1 (PPD1-jas*-AD)、TOPLESS (TPL)、与DBD融合的TPL (TPL-DBD)、NINJA、与AD融合的NINJA (NINJA-AD)、与AD融合的BZR1 (BZR1-AD)、不含与DBD融合的ppd结构域的PPD1 (PPD1- Δ ppd-DBD)、不含与DBD融合的TIFY结构域的PPD1 (PPD1-tify-DBD)、不含与DBD融合的jas结构域的PPD1 (PPD1-jas*-DBD)。

[0344] 用于双分子荧光互补的质粒

[0345] 使用具有N-末端融合体的二元BiFC-Gateway YFP载体pDEST-VYNE (R)^{GW} (Venus氨基酸1-173) 和pDEST-VYCE (R)^{GW} (Venus氨基酸156-239) 构建以下载体:pDESTnYFP-BZR1、pDESTnYFP-NINJA、pDESTnYFP-PPD1、pDESTcYFP-BZR1、pDESTcYFP-PPD1、pDESTcYFP-PPD1 Δ ppd、pDESTcYFP-PPD1 Δ tify和pDESTcYFP-PPD1 Δ jas*。对于与PPD1或BZR相互作用的蛋白在植物中的瞬时表达,将NINJA和TPL Gateway®亚克隆至pRSh1以构建pRSh1-NINJA和pRSh1-TPL。用于免疫共沉淀的质粒:将编码PPD1和3X c-myc C-末端融合体的合成的DNA构建体亚克隆至pRSh1以产生pRSh1-PPD1-3xc-myc,同时将NINJA cDNA序列亚克隆至pB7FWG2,0 (Karimi et al 2002, Trends Plant Sci. 7, 193-195) 以构建pB7FWG2-NINJA-GFP。当被表达时,这些构建体产生序列63、65、73-82中所列的蛋白,包括TOPLESS (TPL)、NINJA、双分子荧光 (BiFC) nYFP、BiFC cYFP、BiFC nYFP-NINJA、BiFC nYFP-BZR1、BiFC cYFP-PPD1、BiFC cYFP-NINJA、BiFC cYFP-BZR1、BiFC cYFP-PPD1-ppd、BiFC cYFP-PPD1-tify、BiFC cYFP-PPD1-jas*。

[0346] 使用ProQuest双杂交体系 (Invitrogen) 分析PPD1、NINJA、TPL和BZR1之间的相互作用。使用诱饵构建体和猎物构建体的组合共转化酵母菌株MaV203 (Invitrogen),在合成缺陷型 (SD) SD/-Leu/-Trp琼脂平板上进行选择。在具有不同浓度的3-氨基三唑 (3-AT) (Sigma) 的SD/-Leu/-Trp/-His平板上使用10 μ l 1:10和1:100稀释度的液滴测试转化菌株的相互作用。

[0347] 使用pDESTnYFP和pDESTcYFP质粒的组合,使用或不使用用于表达NINJA (pRSh1-NINJA) 或TPL (pRSh1-TPL) 和用于本氏烟 (*Nicotiana benthamiana*) 叶的农杆菌-浸润的质粒进行瞬时BiFC实验。对于浸润,从平板上重悬含有该二元载体的根癌农杆菌GV3101菌株并制备以用于如上所述用于LUC测定的转化。将所有的YFP和表达菌株以1:1 (体积/体积) 的比例混合,并添加1/10体积的菌株P19。40h后,从各经浸润的叶中取5个叶盘样品。在取样用于显微镜荧光观察之前2小时,将叶用1 μ g/ml DAPI溶液浸润以染色细胞核。使用Olympus Fluoview FV10i共聚焦激光扫描显微镜检测YFP荧光和DAPI染色。每个实验重复两次。

[0348] 使用PPD1作为诱饵蛋白的Y2H筛选鉴定NINJA为与PPD1的直接相互作用因子。BiFC测定的结果表明PPD1在植物中与NINJA相互作用,并且表明TIFY基序对于该相互作用也是必需的(图7)。可能NINJA作为TPL和PPD1之间的桥梁而发挥功能。使用Y2H,没有观察到PPD1和BZR1之间的直接相互作用(图5)。但是,近来的串联亲和纯化(TAP)实验已表明TPL可能与BZR1相互作用(Wang et al 2013, Mol. Cell. Proteomics 12, 3653-3665),并且在此,Y2H结果确认发生了直接的相互作用(图6)。

[0349] 为了确定PPD蛋白的分子功能,在植物内研究了PPD1、NINJA、TPL和BZR1的相互作用。使用双分子荧光(BiFC)证明在未成熟的本氏烟叶的扁平细胞中,PPD1似乎在细胞核中与BZR1相互作用(图7A、B)。PPD1中的NINJA-结合TIFY基序对于该相互作用是必需的。此外,在完全展开的叶中当nYFP-PPD1和cYFP-BZR1共表达时,未观察到相互作用(图7C)。有趣的是,PPD1和BZR1之间的相互作用在共表达NINJA而非单独的TPL时得到恢复,表明在成熟的叶中缺少相互作用是由于内源NINJA的限制。对于未成熟的叶,当PPD1NINJA-结合TIFY基序缺失时,甚至在NINJA和TPL共表达的存在下,也观察不到PPD1和BZR1之间的相互作用(图7C)。这些结果表明PPD1、NINJA、TPL和BZR1在植物内作为复合物存在,并且表明NINJA是招募PPD1以通过TPL与BZR1相互作用所必需的。

[0350] PPD1似乎并不直接与靶BZR1转录因子相互作用。相反地,PPD1蛋白相互作用实验结果表明了这样一种模式,其中PPD蛋白使用NINJA作为衔接体来招募TPL转录共表达子,该PPD-NINJA-TPL复合物与BZR转录因子的EAR基序相互作用(图8)。因此,在该模式中,拟南芥的PEAPOD1(PPD1)蛋白会充当BR信号传导通路的阻遏物并与NINJA和TPL组合来负调控BZR1。

[0351] 实施例4:PEAPOD可能参与调控赤霉素信号传导通路

[0352] 已知赤霉素(GA)处理降低GA阻遏物DELLA蛋白(包括RGA1)的水平;为了确定PPD、DELLA和GA信号传导通路之间的关系,本申请人进行PPD和DELLA(RGA1)之间的酵母双杂交(Y2H)分析,并将赤霉素(GA)激素和GA生物合成抑制剂(多效唑,PAC)施用至野生型、 Δ ppd突变体和 Δ ppd突变体PPD过表达子(PPD-OX)。

[0353] 将ProQuest双杂交系统(Invitrogen)用于分析PPD1和RGA1之间的相互作用。将PPD1以及PPD1的截短或缺失衍生物(PPD1 Δ ppd、PPD1 Δ tify和PPD1 Δ jas*)的全长编码序列(图4) Gateway亚克隆至pDEST32(N-末端GAL4DBD)或pDEST22(N-末端GAL4AD)。当被翻译时,这些产生了以下肽序列:SEQ ID NO:58、68、69、70、71、72,它们分别为PPD1-DBD、RGA1、RGA1-AD、PPD1-ppd-DBD、PPD1-tify-DBD、PP1-jas*-DBD。

[0354] 使用诱饵构建体和猎物构建体的组合共转化酵母菌株MaV203(Invitrogen),在合成缺陷型(SD)SD/-Leu/-Trp琼脂平板上进行选择。在具有不同浓度的3-氨基三唑(3-AT)(Sigma)的SD/-Leu/-Trp/-His平板上使用10 μ l 1:10和1:100稀释度的液滴测试转化菌株的相互作用。使用PPD1-DBD作为诱饵测试PPD1-RGA1相互作用。将转化的酵母以10倍稀释液点样在对照培养基(-2)或具有15mM 3AT的选择培养基上。对照为空载体、DBD、GAL4DNA结合结构域、AD、GAL4激活结构域(图4)。Y2H结果表明PPD可以直接与DELLA结合(图9)。

[0355] 对于外源施用GA或PAC,用70%乙醇、0.01%Triton X-100对种子进行表面消毒10min,然后用100%乙醇消毒5min,在无菌的滤纸上空气干燥,转移到含有半强度的MS盐、1%蔗糖和0.8%琼脂的培养基平板上。将平板在黑暗中于4℃孵育5天,然后转移到24℃下

的14h光照/10h黑暗的日循环中。通过荧光灯管(Philips TLD 58W/865)以 $100\mu\text{M m}^{-2}\text{s}^{-1}$ 的强度提供光照。使野生型(Co1-0) Δppd 突变体和转基因PPD-0X幼苗在含有不同浓度的GA(图10A)或PAC(图10B)的培养基上生长5天。将GA(ACROS有机物)和PAC(Sigma-Aldrich)分别溶解于乙醇和丙酮中,过滤消毒并添加到培养基平板中。将乙醇或丙酮(0.5%)用于对照处理。使幼苗在24℃下的14h光照/10h黑暗的日循环中生长5天,然后分析下胚轴长度($n=35$)。每次处理重复2次,误差棒=平均值的标准误差。

[0356] DELLA的降低导致促进细胞扩增的DELLA靶基因的转录的增加并可以通过测量生长在含不同水平GA的培养基上的幼苗的下胚轴长度来定量。最低浓度的GA($1\mu\text{M}$)不能促进野生型(WT)下胚轴的伸长,而功能丧失的PPD突变体(Δppd)和过表达苗的转基因PPD(PPD-0X)都显示出增加的下胚轴长度(图10A)。在更高的GA浓度(5-50 μM)下,野生型下胚轴的伸长以剂量依赖的方式发生。在比较中, Δppd 和PPD-0X幼苗分别显示出对最高达5和10 μM GA的超敏感性伸长,其中它们大约达到相同的长度(图10A)。

[0357] 通过施用外源性多效唑(PAC)抑制GA生物合成;这导致DELLA阻遏蛋白的增加和细胞扩增的相应降低。野生型幼苗显示出下胚轴伸长的从0至10 μM PAC呈剂量依赖性降低(图10B)。再次, Δppd 幼苗显示出高敏感性应答,观察到下胚轴伸长在相同范围的PAC施用下降低更大。但是,PPD-0X幼苗是相对不敏感的,直到PAC浓度增加超过0.1 μM ,这之后,它们也显示出下胚轴长度的降低(图10B)。

[0358] Δppd 幼苗对GA的超敏感性应答潜在地反映了不存在通过PPD的转录因子阻遏时,用于降解的增加的DELLA的靶标的组合。类似地,在 Δppd 背景中添加PAC可能导致与WT相比下胚轴伸长的降低更大,因为这种情况在不存在DELLA天然拮抗剂之一-PPD时发生的,表明PPD和GA竞争与DELLA结合。

[0359] 可以预测的是PPD的过表达将导致更高水平的对DELLA的拮抗作用,因此,这些植物的下胚轴伸长应该对GA超敏感;实际上,这是本发明人在PPD-0X幼苗中所观察到的结果。在相反的情况下,当GA水平降低(通过施用PAC)时,PPD-0X幼苗无应答直到PAC浓度大于0.1 μM 。这可能反映了在该点内源GA水平具有足够的降低以观察到未被过表达的PPD拮抗的DELLA蛋白的影响。

[0360] 实施例5:通过增加PEAPOD在植物中的表达来增加根生物量

[0361] 构建体

[0362] 以下描述的是几种用于在不同启动子的控制下表达来自不同物种的PEAPOD序列的构建体,用于在双子叶植物和单子叶植物中表达。

[0363] 用于拟南芥属PPD在双子叶植物中的(根优选的)过表达的pTobRB7 $\Delta 1.3::\text{PPD}$ 双子叶植物构建体的产生

[0364] 合成含有用于在双子叶植物的根中表达的PPD1的编码序列的构建体,核酸序列示于SEQ ID NO:40和126中。PPDORF来自于拟南芥PPD1cDNA(登录号NM_202819)并被修饰以包括修饰的Joshi序列(Joshi 1997, Nucleic Acid Research 15, 6643-6653)、隐性剪接位点的去除,在SEQ ID NO:40的情况中来自多年生黑麦草DGAT1的第3个内含子的加入(SEQ ID NO:39)、BamHI可去除的C-末端V5表位和His标签尾部(编码SEQ ID NO:37)的添加和双终止密码子的添加。优化内含子的位置以用于剪接位点预测,通过NetGene2进行(<http://www.cbs.dtu.dk/services/NetGene2/>)。

[0365] 然后通过 **GATEWAY®** LR 反应将 SEQ ID NO 40 (具有或不具有所述尾部) 的构建体置于 TobRB7 Δ 1.3 启动子 (Yamamoto et al 1991, Plant Cell, 3:371-382) 和 NOS 终止子之间以产生 SEQ ID NO:42 和 SEQ ID NO:44, 其分别编码为 SEQ ID NO:36 和 SEQ ID NO:38。在 SEQ ID NO 126 所示的构建体 (具有或不具有所述 C-末端尾部) 上进行类似的亚克隆步骤, 其分别编码为 SEQ ID NO:108 和 SEQ ID NO:114。

[0366] 用于拟南芥属 PPD 在双子叶植物中的 (根优选的) 过表达的 pTobRB7 Δ 0.6::PPD 双子叶植物构建体的产生

[0367] 合成含有用于在双子叶植物的根中表达的 PPD1 的编码序列的构建体, 核酸序列示于 SEQ ID NO:40 和 126 中。PPD ORF 来自于拟南芥 PPD1cDNA (登录号 NM_202819) 并被修饰以包括修饰的 Joshi 序列 (Joshi 1997, Nucleic Acid Research 15, 6643-6653)、隐性剪接位点的去除, 在 SEQ ID NO:40 的情况中来自多年生黑麦草 DGAT1 的第 3 个内含子的加入 (SEQ ID NO:39)、BamHI 可去除的 C-末端 V5 表位和 His 标签尾部 (编码 SEQ ID NO:37) 的添加和双终止密码子的添加。优化内含子的位置以用于剪接位点预测, 通过 NetGene2 进行 (<http://www.cbs.dtu.dk/services/NetGene2/>)。

[0368] 然后通过 **GATEWAY®** LR 反应将 (具有或不具有所述尾部) 的构建体置于 TobRB7 Δ 0.6 启动子 (Yamamoto et al 1991, Plant Cell, 3:371-382) 和 NOS 终止子之间以产生 SEQ ID NO:43 和 SEQ ID NO:45, 其分别编码为 SEQ ID NO:36 和 SEQ ID NO:38。在 SEQ ID NO 126 所示的构建体 (具有或不具有所述 C-末端尾部) 上进行类似的亚克隆步骤, 其分别编码为 SEQ ID NO:108 和 SEQ ID NO:114。

[0369] 用于拟南芥属 PPD 在双子叶植物中的 (根优选的) 过表达的 pAtWRKY6::PPD 双子叶植物构建体的产生

[0370] 合成含有用于在双子叶植物的根中表达的 PPD1 的编码序列的构建体, 核酸序列示于 SEQ ID NO:41 和 126 中。PPD ORF 来自于拟南芥 PPD1cDNA (登录号 NM_202819) 并被修饰以包括修饰的 Joshi 序列 (Joshi 1997, Nucleic Acid Research 15, 6643-6653)、隐性剪接位点的去除, 在 SEQ ID NO:41 的情况中来自多年生黑麦草 DGAT1 的第 3 个内含子的加入 (SEQ ID NO:39)、BamHI 可去除的 C-末端 V5 表位和 His 标签尾部 (编码 SEQ ID NO:37) 的添加和双终止密码子的添加。优化内含子的位置以用于剪接位点预测, 通过 NetGene2 进行 (<http://www.cbs.dtu.dk/services/NetGene2/>)。

[0371] 然后通过 **GATEWAY®** LR 反应将 (具有或不具有所述尾部) 的构建体置于烟草 AtWRKY6 启动子 (Robatzek and Somssich 2001, The Plant Journal, 28:123-133) 和 NOS 终止子之间以产生 SEQ ID NO:44 和 SEQ ID NO:47, 其分别编码为 SEQ ID NO:36 和 SEQ ID NO:38。在 SEQ ID NO 126 所示的构建体 (具有或不具有所述 C-末端尾部) 上进行类似的亚克隆步骤, 其分别编码为 SEQ ID NO:108 和 SEQ ID NO:114。

[0372] 用于拟南芥属 PPD 在单子叶植物中的 (根优选的) 过表达的 pTobRB7 Δ 1.3::PPD 单子叶植物构建体的产生

[0373] 合成含有用于在单子叶植物的根中表达的 PPD1 的编码序列的构建体, 核酸序列示于 SEQ ID NO:49 和 120 中。优化 PPD ORF 以用于在单子叶植物中表达, 这包括修饰的 Joshi 序列 (Joshi 1997, Nucleic Acid Research 15, 6643-6653)、密码子的优化、mRNA 不稳定性序列的去除、多聚 A 信号序列的去除、隐性剪接位点的去除、在 SEQ ID NO:49 的情况中来自多

年生黑麦草DGAT1的第3个内含子的加入(SEQ ID NO:48)、BamHI可去除的C-末端V5表位和His标签尾部(编码SEQ ID NO:37)的添加和双终止密码子的添加。优化内含子的位置以用于剪接位点预测,通过deepc2进行(<http://www.cbs.dtu.dk/services/NetGene2/>)。

[0374] 然后通过 **GATEWAY®** LR反应将(具有或不具有所述尾部)的构建体置于pTobRB7 Δ 1.3启动子(Yamamoto et al 1991, Plant Cell, 3:371-382)和NOS终止子之间以产生SEQ ID NO:50和SEQ ID NO:53,其分别编码为SEQ ID NO:36和SEQ ID NO:38。在SEQ ID NO 120所示的构建体(具有或不具有所述C-末端尾部)上进行类似的亚克隆步骤,其分别编码为SEQ ID NO:108和SEQ ID NO:114。

[0375] 用于拟南芥属PPD在双子叶植物中的(根优选的)过表达的pTobRB7 Δ 0.6::PPD单子叶植物构建体的产生

[0376] 合成含有用于在单子叶植物的根中表达的PPD1的编码序列的构建体,核酸序列示于SEQ ID NO:49和120中。优化PPD ORF以用于在单子叶植物中表达,这包括修饰的Joshi序列(Joshi 1997, Nucleic Acid Research 15, 6643-6653)、密码子的优化、mRNA不稳定性序列的去除、多聚A信号序列的去除、隐性剪接位点的去除、在SEQ ID NO:49的情况中来自多年生黑麦草DGAT1的第3个内含子的加入(SEQ ID NO:48)、BamHI可去除的C-末端V5表位和His标签尾部(编码SEQ ID NO:37)的添加和双终止密码子的添加。优化内含子的位置以用于剪接位点预测,通过deepc2进行(<http://www.cbs.dtu.dk/services/NetGene2/>)。

[0377] 然后通过 **GATEWAY®** LR反应将(具有或不具有所述尾部)的构建体置于pTobRB7 Δ 0.6启动子(Yamamoto et al 1991, Plant Cell, 3:371-382)和NOS终止子之间以产生SEQ ID NO:51和SEQ ID NO:54,其分别编码为SEQ ID NO:36和SEQ ID NO:38。在SEQ ID NO 120所示的构建体(具有或不具有所述C-末端尾部)上进行类似的亚克隆步骤,其分别编码为SEQ ID NO:108和SEQ ID NO:114。

[0378] 用于拟南芥属PPD在单子叶植物中的(根优选的)过表达的pAtWRKY6::PPD单子叶植物构建体的产生

[0379] 合成含有用于在单子叶植物的根中表达的PPD1的编码序列的构建体,核酸序列示于SEQ ID NO:49和120中。优化PPD ORF以用于在单子叶植物中表达,这包括修饰的Joshi序列(Joshi 1997, Nucleic Acid Research 15, 6643-6653)、密码子的优化、mRNA不稳定性序列的去除、多聚A信号序列的去除、隐性剪接位点的去除、在SEQ ID NO:49的情况中来自多年生黑麦草DGAT1的第3个内含子的加入(SEQ ID NO:48)、BamHI可去除的C-末端V5表位和His标签尾部(编码SEQ ID NO:37)的添加和双终止密码子的添加。优化内含子的位置以用于剪接位点预测,通过deepc2进行(<http://www.cbs.dtu.dk/services/NetGene2/>)。

[0380] 然后通过 **GATEWAY®** LR反应将(具有或不具有所述尾部)的构建体置于pAtWRKY6启动子(Robatzek and Somssich 2001 The Plant Journal, 28:123-133)和NOS终止子之间以产生SEQ ID NO:52和SEQ ID NO:55,其分别编码为SEQ ID NO:36和SEQ ID NO:38。在SEQ ID NO 120所示的构建体(具有或不具有所述C-末端尾部)上进行类似的亚克隆步骤,其分别编码为SEQ ID NO:108和SEQ ID NO:114。

[0381] 含有来自其他植物物种的PPD的表达构建体的产生

[0382] 为了证明可使用来自非拟南芥属的物种的PPD,我们合成了这样的构建体,其在被置于合适的启动子下时会表达来自以下物种的PPD:拟南芥(PPD对照)、豆科植物白三叶草、

原始被子植物无油樟、单子叶植物小果野蕉、松柏类植物挪威云杉和北美云杉以及石松门植物江南卷柏。优化这些构建体以用于在水稻(包括单子叶植物内含子)或拟南芥属(无内含子)中表达。对这些构建体进行改造以包含可去除的尾部,该尾部由编码V5-His标签的核酸序列的任一侧上的两个BamHI位点(编码甘氨酸-丝氨酸)组成。所述肽序列(有和没有C-末端V5-His标签尾部)示于序列108-119中。被优化用于在水稻中表达的相应的核酸序列示于序列120-125中,被优化用于在拟南芥属中表达的相应的核酸序列示于序列126-131中。

[0383] 通过GATEWAY LR反应将拟南芥属优化版本置于双元载体的适当的启动子下游,并通过蘸花方法转化到 Δ ppd拟南芥中。

[0384] 通过GATEWAY LR反应将水稻优化版本置于适当的启动子下游,并通过生物裂解的方式(biolystically)转化到黑麦草中。

[0385] 植物转化

[0386] 拟南芥属的转化

[0387] 按照实施例2所述的方法转化拟南芥属。

[0388] 苜蓿的转化

[0389] 使用改良自Samac et al.2000 (Methods in Molecular Biology 343:301-311)的方法通过农杆菌介导的转化产生过表达Peapod构建体的苜蓿植物。

[0390] 植物组织

[0391] 对Regen-SY的高度可再生基因型(Bingham 1991Crop Sci.31,1098)进行鉴定,其具有在体外条件下从体细胞胚胎形成完整植株的能力。使用该方案选择单一基因型用于植物转化实验。

[0392] 农杆菌菌株和载体

[0393] 使用双元载体pRSh1::PPD-OP和pRSh1::PPD-OP-V5用于所有的转化实验。载体pRSh1是pART27(Gleave 1992 Plant Mol Biol 20:1203-1207)的衍生物并且包含由CaMV 35S启动子表达的bar选择基因和也在CaMV 35S启动子的表达下的禾本科植物优化的Peapod基因。将质粒转移到根癌农杆菌菌株GV310中,并在含有200mg l⁻¹壮观霉素的YM平板上选择转化体。在从细菌培养物中制备质粒DNA之后,通过限制性图谱确认质粒成功转移到农杆菌中。

[0394] 将双元质粒的培养物加入到含有100mg/L壮观霉素的25mL Mannitol Glutamate Luria (MGL)培养基中。使细菌培养物于28℃在旋转振荡器上生长过夜(16个小时)。通过离心(3000x g)收集培养物,去除上清液并将细胞重悬于溶于用于植物转化的制品中的5mL 10mM MgSO₄溶液中。

[0395] 植物转化

[0396] 收集来自Regen-SY的组织培养物相应基因型的第二和第三完全展开叶,并在50%乙醇溶液中冲洗30秒,然后在无菌蒸馏水中洗涤,接着在次氯酸钠溶液(3%可用氯)中进行表面消毒。然后将无菌叶切成叶蝶,接着将它们浮于SH1.5D愈伤组织诱导培养基的溶液上。对于叶蝶的转化,将约3mL农杆菌培养液加入到12mL SH1.5D中,并孵育15分钟,偶尔搅拌以将叶用农杆菌全部覆盖。然后去除细菌溶液,将叶小片在无菌滤纸上稍微印干,然后使近轴侧朝上置于用0.8%植物用琼脂凝胶化的SH1.5D上。接着将平板于25℃在黑暗中孵育5天。

[0397] 接种后,从平板上去除叶小片,并在无菌SH1.5D液体培养基中冲洗3-4次,在无菌

滤纸上印干,放置于补充有用于筛选的5mg/L草铵膦和100mg/L头孢噻肟和特美汀的SH1.5D平板上,并于25℃在黑暗中孵育15天。然后将在叶小片上形成的愈伤组织传代至新鲜的SH1.5D选择培养基中,再持续15天。

[0398] 然后将转化的愈伤组织转移到补充有草甘膦、头孢噻肟和特美汀的BL0培养基中用于形成体细胞胚胎。一旦胚胎形成,将它们解剖并转移到用于发芽筛选的半强度MSN培养基中。将整个生根植株转移并使其在温室中定植(establish)。

[0399] PCR分析

[0400] 进行PCR分析以确认T-DNA稳定整合至从转化实验中回收的植物的基因组中。使用基因组DNA Mini试剂盒(Geneaid)从约50mg的体外生长的叶中提取基因组DNA。

[0401] 使用特异性针对nos3'聚腺苷酸化信号的引物对(ocs3'-1f,5'-GATATGCGAGACGCCTATGA-3'[SEQ ID NO:132];ocs3'-1r,5'-GAGTTCCTTCAGTGAACGT-3'[SEQ ID NO:133])产生439bp的扩增产物。也包括仅包含质粒DNA模板、未转化的植物DNA或水的对照反应。PCR反应的方案由如下组成:94℃下起始变性5分钟,30个循环的95℃30s、55℃15s、72℃1min,和72℃延伸10min。在TAE缓冲液中通过凝胶电泳将扩增产物在1.0%琼脂糖凝胶上进行分析并使用Bio-Rad Gel Doc成像系统进行显影。

[0402] 用于苜蓿转化的培养基的一般组成

[0403] A.MGL

- 甘露醇 5.0 g/L
- L 谷氨酸 1.0 g/L
- KH_2PO_4 250 mg/L

- MgSO_4 100 mg/L

[0404] • NaCl 100 mg/L

- 生物素 100 mg/L

- 蛋白胨 5.0 g/L
- 酵母提取物 2.5 g/L
- pH7.0 (NaOH)

[0405] B.SH1.5D

- SH 大量和微量营养元素 (Duchefa)

[0406]

- SH 维生素 (Duchefa)

- K_2SO_4 4.34 g/L
 - 脯氨酸 288 g/L
 - 硫代脯氨酸 53 mg/L
- [0407]
- 蔗糖 30 g/L
 - pH 5.8 (KOH)
 - 植物用琼脂 8.0 g/L
- 激动素 0.2 mg/L (在高压灭菌后加入)
 - 2,4D 1.5 mg/L (在高压灭菌后加入)
- [0408] C.BL0 (Blaydes培养基)
- [0409] • Blaydes改良的基础培养基 (PhytoTechnology Laboratories)
- L 肌醇 100 mg/
- [0410]
- 酵母提取物 2.0 g/L
 - pH 5.8 (KOH)
 - 植物用凝胶 2.5 g/L
- [0411] D. 半MSN培养基
- [0412] • MS大量和微量营养元素, 半强度 (Duchefa)
- Nitch & Nitch 维生素 (Duchefa)
- [0413]
- 蔗糖 15 g/L
 - pH 5.8 (KOH)
 - 植物用琼脂 8.0 g/L
- [0414] 黑麦草的转化
- [0415] 使用改良自Altpeter et al.2000 (Molecular Breeding 6:519-528) 的方法通过微粒轰击产生过表达Peapod构建体的黑麦草植物。
- [0416] 从高达7mm的不成熟花序诱导用于转化的愈伤组织。收获花分蘖, 在次氯酸钠溶液 (4%可用氯) 中进行表面消毒, 解剖, 然后在黑暗中于25℃下培养4至6周, 接着在含大量营养物、微量营养物和维生素的Murashige和Skoog (MS) 基础培养基 (1962Physiol Plant.15: 473-497) 上进行转化, 该培养基补充有30g/L麦芽糖、5mg/L 2,4-D, pH被调节至5.8, 并用6g/L琼脂糖固化。
- [0417] 使用Invitrogen Pure Link Hi Pure Plasmid Maxiprep试剂盒制备质粒, 浓度

调节至1 μ g/ μ L。将质粒pAcH1用于选择,该质粒包含含有由具有第一个内含子的水稻肌动蛋白启动子表达并终止于nos 3'聚腺苷酸化信号的嵌合潮霉素转移酶(HPH)基因(Bilang et al.1991 Gene 100:247-250)的表达盒。将含有PPD表达盒的质粒以1:1的摩尔比与pAcH1混合。

[0418] 使用Sanford et al.1993的方法(Meth.Enzymol.217:483-509.)将质粒DNA包被于M17钨颗粒(1.4 μ M平均直径分布)上,并使用DuPont PDS-1000/氦生物弹射粒子输送系统转化至靶组织中。在转化前高达6小时,将愈伤组织传代培养至含有64g/L甘露醇的愈伤组织起始培养基上。转化后(约16小时),然后将转化的愈伤组织转移到补充有2mg/L 2,4-D、不含甘露醇的MS基础培养基中。2天后,将愈伤组织转移至含有200mg/L潮霉素的相同培养基中,并在黑暗中培养4小时用于转基因株系的选择。在补充有0.2mg/L激动素、30g/L蔗糖、和50mg/L潮霉素,被调节至pH5.8并用8g/L植物用琼脂固化的MS基础培养基上在光照下发生来自体细胞胚胎的整株植物的再生。将转化的植物转移到含有温室的环境中用于分析。

[0419] 转化体的PCR分析

[0420] 进行PCR分析以确认HPH和PPD转基因稳定整合至从转化实验中回收的植物的基因组中。使用基因组DNA Mini试剂盒(Geneaid)从约50mg的体外生长的叶中提取基因组DNA。分别使用特异性针对HPH gene的引物对(hpt-1,5'-GCTGGGGCGTCGGTTTCCACTATCCG-3' [SEQ ID NO:134];hpt-2,5'-CGCATAACAGCGGTCATTGACTGGAGC-3') [SEQ ID NO:135];和特异性针对nos3'聚腺苷酸化信号的引物对(nos3'-1f,5'-CTGTTGCCGGTCTTGCGATG-3' -[SEQ ID NO:136];nos3'-1r,5'-GTCACATAGATGACACCGCG-3' -[SEQ ID NO:137])产生375bp和202bp的扩增产物。也包括仅包含质粒DNA模板、未转化的植物DNA或水的对照反应。PCR反应的方案由如下组成:94 $^{\circ}$ C下起始变性5分钟,30个循环的95 $^{\circ}$ C 30s、55 $^{\circ}$ C 15s、72 $^{\circ}$ C 1min,和72 $^{\circ}$ C延伸10min。在TAE缓冲液中通过凝胶电泳将扩增产物在1.0%琼脂糖凝胶上进行分析并使用Bio-Rad Gel Doc成像系统进行显影。

[0421] 禾本科植物转化体的DNA印迹分析

[0422] 使用DNA印迹杂交评估每个株系的转基因拷贝数。使用Doyle J和Doyle J 1990的方法(Focus,12:13-15)从温室生长的植物的叶材料提取基因组DNA用于DNA印迹杂交。将DNA(20 μ g)消化,并在0.8%琼脂糖凝胶上进行分离并使用0.4N NaOH通过毛细管转移将其转移至尼龙膜(Roche)上。当探测HPH和PPD转基因时,分别用XbaI或HindIII消化基因组DNA。使用DIG PCR合成试剂盒制备探针。分别使用特异性针对HPH基因的引物对(rgh1,5'-CTCGTGCTTTTCAGCTTCGATGTAG-3' [SEQ ID NO:138];rgh5,5'-GCTGGGGCGTCGGTTTCCACTATCGG-3' [SEQ ID NO:139])和特异性针对PPD的引物对(GrPPD1F,5'-CACAGGATGGATTCTCCAAGG-3' [SEQ ID NO:140];GrPPD1R,5'-TAAGGTCCACGGAGAGGTTC-3' [SEQ ID NO:141])产生906bp和586bp的扩增产物用于探针。使用标准缓冲液(Roche)在45 $^{\circ}$ C下进行预杂交(1小时)和杂交(12小时)。使用CDP-Star作为化学发光底物根据制造商的方案使用非放射性方法实现检测。使用Bio-Rad ChemiDoc MP系统和软件检测光信号。

[0423] 抗PPD1蛋白的多克隆抗体的产生和免疫印迹

[0424] 使用全长的拟南芥PPD1蛋白通过GenScript产生定制的抗PPD1亲和纯化的兔多克隆抗体。在1:5000的稀释度下所述抗体能够通过免疫印迹检测低于10ng的经纯化的PPD蛋白。将植物组织冷冻在液氮中,并研磨成细粉。将冷冻的组织粉末以1.0/1.5(重量/体积)的

比例加入到含有50mM Tris pH 7.5、150mM NaCl、1mM EDTA、10% (体积/体积) 甘油、5mM DTT、1% (体积/体积) 完全蛋白酶抑制剂混合物 (Sigma) 和1% (体积/体积) Triton X-100的提取缓冲液中,均质化直到解冻,然后以16,300g在4℃下离心12min。通过Bradford测定 (Coomassie Plus, Thermo Scientific) 对上清液中的总的溶解蛋白定量,进行调节以得到等同的总蛋白浓度/样品 (通常在10-40μg之间),在1XNuPAGE LDS样品缓冲液 (Invitrogen) 中变性并在4-12% Bis-Tris SDS/PAGE凝胶 (Novex) 上运行。在使用iBlot装置 (Invitrogen) 印迹到PVDF膜上后,使用1:5,000稀释度的1⁰抗PPD1多克隆抗体,然后使用1:5,000稀释度的2⁰山羊抗兔HRP抗体 (Sigma),用Western Bright ECL试剂 (Advansta) 检测蛋白,并使用ChemiDoc™仪器 (BioRad) 捕获图像。

[0425] 转化体的根生物量分析

[0426] 从野生型和用在组成型启动子下的拟南芥属PPD转化的黑麦草植物中取等数量 (通常为4-10个) 的分蘖。将分蘖种植到含有盆栽混合物的塑料生长袋中并修剪至相同的高度。使植物在温室中生长约6周,通过观察生长袋外的根生长可以看出,与野生型植物相比,用在组成型启动子下的拟南芥属PPD转化的黑麦草植物的根生物量/生长/长度/分支增加 (图11)。可以通过在流水下冲洗根、去除所连接的地上部分 (叶和茎) 并在65℃下干燥根48小时,然后对于重进行称重来对根生物量的增加进行定量 (表2)。

[0427] 表2

	Wt 黑麦草	载体对照	组成型启动子::拟南芥属 PPD 株系 1	组成型启动子::拟南芥属 PPD 株系 2	组成型启动子::拟南芥属 PPD 株系 3	组成型启动子::拟南芥属 PPD 株系 4
[0428] Av 根重量(g) (n=12)	0.0733	0.0387	0.4506	0.5657	0.3077	0.3503
SE	0.0138	0.0125	0.0428	0.0625	0.0426	0.0638

[0429] 从野生型和用在三个根启动子之一的拟南芥属PPD转化的黑麦草植物中取等数量 (通常为4-10个) 的分蘖。将分蘖种植到含有盆栽混合物的塑料生长袋中并修剪至相同的高度。使植物在温室中生长约6周,通过观察生长袋外的根生长可以看出,与野生型植物相比,用在组成型启动子下的拟南芥属PPD转化的黑麦草植物的根生物量/生长/长度/分支增加 (图12)。可以通过在流水下冲洗根、去除所连接的地上部分 (叶和芽) 并在65℃下干燥根48小时,然后对于重进行称重来对根生物量的增加进行定量 (表3和表4)。

[0430] 表3

	Wt 黑麦草	载体对照	pTobRB7Δ0.6::拟南芥属 PPD 株系 1	pTobRB7Δ0.6::拟南芥属 PPD 株系 2	pTobRB7Δ0.6::拟南芥属 PPD 株系 3
[0431] Av 根重量(g) (n=12)	0.0733	0.0387	0.2338	0.3686	0.3704
SE	0.0138	0.0125	0.0357	0.0356	0.0611

[0432] 表4

	Wt 黑 麦草	载体 对照	pTobRB7Δ1.3:: 拟南芥属 PPD 株系 1	pTobRB7Δ1.3:: 拟南芥属 PPD 株系 2	pTobRB7Δ1.3:: 拟南芥属 PPD 株系 3	pTobRB7Δ1.3:: 拟南芥属 PPD 株系 4
[0433] Av 根 重量(g) (n=12)	0.0733	0.0387	0.3227	0.2338	0.2720	0.4014
SE	0.0138	0.0125	0.0556	0.0191	0.0581	0.0445

[0434] 从野生型和用在组成型启动子下的拟南芥属PPD转化的苜蓿植物(品种USD5)中取约相大小的插条。将插条种植到含有盆栽混合物的塑料生长袋中。使植物在具有x小时日长的生长室中生长约6周;通过观察生长袋外的根生长可以看出,与野生型植物相比,用在组成型启动子下的拟南芥属PPD转化的苜蓿植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根(对这些进行拍照,图12),然后去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后进行称重来对根生物量的增加进行定量(表5)。

[0435] 表5

[0436]	植物株系	合并的来自10个插条的干根重 (mg)
	未转化的对照	43
	转化的株系1	110
	转化的株系2	54
	转化的株系3	160
	转化的株系4	59
	转化的株系5	53
	转化的株系6	85
	转化的株系7	148

[0437] 从野生型和用在组成型启动子下的白三叶草PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物的塑料生长袋中并修剪至相同的高度。使植物在温室中生长约6周,通过观察生长袋外的根生长可以看出,与野生型植物相比,用在组成型启动子下的白三叶草PPD转化的黑麦草植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后对干重进行称重来对根生物量的增加进行定量。

[0438] 从野生型和用在三个根启动子之一的白三叶草PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物的塑料生长袋中并修剪至相同的高度。使植物在温室中生长约6周,通过观察生长袋外的根生长可以看出,与野生型植物相比,用在组成型启动子下的白三叶草PPD转化的黑麦草植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后对干重进行称重来对根生物量的增加进行定量。

[0439] 从野生型和用在组成型启动子下的无油樟PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物的塑料生长袋中并修剪至相同的高度。使植物在温室中生长约6周,通过观察生长袋外的根生长可以看出,与野生型植物相比,用在组成型启动子下的无油樟PPD转化的黑麦草植物的根生物量/生长/长度/分支增加(图11)。可以通过在流水下冲洗根、去除所连接的地上部分(叶和茎)并在65℃下干燥根48小

时,然后对干重进行称重来对根生物量的增加进行定量。

[0440] 从野生型和用在三个根启动子之一的无油樟PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物的塑料生长袋中并修剪至相同的高度。使植物在温室中生长约6周,通过观察生长袋外的根生长可以看出,与野生型植物相比,用在组成型启动子下的无油樟PPD转化的黑麦草植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后对干重进行称重来对根生物量的增加进行定量。

[0441] 从野生型和用在组成型启动子下的小果野蕉PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物的塑料生长袋中并修剪至相同的高度。使植物在温室中生长约6周,通过观察生长袋外的根生长可以看出,与野生型植物相比,用在组成型启动子下的小果野蕉PPD转化的黑麦草植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后对干重进行称重来对根生物量的增加进行定量。

[0442] 从野生型和用在三个根启动子之一的小果野蕉PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物的塑料生长袋中并修剪至相同的高度。使植物在温室中生长约6周,通过观察生长袋外的根生长可以看出,与野生型植物相比,用在组成型启动子下的小果野蕉PPD转化的黑麦草植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后对干重进行称重来对根生物量的增加进行定量。

[0443] 从野生型和用在组成型启动子下的北美云杉PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物的塑料生长袋中并修剪至相同的高度。使植物在温室中生长约6周,通过观察生长袋外的根生长可以看出,与野生型植物相比,用在组成型启动子下的北美云杉PPD转化的黑麦草植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后对干重进行称重来对根生物量的增加进行定量。

[0444] 从野生型和用在三个根启动子之一的北美云杉PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物的塑料生长袋中并修剪至相同的高度。使植物在温室中生长约6周,通过观察生长袋外的根生长可以看出,与野生型植物相比,用在组成型启动子下的北美云杉PPD转化的黑麦草植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后对干重进行称重来对根生物量的增加进行定量。

[0445] 从野生型和用在组成型启动子下的江南卷柏PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物的塑料生长袋中并修剪至相同的高度。使植物在温室中生长约6周,通过观察生长袋外的根生长可以看出,与野生型植物相比,用在组成型启动子下的江南卷柏PPD转化的黑麦草植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后对干重进行称重来对根生物量的增加进行定量。

[0446] 从野生型和用在三个根启动子之一的江南卷柏PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物的塑料生长袋中并修剪至相同的

高度。使植物在温室中生长约6周,通过观察生长袋外的根生长可以看出,与野生型植物相比,用在组成型启动子下的江南卷柏PPD转化的黑麦草植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后对干重进行称重来对根生物量的增加进行定量。

[0447] 将来自野生型和用在组成型启动子下的白三叶草PPD转化的 Δ ppd拟南芥属植物的种子划破并播种于盆栽混合物中。使植物在具有x小时日长的生长室中生长约6周;通过观察盆外的根生长可以看出,与野生型植物相比,用在组成型启动子下的白三叶草PPD转化的拟南芥属植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、然后去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后进行称重来对根生物量的增加进行定量。

[0448] 将来自野生型和用在组成型启动子下的小果野蕉PPD转化的 Δ ppd拟南芥属植物的种子划破并播种于盆栽混合物中。使植物在具有x小时日长的生长室中生长约6周;通过观察盆外的根生长可以看出,与野生型植物相比,用在组成型启动子下的小果野蕉PPD转化的拟南芥属植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、然后去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后进行称重来对根生物量的增加进行定量。

[0449] 将来自野生型和用在组成型启动子下的无油樟PPD转化的 Δ ppd拟南芥属植物的种子划破并播种于盆栽混合物中。使植物在具有x小时日长的生长室中生长约6周;通过观察盆外的根生长可以看出,与野生型植物相比,用在组成型启动子下的无油樟PPD转化的拟南芥属植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、然后去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后进行称重来对根生物量的增加进行定量。

[0450] 将来自野生型和用在组成型启动子下的挪威云杉PPD转化的 Δ ppd拟南芥属植物的种子划破并播种于盆栽混合物中。使植物在具有x小时日长的生长室中生长约6周;通过观察盆外的根生长可以看出,与野生型植物相比,用在组成型启动子下的挪威云杉PPD转化的拟南芥属植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、然后去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后进行称重来对根生物量的增加进行定量。

[0451] 将来自野生型和用在组成型启动子下的江南卷柏PPD转化的 Δ ppd拟南芥属植物的种子划破并播种于盆栽混合物中。使植物在具有x小时日长的生长室中生长约6周;通过观察盆外的根生长可以看出,与野生型植物相比,用在组成型启动子下的江南卷柏PPD转化的拟南芥属植物的根生物量/生长/长度/分支增加。可以通过在流水下冲洗根、然后去除所连接的地上部分(叶和茎)并在65℃下干燥根48小时,然后进行称重来对根生物量的增加进行定量。

[0452] 对转化体的耐旱性分析

[0453] 从野生型和用在组成型启动子下的拟南芥属PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植

物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0454] 从野生型和用在组成型启动子下的白三叶草PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0455] 从野生型和用在组成型启动子下的小果野蕉PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0456] 从野生型和用在组成型启动子下的无油樟PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0457] 从野生型和用在组成型启动子下的北美云杉PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0458] 从野生型和用在组成型启动子下的江南卷柏PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0459] 从野生型和用在三个根启动子之一的拟南芥属PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0460] 从野生型和用在三个根启动子之一的白三叶草PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0461] 从野生型和用在三个根启动子之一的小果野蕉PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎

点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0462] 从野生型和用在三个根启动子之一的无油樟PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0463] 从野生型和用在三个根启动子之一的北美云杉PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0464] 从野生型和用在三个根启动子之一的江南卷柏PPD转化的黑麦草植物中取等数量(通常为4-10个)的分蘖。将分蘖种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0465] 将相同数目(通常为4-10)的野生型和用在组成型启动子下的拟南芥属PPD转化的 Δ ppd拟南芥属幼苗种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0466] 将相同数目(通常为4-10)的野生型和用在组成型启动子下的白三叶草PPD转化的 Δ ppd拟南芥属幼苗种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0467] 将相同数目(通常为4-10)的野生型和用在组成型启动子下的小果野蕉PPD转化的 Δ ppd拟南芥属幼苗种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0468] 将相同数目(通常为4-10)的野生型和用在组成型启动子下的无油樟PPD转化的 Δ ppd拟南芥属幼苗种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0469] 将相同数目(通常为4-10)的野生型和用在组成型启动子下的挪威云杉PPD转化的 Δ ppd拟南芥属幼苗种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将

一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

[0470] 将相同数目(通常为4-10)的野生型和用在组成型启动子下的江南卷柏PPD转化的 Δ ppd拟南芥属幼苗种植到含有盆栽混合物和土壤的大盆中。使植物在温室中生长,然后将一半的各类型植物进行水胁迫(通常为12%的重量含水量,正好在永久凋萎点之上),同时使另一半保持含水(通常为22%的重量含水量,约为田间持水量)。通过与WT植物比较根和芽生物量可以对过表达PPD的植物的增加的干旱胁迫耐受性进行定量。

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140,1615-1620 (2013) .

[0511] 序列汇总

[0512]

SEQ ID NO:	序列类型	物种/来源	参考
1	多肽	拟南芥 (<i>Arabidopsis thaliana</i>)	PEAPOD 1 蛋白
2	多肽	拟南芥	PEAPOD 2 蛋白
3	多肽	毛果杨 (<i>Populus trichocarpa</i>)	PEAPOD 蛋白
4	多肽	挪威云杉 (<i>Picea abies</i>)	PEAPOD 蛋白
5	多肽	北美云杉 (<i>Picea sitchensis</i>)	PEAPOD 蛋白
6	多肽	雷蒙德氏棉 (<i>Gossypium raimondii</i>)	PEAPOD 蛋白
7	多肽	洛矶山耧斗菜 (<i>Aquilegia coerulea</i>)	PEAPOD 蛋白 1
8	多肽	洛矶山耧斗菜	PEAPOD 蛋白 2
9	多肽	蒺藜状苜蓿 (<i>Medicago truncatula</i>)	PEAPOD 蛋白
10	多肽	番茄 (<i>Solanum lycopersicum</i>)	PEAPOD 蛋白
11	多肽	白三叶草 (<i>Trifolium repens</i>)	PEAPOD 蛋白
12	多肽	无油樟 (<i>Amborella trichopoda</i>)	PEAPOD 蛋白
13	多肽	江南卷柏 (<i>Selaginella moellendorffii</i>)	PEAPOD 蛋白 1
14	多肽	江南卷柏	PEAPOD 蛋白 2
15	多肽	烟草 (<i>Nicotiana tabacum</i>)	PEAPOD 蛋白
16	多肽	马铃薯 (<i>Solanum tuberosum</i>)	PEAPOD 蛋白
17	多肽	大豆 (<i>Glycine max</i>)	PEAPOD 蛋白
18	多肽	克莱门氏柑桔 (<i>Citrus clementine</i>)	PEAPOD 蛋白
19	多肽	蓖麻 (<i>Ricinus communis</i>)	PEAPOD 蛋白
20	多肽	葡萄 (<i>Vitis vinifera</i>)	PEAPOD 蛋白
21	多肽	川桑 (<i>Morus notabilis</i>)	PEAPOD 蛋白
22	多肽	海枣 (<i>Phoenix dactylifera</i>)	PEAPOD 蛋白
23	多肽	可可 (<i>Theobroma cacao</i>)	PEAPOD 蛋白
24	多肽	紫萍 (<i>Spirodela polyrhiza</i>)	PEAPOD 蛋白
25	多肽	芭蕉属 (<i>Musa</i>) 物种	PEAPOD 蛋白
26	多肽	白蝴蝶兰 (<i>Phalaenopsis aphrodite</i>)	PEAPOD 蛋白
27	多肽	人工	内部 46 个氨基酸拟南芥属 PPD1 区域
28	多肽	人工	内部 46 个氨基酸共有基序 1, 相同的残基
29	多肽	人工	内部 46 个氨基酸共有基序 2, 可变残基
30	多肽	人工	内部 27 个氨基酸拟南芥属 PPD1 区域

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31	多肽	人工	内部 27 个氨基酸共有基序 1, 相同的残基
32	多肽	人工	内部 27 个氨基酸共有基序 2, 可变残基
33	多肽	人工	来自拟南芥属 PPD1 的 6 个氨基酸 TIFY 基序
34	多肽	人工	6 个氨基酸 TIFY 共有基序 1, 相同的残基
35	多肽	人工	6 个氨基酸 TIFY 共有基序 1, 可变残基
36	多肽	人工	在根中表达的 PPD1 V5-HIS 尾部肽序列
37	多肽	人工	接头盒 V5-His 尾部肽序列
38	多肽	人工	在根中表达的 PPD1 (无尾部) 肽序列
39	多核苷酸		拟南芥 DGAT1 内含子 3 核酸序列
40	多核苷酸	人工	用于在烟草 TobRB7 Δ 1.3 根启动子下在双子叶植物根中表达的 GenScript 合成的优化的拟南芥属 PPD1 编码区域 (有内含子) 核酸序列; 烟草 TobRB7 Δ 0.6 根启动子
41	多核苷酸	人工	用于在拟南芥属 AtWRKY6 根启动子下在双子叶植物根中表达的 GenScript 合成的优化的拟南芥属 PPD1 编码区域 (有内含子) 核酸序列
42	多核苷酸	人工	TobRB7 Δ 1.3 启动子::attB1:: 优化的拟南芥属 PPD-V5-His(INTRON)::attB2::nos 终止子表达盒核酸序列
43	多核苷酸	人工	TobRB7 Δ 0.6 启动子::attB1:: 优化的拟南芥属 PPD-V5-His(INTRON)::attB2::nos 终止子表达盒核酸序列
44	多核苷酸	人工	AtWRKY6 启动子::attB1:: 优化的拟南芥属 PPD-V5-His(INTRON)::attB2::nos 终止子表达盒核酸序列
45	多核苷酸	人工	TobRB7 Δ 1.3 启动子::attB1:: 优化的拟南芥属 PPD (INTRON)::attB2::nos 终止子表达盒
46	多核苷酸	人工	TobRB7 Δ 0.6 启动子::attB1:: 优化的拟南芥属 PPD (INTRON)::attB2::nos 终止子表达盒
47	多核苷酸	人工	AtWRKY6 启动子::attB1:: 优化的拟

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			南芥属 PPD (INTRON)::attB2::nos 终止子表达盒.
48	多核苷酸		多年生黑麦草 DGAT1 内含子 3 核酸序列
49	多核苷酸	人工	用于使用烟草 TobRB7 Δ1.3 根启动子在禾本科植物根表达的 GENEART 合成的水稻优化的 PPD1 编码区域 (有内含子) 核酸序列
50	多核苷酸	人工	TobRB7 Δ1.3 启动子::attB1::水稻优化的 PPD-V5-His(INTRON)::attB2::nos 终止子表达盒核酸序列
51	多核苷酸	人工	TobRB7 Δ0.6 启动子::attB1::水稻优化的 PPD-V5-His(INTRON)::attB2::nos 终止子表达盒核酸序列
52	多核苷酸	人工	AtWRKY6 启动子::attB1::水稻优化的 PPD-V5-His(INTRON)::attB2::nos 终止子表达盒核酸序列
53	多核苷酸	人工	TobRB7 Δ1.3 启动子::attB1::水稻优化的 PPD (INTRON)::attB2::nos 终止子表达盒
54	多核苷酸	人工	TobRB7 Δ0.6 启动子::attB1::水稻优化的 PPD (INTRON)::attB2::nos 终止子表达盒
55	多核苷酸	人工	AtWRKY6 启动子::attB1::水稻优化的 PPD (INTRON)::attB2::nos 终止子表达盒
56	多肽	人工	酵母双杂交 (Y2H) DNA 结合结构域 (DBD) 肽序列
57	多肽	人工	Y2H 激活结构域 (AD) 肽序列
58	多肽	人工	Y2H PPD1-DBD 肽序列
59	多肽	人工	Y2H PPD1-AD 肽序列
60	多肽	人工	Y2H PPD1-ppd-AD 肽序列
61	多肽	人工	Y2H PPD1-tify-AD 肽序列
62	多肽	人工	Y2H PPD1-jas*-AD 肽序列
63	多肽	人工	TPL 肽序列
64	多肽	人工	Y2H TPL-DBD 肽序列
65	多肽	人工	NINJA 肽序列
66	多肽	人工	Y2H NINJA-AD 肽序列
67	多肽	人工	Y2H BZR1-AD 肽序列
68	多肽	人工	Y2H RGA1 肽序列
69	多肽	人工	Y2H RGA1-AD 肽序列
70	多肽	人工	Y2H PPD1-ppd-DBD 肽序列

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71	多肽	人工	Y2H PPD1-tify-DBD 肽序列
72	多肽	人工	Y2H PPD1-jas*-DBD 肽序列
73	多肽	人工	双分子荧光(BiFC) nYFP 肽序列
74	多肽	人工	BiFC cYFP 肽序列
75	多肽	人工	BiFC nYFP-NINJA 肽序列
76	多肽	人工	BiFC nYFP-BZR1 肽序列
77	多肽	人工	BiFC cYFP-PPD1 肽序列
78	多肽	人工	BiFC cYFP-NINJA 肽序列
79	多肽	人工	BiFC cYFP-BZR1 肽序列
80	多肽	人工	BiFC cYFP-PPD1-ppd 肽序列
81	多肽	人工	BiFC cYFP-PPD1-tify 肽序列
82	多肽	人工	BiFC cYFP-PPD1-jas* 肽序列
83	多核苷酸	拟南芥	拟南芥 PPD1 编码序列
84	多核苷酸	拟南芥	拟南芥 PPD2 编码序列
85	多核苷酸	毛果杨	毛果杨, PPD 编码序列
86	多核苷酸	挪威云杉	挪威云杉, PPD 基因组序列
87	多核苷酸	雷蒙德氏棉	雷蒙德氏棉, PPD 编码序列
88	多核苷酸	洛矶山耧斗菜	洛矶山耧斗菜, PPD 编码序列 1
89	多核苷酸	洛矶山耧斗菜	洛矶山耧斗菜, PPD 编码序列 2
90	多核苷酸	蒺藜状苜蓿	蒺藜状苜蓿, PPD 编码序列
91	多核苷酸	番茄	番茄, PPD 编码序列
92	多核苷酸	白三叶草	白三叶草, PPD 编码序列
93	多核苷酸	无油樟	无油樟, PPD 编码序列
94	多核苷酸	江南卷柏	江南卷柏, PPD 编码序列 1
95	多核苷酸	江南卷柏	江南卷柏, PPD 编码序列 2
96	多核苷酸	烟草	烟草, PPD 编码序列
97	多核苷酸	马铃薯	马铃薯, PPD 编码序列
98	多核苷酸	大豆	大豆, PPD 编码序列
99	多核苷酸	克莱门氏柑桔	克莱门氏柑桔, PPD 编码序列
100	多核苷酸	蓖麻	蓖麻, PPD 编码序列
101	多核苷酸	葡萄	葡萄, PPD 编码序列
102	多核苷酸	川桑	川桑, PPD 编码序列
103	多核苷酸	海枣	海枣, PPD 编码序列
104	多核苷酸	可可	可可, PPD 编码序列
105	多核苷酸	紫萍	紫萍, PPD 基因组序列
106	多核苷酸	芭蕉属物种	芭蕉属物种, PPD 编码序列
107	多核苷酸	白蝴蝶兰	白蝴蝶兰, PPD 编码序列
108	多肽	人工	拟南芥 PPD1+ V5-His 标签
109	多肽	人工	白三叶草 PPD + V5-His 标签
110	多肽	人工	无油樟 PPD + V5-His 标签
111	多肽	人工	小果野蕉 PPD + V5-His 标签
112	多肽	人工	北美云杉 PPD + V5-His 标签
113	多肽	人工	江南卷柏 PPD + V5-His 标签
114	多肽	人工	拟南芥 PPD - 无标签
115	多肽	人工	白三叶草 PPD - 无标签

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116	多肽	人工	无油樟 PPD – 无标签
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123	多核苷酸	人工	小果野蕉 PPD – 单子叶植物优化的核酸序列
124	多核苷酸	人工	北美云杉 PPD – 单子叶植物优化的核酸序列
125	多核苷酸	人工	江南卷柏 PPD – 单子叶植物优化的核酸序列
126	多核苷酸	人工	拟南芥 PPD – 双子叶植物优化的核酸序列
127	多核苷酸	人工	白三叶草 PPD – 双子叶植物优化的核酸序列
128	多核苷酸	人工	无油樟 PPD – 双子叶植物优化的核酸序列
129	多核苷酸	人工	小果野蕉 PPD – 双子叶植物优化的核酸序列
130	多核苷酸	人工	挪威云杉 PPD – 双子叶植物优化的核酸序列
131	多核苷酸	人工	江南卷柏 PPD – 双子叶植物优化的核酸序列
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133	多核苷酸	人工	引物, ocs3'-1r
134	多核苷酸	人工	引物, hpt-1
135	多核苷酸	人工	引物, hpt-2
136	多核苷酸	人工	引物, nos3'-1f
137	多核苷酸	人工	引物, nos3'-1r
138	多核苷酸	人工	引物, rgh1
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141	多核苷酸	人工	引物, GrPPD1R

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[0535]	Glu Glu Leu Thr Glu Glu Asp Ile Ser Gln Leu Thr Arg Glu Asp Cys
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[0537]	Arg Arg Tyr Leu Lys Glu Lys Gly Met Arg Arg Pro Ser Trp Asn Lys
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[0539]	Tyr Gln Ala Ile Gln Gln Val Leu Ser Leu Lys Gly Leu Leu Glu Gly
[0540]	50 55 60
[0541]	Lys Pro Cys Asp Asp Asn Ser Asp Val Phe Ser His Arg Ser Pro Ile
[0542]	65 70 75 80
[0543]	Thr Val Ile Pro Asn Val Gly Ser Met Arg Glu Lys Glu Lys Ala Val
[0544]	85 90 95
[0545]	Asn Ile Ala Asp Pro Glu Ile Ser Gly Ser His Gln Pro Asn Phe Arg

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[0549]	Trp Pro Pro Ser Gln Glu Pro Val Ser Gln Met Thr Ile Phe Tyr Ala		
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[0551]	Gly Ala Val Asn Val Tyr Asn Asp Ile Pro Glu Asp Lys Val Gln Ala		
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[0553]	Ile Ile Tyr Leu Ala Gly Lys Ser Asp Ser Leu Gln Gln Thr Asn Val		
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[0555]	Ile Arg Thr Gly Pro Asp Gln Cys Ile Ala Ser Ala Ala Ser Pro Ser		
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[0557]	Leu Asn Asp Leu His Ser Arg Arg Ile His Pro Thr Ser Asn Ile Thr		
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[0559]	Thr Ser Gln Ser Leu Arg Val Ala Thr Ser Leu Pro Val Gly Pro His		
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[0563]	Lys Arg Lys Asp Arg Gly Arg Leu Lys Gly Thr Leu Ala Ser Gly Gly		
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[0565]	Ser Ser Lys Arg Gly Ser Ser Cys Leu Glu Leu Tyr Ala Thr Ser Arg		
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[0567]	Leu Lys Ser Glu Gly Val Ala Thr Thr Thr Thr Gln Ser Asn Met Asn		
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[0569]	Asn Val Val Val Ser Pro Ser Asn Pro Arg Met Pro Leu Asn Pro Gly		
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[0596]	Leu Ala Pro Thr Leu Ser Val Leu Asn Pro Asp Ala Lys Arg Asn Pro		
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[0598]	Leu Ser Ser Lys Pro Ala Ser Thr Thr Lys Pro His Ser Ala Gln Leu		
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[0602]	Asp Lys Ala Gln Ala Ile Met Leu Leu Ala Ala Ser Lys Thr Phe His		
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[0604]	Val Pro Thr Ser Ser Val Pro Gly His Pro Pro Phe Thr Ser Ala Thr		
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[0606]	Gln Gln Gln Gln Gln Gln Gln Arg Glu Leu Asn Gln Gln Thr Glu Ala		
[0607]	225	230	235
[0608]	Thr Gln Lys Tyr Pro Met Gln His Gln Gln Ala Pro Gln Ile Tyr Leu		
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[0610]	Ser Ser Gly Ser Ala Leu Pro Asp Glu Ser Cys Thr Glu Pro Gly Leu		
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[0618]	Phe Gln Phe Pro Pro Ser Ala Arg Thr Ser Gln Val Leu Arg Tyr Ser		
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[0655]	Ala	Ala	Asn	Thr	Asn	Lys	Val	Val	Ile	Ser	Ser	Tyr	Glu	Leu	Pro	Gln
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[0657]	Ala	Arg	Lys	Ala	Ser	Leu	Gln	Arg	Phe	Leu	Gln	Arg	Arg	Arg	Glu	Lys
[0658]					180					185					190	
[0659]	Thr	Ala	Lys	Glu	Ala	Ala	Ser	Lys	Gly	Asn	Ser	Asn	Lys	Ser	Pro	Cys
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[0674]	Arg Arg Tyr Leu Lys Glu Lys Gly Met Arg Arg Pro Ser Trp Asn Lys	
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[0676]	Ser Gln Ala Ile Gln Gln Val Ile Ser Leu Lys Ala Leu Leu Glu Thr	
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[0678]	Thr Pro Asp Ser Asp Thr Gly Pro Arg Arg Lys Leu His Ile Pro Arg	
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[0680]	Pro Asp Thr Arg Val Gln Gln Val Gln Lys Gly Thr Asp Thr Asp Ala	
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[0682]	Glu Phe Ser Lys Ser Ala Glu Gly Met Val Pro Tyr Gly Arg Lys His	
[0683]	100	105 110
[0684]	Ser Lys Lys Pro Asp Ile Pro Gly Asp Ile Ala Ala Gly Ser Val Ala	
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[0686]	Val Ala Ala Gly Asn Asn Leu Ala Pro Ser Arg Thr Thr Asp Leu Gly	
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[0688]	Asn Thr Pro Ala Ser Gln Leu Thr Ile Phe Tyr Cys Gly Lys Val Asn	
[0689]	145	150 155 160
[0690]	Val Tyr Asp Asp Val Pro Ala Glu Lys Ala Gln Ala Ile Met His Leu	
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[0692]	Ala Ala Thr Pro Leu Phe Val Pro Ser Glu Thr Pro Leu Gly Ala Thr	
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[0694]	Leu Ala Ala Arg His Ser Glu Cys His Leu Gln Ala Ala Ser Val Lys	
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[0696]	Gln Gly Pro Asp Ser Ala Met Val Leu Met Pro Thr Met Gln Thr Gly	
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[0703]	260 265 270
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[0713]	Arg Arg Tyr Leu Lys Gln Lys Gly Met Arg Lys Pro Ser Trp Asn Lys
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[0717]	Asp Thr Asp Ala Gly Thr Arg Lys Lys Leu His Ile Pro Arg Ala Asp
[0718]	65 70 75 80
[0719]	Thr His Val Gln Ser Gly Lys Asn Thr Tyr Gly Glu Pro Ser Glu Pro
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[0721]	Val Pro Asp Arg Arg Asn Gln Gln Asp Arg Pro Asp Leu Ser Ser His
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[0723]	Ile Thr Ala Leu Pro Val Ala Val Val Asp Asn Ser Ala Pro Ser Arg
[0724]	115 120 125
[0725]	Thr Ile Gly Ser Ala Asp Lys Pro Val Gly Gln Met Thr Ile Phe Tyr
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[0727]	Arg Gly Lys Val Asn Val Tyr Asp Asp Val Pro Ala Asp Lys Ala Gln
[0728]	145 150 155 160
[0729]	Lys Ile Met Cys Leu Ala Ser Ser Pro Leu Cys Val Pro Ser Glu Thr
[0730]	165 170 175
[0731]	Pro Ser Asn Ala Thr Val Ala Ala Arg His Ser Ala Cys Cys Leu Gln
[0732]	180 185 190
[0733]	Ala Ala Asn Ser Lys Leu Arg Leu Asp Thr Asn Ile Val Pro Thr Ile
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[0735]	Gln Thr Val Lys Met Ser Glu Val Ser Arg Val Pro Ile Glu Glu Ser
[0736]	210 215 220
[0737]	Asn Arg Leu Tyr Asn Asp Asn Pro Glu Ala Val Glu Ser Pro Ala Ser
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[0739]	Arg Lys Ala Ser Val Gln Arg Tyr Leu Glu Lys Arg Lys Glu Arg Phe
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[0759]					20					25					30	
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[0763]					50					55					60	
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[0766]	Ser	His	Ala	Pro	Pro	Pro	Pro	Pro	Gln	Pro	Gln	Ser	Gln	Val	Asn	Leu
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[0768]	Thr	Glu	Pro	Pro	Pro	Pro	Pro	Lys	Ala	Pro	Pro	Pro	Glu	Glu	Ser	Ser
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[0770]	Phe	His	Ala	Ala	Glu	Asp	Ile	Gln	Lys	Pro	Ala	Ser	Ser	Gly	Glu	Lys
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[0782]	Lys Asp Val Leu Ile Pro Val Asp Thr Thr Ile Leu Gln Val Ala Gln
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[0784]	Ser Asp Lys Met Met Glu Tyr Pro Leu Gln Tyr Arg Glu Lys Gly Ser
[0785]	225 230 235 240
[0786]	Ile Ala Arg Asp Ala Glu Gly Gln Ala Ser Arg Lys Val Ser Leu Gln
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[0790]	Leu Thr Gly Ile Thr Ser Ser Asn Phe Glu Met Tyr Leu Asn Leu Pro
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[0792]	Val Lys Val His Ala Ser Asn Gly Asn Ser Ser Arg Ser Ser Thr Ser
[0793]	290 295 300
[0794]	Ser Pro Pro Gln Pro Arg Leu Pro Leu Val Ser Ser Gly Ser Ala Asp
[0795]	305 310 315 320
[0796]	Asn Gln Leu Lys Val Ala Leu Pro Ile Asp Leu Asn Asp Lys Val Ser
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[0815]	Ser Pro Pro Glu Ser Met Pro Pro Arg Val Asn Val Thr Ser Asn Ser
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[0817]	Ala Asp Leu Val Lys Glu Pro Thr Ile Ser Val Ser Gly Asp Gln Asn
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[0825]	Val	Pro	Thr	Asp	Lys	Ala	Gln	Glu	Ile	Met	His	Leu	Ala	Ala	Thr	Pro
[0826]						165				170					175	
[0827]	Ile	Asp	Phe	Ser	Gln	Asn	Gly	Ser	Phe	Gly	Gly	Ile	Thr	Ala	Tyr	Arg
[0828]						180				185					190	
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[0867]	Lys Ser Pro Pro Pro Ala Pro Ser Leu Asp Cys Pro Leu Glu Glu Ala		
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[0869]	Asp Asn Lys Val Ile Ser Ser Arg Ser Pro Gly Ala Thr Asp Gly Leu		
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[0871]	Val Gly Gln Met Thr Ile Phe Tyr Cys Gly Lys Val Asn Val Tyr Asp		
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[0873]	Gly Val Pro Pro Asp Lys Ala Gln Ala Ile Met His Leu Ala Ala Thr		
[0874]	165	170	175
[0875]	Pro Ile His Ser Pro Leu Asp Asp Pro Ile Arg Arg Pro Val Phe Ala		
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[0877]	Phe Pro Tyr His Leu Gln Thr Pro Ser Asp Lys His Val Phe Val Pro		
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[0879]	Ser Asn Ala Ala Ile Ser Pro Thr Thr Pro Thr Glu Lys Val Thr Glu		
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[0881]	Tyr Ser Gln Gln Cys Arg Glu Lys Gly Asn Val Thr Tyr Asp His Asp		
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[0883]	Val Glu Gly Gln Ala Asn Arg Lys Met Ser Leu Gln Arg Tyr Leu Glu		
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[0885]	Lys Lys Lys Asp Arg Gly Arg Phe Lys Gly Arg Lys Asn Leu Gly Pro		
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[0887]	Asn Ser Ser Ser Leu Asp Ala Tyr Leu Asn His Gln Met Arg Thr His		
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[0889]	Ile Ser Asn Glu Gln Ser Thr Arg Ser Ser Thr Ser Ser Pro Thr Gln		
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[0891]	Pro Gly Val Pro His Thr Ser Ser Asn Ser Ala Glu Asp Gln Leu Lys		
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[2432]	Ala	Thr	Ser	Ser	Ser	Glu	Glu	Ser	Ser	Asn	Lys	Gly	Gln	Arg	Gln	Leu
[2433]		130						135					140			
[2434]	Thr	Val	Ser	Ser	Arg	Ser	Asn	Gln	Thr	Ser	Leu	Tyr	Lys	Lys	Ala	Gly
[2435]	145					150					155					160
[2436]	Ser	Met	Asp	Val	Gly	Val	Ser	Pro	Ala	Lys	Ser	Ile	Leu	Ala	Lys	Pro
[2437]				165						170					175	
[2438]	Leu	Lys	Leu	Leu	Thr	Glu	Glu	Asp	Ile	Ser	Gln	Leu	Thr	Arg	Glu	Asp
[2439]				180						185					190	
[2440]	Cys	Arg	Lys	Phe	Leu	Lys	Asp	Lys	Gly	Met	Arg	Arg	Pro	Ser	Trp	Asn
[2441]			195						200					205		
[2442]	Lys	Ser	Gln	Ala	Ile	Gln	Gln	Val	Leu	Ser	Leu	Lys	Ala	Leu	Tyr	Glu
[2443]		210					215						220			
[2444]	Pro	Gly	Asp	Asp	Ser	Gly	Ala	Gly	Ile	Phe	Arg	Lys	Ile	Leu	Val	Ser
[2445]	225					230					235					240
[2446]	Gln	Pro	Val	Asn	Pro	Pro	Arg	Val	Thr	Thr	Thr	Leu	Ile	Glu	Pro	Ser
[2447]				245						250					255	
[2448]	Asn	Glu	Leu	Glu	Ala	Cys	Gly	Arg	Val	Ser	Tyr	Pro	Glu	Asp	Asn	Gly
[2449]				260						265					270	
[2450]	Ala	Cys	His	Arg	Met	Asp	Ser	Pro	Arg	Ser	Ala	Glu	Phe	Ser	Gly	Gly
[2451]			275						280				285			
[2452]	Ser	Gly	His	Phe	Val	Ser	Glu	Lys	Asp	Gly	His	Lys	Thr	Thr	Ile	Ser
[2453]		290						295					300			
[2454]	Pro	Arg	Ser	Pro	Ala	Glu	Thr	Ser	Glu	Leu	Val	Gly	Gln	Met	Thr	Ile
[2455]	305					310					315					320
[2456]	Phe	Tyr	Ser	Gly	Lys	Val	Asn	Val	Tyr	Asp	Gly	Ile	Pro	Pro	Glu	Lys

[2457]		325		330		335
[2458]	Ala Arg Ser Ile Met His Phe Ala Ala Asn Pro Ile Asp Leu Pro Glu					
[2459]		340		345		350
[2460]	Asn Gly Ile Phe Ala Ser Ser Arg Met Ile Ser Lys Leu Ile Ser Lys					
[2461]		355		360		365
[2462]	Glu Lys Met Met Glu Leu Pro Gln Lys Gly Leu Glu Lys Ala Asn Ser					
[2463]		370		375		380
[2464]	Ser Arg Asp Ser Gly Met Glu Gly Gln Ala Asn Arg Lys Val Ser Leu					
[2465]	385		390		395	400
[2466]	Gln Arg Tyr Arg Glu Lys Arg Lys Asp Arg Lys Phe Ser Lys Ala Lys					
[2467]		405		410		415
[2468]	Lys Cys Pro Gly Val Ala Ser Ser Ser Leu Glu Met Phe Leu Asn Cys					
[2469]		420		425		430
[2470]	Gln Pro Arg Met Lys Ala Ala Tyr Ser Gln Asn Leu Gly Cys Thr Gly					
[2471]		435		440		445
[2472]	Ser Pro Leu His Ser Gln Ser Pro Glu Ser Gln Thr Lys Ser Pro Asn					
[2473]		450		455		460
[2474]	Leu Ser Val Asp Leu Asn Ser Glu Gly Ile					
[2475]	465		470			
[2476]	<210> 59					
[2477]	<211> 452					
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[2480]	<220>					
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[2482]	<400> 59					
[2483]	Met Pro Lys Lys Lys Arg Lys Val Ser Ser Gly Ala Asn Phe Asn Gln					
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[2485]	Ser Gly Asn Ile Ala Asp Ser Ser Leu Ser Phe Thr Phe Thr Asn Ser					
[2486]		20		25		30
[2487]	Ser Asn Gly Pro Asn Leu Ile Thr Thr Gln Thr Asn Ser Gln Ala Leu					
[2488]		35		40		45
[2489]	Ser Gln Pro Ile Ala Ser Ser Asn Val His Asp Asn Phe Met Asn Asn					
[2490]		50		55		60
[2491]	Glu Ile Thr Ala Ser Lys Ile Asp Asp Gly Asn Asn Ser Lys Pro Leu					
[2492]	65		70		75	80
[2493]	Ser Pro Gly Trp Thr Asp Gln Thr Ala Tyr Asn Ala Phe Gly Ile Thr					
[2494]		85		90		95
[2495]	Thr Gly Met Phe Asn Thr Thr Thr Met Asp Asp Val Tyr Asn Tyr Leu					

[2496]		100		105		110											
[2497]	Phe	Asp	Asp	Glu	Asp	Thr	Pro	Pro	Asn	Pro	Lys	Lys	Glu	Gly	Gly	Ser	
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[2499]	Asn	Gln	Thr	Ser	Leu	Tyr	Lys	Lys	Ala	Gly	Ser	Met	Asp	Val	Gly	Val	
[2500]		130		135		140											
[2501]	Ser	Pro	Ala	Lys	Ser	Ile	Leu	Ala	Lys	Pro	Leu	Lys	Leu	Leu	Thr	Glu	
[2502]	145			150		155											
[2503]	Glu	Asp	Ile	Ser	Gln	Leu	Thr	Arg	Glu	Asp	Cys	Arg	Lys	Phe	Leu	Lys	
[2504]		165		170		175											
[2505]	Asp	Lys	Gly	Met	Arg	Arg	Pro	Ser	Trp	Asn	Lys	Ser	Gln	Ala	Ile	Gln	
[2506]		180		185		190											
[2507]	Gln	Val	Leu	Ser	Leu	Lys	Ala	Leu	Tyr	Glu	Pro	Gly	Asp	Asp	Ser	Gly	
[2508]		195		200		205											
[2509]	Ala	Gly	Ile	Phe	Arg	Lys	Ile	Leu	Val	Ser	Gln	Pro	Val	Asn	Pro	Pro	
[2510]		210		215		220											
[2511]	Arg	Val	Thr	Thr	Thr	Leu	Ile	Glu	Pro	Ser	Asn	Glu	Leu	Glu	Ala	Cys	
[2512]	225			230		235											
[2513]	Gly	Arg	Val	Ser	Tyr	Pro	Glu	Asp	Asn	Gly	Ala	Cys	His	Arg	Met	Asp	
[2514]		245		250		255											
[2515]	Ser	Pro	Arg	Ser	Ala	Glu	Phe	Ser	Gly	Gly	Ser	Gly	His	Phe	Val	Ser	
[2516]		260		265		270											
[2517]	Glu	Lys	Asp	Gly	His	Lys	Thr	Thr	Ile	Ser	Pro	Arg	Ser	Pro	Ala	Glu	
[2518]		275		280		285											
[2519]	Thr	Ser	Glu	Leu	Val	Gly	Gln	Met	Thr	Ile	Phe	Tyr	Ser	Gly	Lys	Val	
[2520]		290		295		300											
[2521]	Asn	Val	Tyr	Asp	Gly	Ile	Pro	Pro	Glu	Lys	Ala	Arg	Ser	Ile	Met	His	
[2522]	305			310		315											
[2523]	Phe	Ala	Ala	Asn	Pro	Ile	Asp	Leu	Pro	Glu	Asn	Gly	Ile	Phe	Ala	Ser	
[2524]		325		330		335											
[2525]	Ser	Arg	Met	Ile	Ser	Lys	Leu	Ile	Ser	Lys	Glu	Lys	Met	Met	Glu	Leu	
[2526]		340		345		350											
[2527]	Pro	Gln	Lys	Gly	Leu	Glu	Lys	Ala	Asn	Ser	Ser	Arg	Asp	Ser	Gly	Met	
[2528]		355		360		365											
[2529]	Glu	Gly	Gln	Ala	Asn	Arg	Lys	Val	Ser	Leu	Gln	Arg	Tyr	Arg	Glu	Lys	
[2530]		370		375		380											
[2531]	Arg	Lys	Asp	Arg	Lys	Phe	Ser	Lys	Ala	Lys	Lys	Cys	Pro	Gly	Val	Ala	
[2532]	385			390		395											
[2533]	Ser	Ser	Ser	Leu	Glu	Met	Phe	Leu	Asn	Cys	Gln	Pro	Arg	Met	Lys	Ala	
[2534]		405		410		415											

[2535]	Ala Tyr Ser Gln Asn Leu Gly Cys Thr Gly Ser Pro Leu His Ser Gln
[2536]	420 425 430
[2537]	Ser Pro Glu Ser Gln Thr Lys Ser Pro Asn Leu Ser Val Asp Leu Asn
[2538]	435 440 445
[2539]	Ser Glu Gly Ile
[2540]	450
[2541]	<210> 60
[2542]	<211> 391
[2543]	<212> PRT
[2544]	<213> Artificial Sequence
[2545]	<220>
[2546]	<223> Consensus sequence
[2547]	<400> 60
[2548]	Met Pro Lys Lys Lys Arg Lys Val Ser Ser Gly Ala Asn Phe Asn Gln
[2549]	1 5 10 15
[2550]	Ser Gly Asn Ile Ala Asp Ser Ser Leu Ser Phe Thr Phe Thr Asn Ser
[2551]	20 25 30
[2552]	Ser Asn Gly Pro Asn Leu Ile Thr Thr Gln Thr Asn Ser Gln Ala Leu
[2553]	35 40 45
[2554]	Ser Gln Pro Ile Ala Ser Ser Asn Val His Asp Asn Phe Met Asn Asn
[2555]	50 55 60
[2556]	Glu Ile Thr Ala Ser Lys Ile Asp Asp Gly Asn Asn Ser Lys Pro Leu
[2557]	65 70 75 80
[2558]	Ser Pro Gly Trp Thr Asp Gln Thr Ala Tyr Asn Ala Phe Gly Ile Thr
[2559]	85 90 95
[2560]	Thr Gly Met Phe Asn Thr Thr Thr Met Asp Asp Val Tyr Asn Tyr Leu
[2561]	100 105 110
[2562]	Phe Asp Asp Glu Asp Thr Pro Pro Asn Pro Lys Lys Glu Gly Gly Ser
[2563]	115 120 125
[2564]	Asn Gln Thr Ser Leu Tyr Lys Lys Ala Gly Ser Tyr Glu Pro Gly Asp
[2565]	130 135 140
[2566]	Asp Ser Gly Ala Gly Ile Phe Arg Lys Ile Leu Val Ser Gln Pro Val
[2567]	145 150 155 160
[2568]	Asn Pro Pro Arg Val Thr Thr Thr Leu Ile Glu Pro Ser Asn Glu Leu
[2569]	165 170 175
[2570]	Glu Ala Cys Gly Arg Val Ser Tyr Pro Glu Asp Asn Gly Ala Cys His
[2571]	180 185 190
[2572]	Arg Met Asp Ser Pro Arg Ser Ala Glu Phe Ser Gly Gly Ser Gly His
[2573]	195 200 205

[2574]	Phe Val Ser Glu Lys Asp Gly His Lys Thr Thr Ile Ser Pro Arg Ser
[2575]	210 215 220
[2576]	Pro Ala Glu Thr Ser Glu Leu Val Gly Gln Met Thr Ile Phe Tyr Ser
[2577]	225 230 235 240
[2578]	Gly Lys Val Asn Val Tyr Asp Gly Ile Pro Pro Glu Lys Ala Arg Ser
[2579]	245 250 255
[2580]	Ile Met His Phe Ala Ala Asn Pro Ile Asp Leu Pro Glu Asn Gly Ile
[2581]	260 265 270
[2582]	Phe Ala Ser Ser Arg Met Ile Ser Lys Leu Ile Ser Lys Glu Lys Met
[2583]	275 280 285
[2584]	Met Glu Leu Pro Gln Lys Gly Leu Glu Lys Ala Asn Ser Ser Arg Asp
[2585]	290 295 300
[2586]	Ser Gly Met Glu Gly Gln Ala Asn Arg Lys Val Ser Leu Gln Arg Tyr
[2587]	305 310 315 320
[2588]	Arg Glu Lys Arg Lys Asp Arg Lys Phe Ser Lys Ala Lys Lys Cys Pro
[2589]	325 330 335
[2590]	Gly Val Ala Ser Ser Ser Leu Glu Met Phe Leu Asn Cys Gln Pro Arg
[2591]	340 345 350
[2592]	Met Lys Ala Ala Tyr Ser Gln Asn Leu Gly Cys Thr Gly Ser Pro Leu
[2593]	355 360 365
[2594]	His Ser Gln Ser Pro Glu Ser Gln Thr Lys Ser Pro Asn Leu Ser Val
[2595]	370 375 380
[2596]	Asp Leu Asn Ser Glu Gly Ile
[2597]	385 390
[2598]	<210> 61
[2599]	<211> 427
[2600]	<212> PRT
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[2603]	<223> Consensus sequence
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[2605]	Met Pro Lys Lys Lys Arg Lys Val Ser Ser Gly Ala Asn Phe Asn Gln
[2606]	1 5 10 15
[2607]	Ser Gly Asn Ile Ala Asp Ser Ser Leu Ser Phe Thr Phe Thr Asn Ser
[2608]	20 25 30
[2609]	Ser Asn Gly Pro Asn Leu Ile Thr Thr Gln Thr Asn Ser Gln Ala Leu
[2610]	35 40 45
[2611]	Ser Gln Pro Ile Ala Ser Ser Asn Val His Asp Asn Phe Met Asn Asn
[2612]	50 55 60

[2613]	Glu Ile Thr Ala Ser Lys Ile Asp Asp Gly Asn Asn Ser Lys Pro Leu
[2614]	65 70 75 80
[2615]	Ser Pro Gly Trp Thr Asp Gln Thr Ala Tyr Asn Ala Phe Gly Ile Thr
[2616]	85 90 95
[2617]	Thr Gly Met Phe Asn Thr Thr Thr Met Asp Asp Val Tyr Asn Tyr Leu
[2618]	100 105 110
[2619]	Phe Asp Asp Glu Asp Thr Pro Pro Asn Pro Lys Lys Glu Gly Gly Ser
[2620]	115 120 125
[2621]	Asn Gln Thr Ser Leu Tyr Lys Lys Ala Gly Ser Met Asp Val Gly Val
[2622]	130 135 140
[2623]	Ser Pro Ala Lys Ser Ile Leu Ala Lys Pro Leu Lys Leu Leu Thr Glu
[2624]	145 150 155 160
[2625]	Glu Asp Ile Ser Gln Leu Thr Arg Glu Asp Cys Arg Lys Phe Leu Lys
[2626]	165 170 175
[2627]	Asp Lys Gly Met Arg Arg Pro Ser Trp Asn Lys Ser Gln Ala Ile Gln
[2628]	180 185 190
[2629]	Gln Val Leu Ser Leu Lys Ala Leu Tyr Glu Pro Gly Asp Asp Ser Gly
[2630]	195 200 205
[2631]	Ala Gly Ile Phe Arg Lys Ile Leu Val Ser Gln Pro Val Asn Pro Pro
[2632]	210 215 220
[2633]	Arg Val Thr Thr Thr Leu Ile Glu Pro Ser Asn Glu Leu Glu Ala Cys
[2634]	225 230 235 240
[2635]	Gly Arg Val Ser Tyr Pro Glu Asp Asn Gly Ala Cys His Arg Met Asp
[2636]	245 250 255
[2637]	Ser Pro Arg Ser Ala Glu Phe Ser Gly Gly Ser Gly His Phe Val Ser
[2638]	260 265 270
[2639]	Glu Lys Asp Gly His Lys Thr Thr Ile Ser Pro Arg Ser Pro Ala Glu
[2640]	275 280 285
[2641]	Thr Ser Glu Leu Ile Met His Phe Ala Ala Asn Pro Ile Asp Leu Pro
[2642]	290 295 300
[2643]	Glu Asn Gly Ile Phe Ala Ser Ser Arg Met Ile Ser Lys Leu Ile Ser
[2644]	305 310 315 320
[2645]	Lys Glu Lys Met Met Glu Leu Pro Gln Lys Gly Leu Glu Lys Ala Asn
[2646]	325 330 335
[2647]	Ser Ser Arg Asp Ser Gly Met Glu Gly Gln Ala Asn Arg Lys Val Ser
[2648]	340 345 350
[2649]	Leu Gln Arg Tyr Arg Glu Lys Arg Lys Asp Arg Lys Phe Ser Lys Ala
[2650]	355 360 365
[2651]	Lys Lys Cys Pro Gly Val Ala Ser Ser Ser Leu Glu Met Phe Leu Asn

[2652]	370	375	380
[2653]	Cys Gln Pro Arg Met Lys Ala Ala Tyr Ser Gln Asn Leu Gly Cys Thr		
[2654]	385	390	395
[2655]	Gly Ser Pro Leu His Ser Gln Ser Pro Glu Ser Gln Thr Lys Ser Pro		
[2656]	405	410	415
[2657]	Asn Leu Ser Val Asp Leu Asn Ser Glu Gly Ile		
[2658]	420	425	
[2659]	<210> 62		
[2660]	<211> 368		
[2661]	<212> PRT		
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[2663]	<220>		
[2664]	<223> Consensus sequence		
[2665]	<400> 62		
[2666]	Met Pro Lys Lys Lys Arg Lys Val Ser Ser Gly Ala Asn Phe Asn Gln		
[2667]	1	5	10
[2668]	Ser Gly Asn Ile Ala Asp Ser Ser Leu Ser Phe Thr Phe Thr Asn Ser		
[2669]	20	25	30
[2670]	Ser Asn Gly Pro Asn Leu Ile Thr Thr Gln Thr Asn Ser Gln Ala Leu		
[2671]	35	40	45
[2672]	Ser Gln Pro Ile Ala Ser Ser Asn Val His Asp Asn Phe Met Asn Asn		
[2673]	50	55	60
[2674]	Glu Ile Thr Ala Ser Lys Ile Asp Asp Gly Asn Asn Ser Lys Pro Leu		
[2675]	65	70	75
[2676]	Ser Pro Gly Trp Thr Asp Gln Thr Ala Tyr Asn Ala Phe Gly Ile Thr		
[2677]	85	90	95
[2678]	Thr Gly Met Phe Asn Thr Thr Thr Met Asp Asp Val Tyr Asn Tyr Leu		
[2679]	100	105	110
[2680]	Phe Asp Asp Glu Asp Thr Pro Pro Asn Pro Lys Lys Glu Gly Gly Ser		
[2681]	115	120	125
[2682]	Asn Gln Thr Ser Leu Tyr Lys Lys Ala Gly Ser Met Asp Val Gly Val		
[2683]	130	135	140
[2684]	Ser Pro Ala Lys Ser Ile Leu Ala Lys Pro Leu Lys Leu Leu Thr Glu		
[2685]	145	150	155
[2686]	Glu Asp Ile Ser Gln Leu Thr Arg Glu Asp Cys Arg Lys Phe Leu Lys		
[2687]	165	170	175
[2688]	Asp Lys Gly Met Arg Arg Pro Ser Trp Asn Lys Ser Gln Ala Ile Gln		
[2689]	180	185	190
[2690]	Gln Val Leu Ser Leu Lys Ala Leu Tyr Glu Pro Gly Asp Asp Ser Gly		

[2691]	195	200	205
[2692]	Ala Gly Ile Phe Arg Lys Ile Leu Val Ser Gln Pro Val Asn Pro Pro		
[2693]	210	215	220
[2694]	Arg Val Thr Thr Thr Leu Ile Glu Pro Ser Asn Glu Leu Glu Ala Cys		
[2695]	225	230	235
[2696]	Gly Arg Val Ser Tyr Pro Glu Asp Asn Gly Ala Cys His Arg Met Asp		
[2697]	245	250	255
[2698]	Ser Pro Arg Ser Ala Glu Phe Ser Gly Gly Ser Gly His Phe Val Ser		
[2699]	260	265	270
[2700]	Glu Lys Asp Gly His Lys Thr Thr Ile Ser Pro Arg Ser Pro Ala Glu		
[2701]	275	280	285
[2702]	Thr Ser Glu Leu Val Gly Gln Met Thr Ile Phe Tyr Ser Gly Lys Val		
[2703]	290	295	300
[2704]	Asn Val Tyr Asp Gly Ile Pro Pro Glu Lys Ala Arg Ser Ile Met His		
[2705]	305	310	315
[2706]	Phe Ala Ala Asn Pro Ile Asp Leu Pro Glu Asn Gly Ile Phe Ala Ser		
[2707]	325	330	335
[2708]	Ser Arg Met Ile Ser Lys Leu Ile Ser Lys Glu Lys Met Met Glu Leu		
[2709]	340	345	350
[2710]	Pro Gln Lys Gly Leu Glu Lys Ala Asn Ser Ser Arg Asp Ser Gly Met		
[2711]	355	360	365
[2712]	<210> 63		
[2713]	<211> 1131		
[2714]	<212> PRT		
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[2716]	<220>		
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[2718]	<400> 63		
[2719]	Met Ser Ser Leu Ser Arg Glu Leu Val Phe Leu Ile Leu Gln Phe Leu		
[2720]	1	5	10
[2721]	Asp Glu Glu Lys Phe Lys Glu Thr Val His Lys Leu Glu Gln Glu Ser		
[2722]	20	25	30
[2723]	Gly Phe Phe Phe Asn Met Lys Tyr Phe Glu Asp Glu Val His Asn Gly		
[2724]	35	40	45
[2725]	Asn Trp Asp Glu Val Glu Lys Tyr Leu Ser Gly Phe Thr Lys Val Asp		
[2726]	50	55	60
[2727]	Asp Asn Arg Tyr Ser Met Lys Ile Phe Phe Glu Ile Arg Lys Gln Lys		
[2728]	65	70	75
[2729]	Tyr Leu Glu Ala Leu Asp Lys His Asp Arg Pro Lys Ala Val Asp Ile		

[2730]		85		90		95
[2731]	Leu Val Lys Asp Leu Lys Val Phe Ser Thr Phe Asn Glu Glu Leu Phe					
[2732]		100		105		110
[2733]	Lys Glu Ile Thr Gln Leu Leu Thr Leu Glu Asn Phe Arg Glu Asn Glu					
[2734]		115		120		125
[2735]	Gln Leu Ser Lys Tyr Gly Asp Thr Lys Ser Ala Arg Ala Ile Met Leu					
[2736]		130		135		140
[2737]	Val Glu Leu Lys Lys Leu Ile Glu Ala Asn Pro Leu Phe Arg Asp Lys					
[2738]		145		150		155
[2739]	Leu Gln Phe Pro Thr Leu Arg Asn Ser Arg Leu Arg Thr Leu Ile Asn					
[2740]		165		170		175
[2741]	Gln Ser Leu Asn Trp Gln His Gln Leu Cys Lys Asn Pro Arg Pro Asn					
[2742]		180		185		190
[2743]	Pro Asp Ile Lys Thr Leu Phe Val Asp His Ser Cys Gly Pro Pro Asn					
[2744]		195		200		205
[2745]	Gly Ala Arg Ala Pro Ser Pro Val Asn Asn Pro Leu Leu Gly Gly Ile					
[2746]		210		215		220
[2747]	Pro Lys Ala Gly Gly Phe Pro Pro Leu Gly Ala His Gly Pro Phe Gln					
[2748]		225		230		235
[2749]	Pro Thr Ala Ser Pro Val Pro Thr Pro Leu Ala Gly Trp Met Ser Ser					
[2750]		245		250		255
[2751]	Pro Ser Ser Val Pro His Pro Ala Val Ser Ala Gly Ala Ile Ala Leu					
[2752]		260		265		270
[2753]	Gly Gly Pro Ser Ile Pro Ala Ala Leu Lys His Pro Arg Thr Pro Pro					
[2754]		275		280		285
[2755]	Thr Asn Ala Ser Leu Asp Tyr Pro Ser Ala Asp Ser Glu His Val Ser					
[2756]		290		295		300
[2757]	Lys Arg Thr Arg Pro Met Gly Ile Ser Asp Glu Val Asn Leu Gly Val					
[2758]		305		310		315
[2759]	Asn Met Leu Pro Met Ser Phe Ser Gly Gln Ala His Gly His Ser Pro					
[2760]		325		330		335
[2761]	Ala Phe Lys Ala Pro Asp Asp Leu Pro Lys Thr Val Ala Arg Thr Leu					
[2762]		340		345		350
[2763]	Ser Gln Gly Ser Ser Pro Met Ser Met Asp Phe His Pro Ile Lys Gln					
[2764]		355		360		365
[2765]	Thr Leu Leu Leu Val Gly Thr Asn Val Gly Asp Ile Gly Leu Trp Glu					
[2766]		370		375		380
[2767]	Val Gly Ser Arg Glu Arg Leu Val Gln Lys Thr Phe Lys Val Trp Asp					
[2768]		385		390		395
						400

[2769]	Leu	Ser	Lys	Cys	Ser	Met	Pro	Leu	Gln	Ala	Ala	Leu	Val	Lys	Glu	Pro
[2770]					405					410					415	
[2771]	Val	Val	Ser	Val	Asn	Arg	Val	Ile	Trp	Ser	Pro	Asp	Gly	Ser	Leu	Phe
[2772]					420					425					430	
[2773]	Gly	Val	Ala	Tyr	Ser	Arg	His	Ile	Val	Gln	Leu	Tyr	Ser	Tyr	His	Gly
[2774]					435					440					445	
[2775]	Gly	Glu	Asp	Met	Arg	Gln	His	Leu	Glu	Ile	Asp	Ala	His	Val	Gly	Gly
[2776]					450					455					460	
[2777]	Val	Asn	Asp	Ile	Ser	Phe	Ser	Thr	Pro	Asn	Lys	Gln	Leu	Cys	Val	Ile
[2778]	465					470					475					480
[2779]	Thr	Cys	Gly	Asp	Asp	Lys	Thr	Ile	Lys	Val	Trp	Asp	Ala	Ala	Thr	Gly
[2780]					485						490					495
[2781]	Val	Lys	Arg	His	Thr	Phe	Glu	Gly	His	Glu	Ala	Pro	Val	Tyr	Ser	Val
[2782]					500						505				510	
[2783]	Cys	Pro	His	Tyr	Lys	Glu	Asn	Ile	Gln	Phe	Ile	Phe	Ser	Thr	Ala	Leu
[2784]					515						520				525	
[2785]	Asp	Gly	Lys	Ile	Lys	Ala	Trp	Leu	Tyr	Asp	Asn	Met	Gly	Ser	Arg	Val
[2786]					530						535				540	
[2787]	Asp	Tyr	Asp	Ala	Pro	Gly	Arg	Trp	Cys	Thr	Thr	Met	Ala	Tyr	Ser	Ala
[2788]	545					550					555					560
[2789]	Asp	Gly	Thr	Arg	Leu	Phe	Ser	Cys	Gly	Thr	Ser	Lys	Asp	Gly	Glu	Ser
[2790]					565						570				575	
[2791]	Phe	Ile	Val	Glu	Trp	Asn	Glu	Ser	Glu	Gly	Ala	Val	Lys	Arg	Thr	Tyr
[2792]					580						585				590	
[2793]	Gln	Gly	Phe	His	Lys	Arg	Ser	Leu	Gly	Val	Val	Gln	Phe	Asp	Thr	Thr
[2794]					595						600				605	
[2795]	Lys	Asn	Arg	Tyr	Leu	Ala	Ala	Gly	Asp	Asp	Phe	Ser	Ile	Lys	Phe	Trp
[2796]					610						615				620	
[2797]	Asp	Met	Asp	Ala	Val	Gln	Leu	Leu	Thr	Ala	Ile	Asp	Gly	Asp	Gly	Gly
[2798]	625					630					635					640
[2799]	Leu	Gln	Ala	Ser	Pro	Arg	Ile	Arg	Phe	Asn	Lys	Glu	Gly	Ser	Leu	Leu
[2800]					645						650				655	
[2801]	Ala	Val	Ser	Gly	Asn	Glu	Asn	Val	Ile	Lys	Ile	Met	Ala	Asn	Ser	Asp
[2802]					660						665				670	
[2803]	Gly	Leu	Arg	Leu	Leu	His	Thr	Phe	Glu	Asn	Ile	Ser	Ser	Glu	Ser	Ser
[2804]					675						680				685	
[2805]	Lys	Pro	Ala	Ile	Asn	Ser	Ile	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Thr
[2806]					690						695				700	
[2807]	Ser	Ala	Gly	His	Ala	Asp	Arg	Ser	Ala	Asn	Val	Val	Ser	Ile	Gln	Gly

[2808]	705	710	715	720
[2809]	Met Asn Gly Asp Ser Arg Asn Met Val Asp Val Lys Pro Val Ile Thr			
[2810]		725	730	735
[2811]	Glu Glu Ser Asn Asp Lys Ser Lys Ile Trp Lys Leu Thr Glu Val Ser			
[2812]		740	745	750
[2813]	Glu Pro Ser Gln Cys Arg Ser Leu Arg Leu Pro Glu Asn Leu Arg Val			
[2814]		755	760	765
[2815]	Ala Lys Ile Ser Arg Leu Ile Phe Thr Asn Ser Gly Asn Ala Ile Leu			
[2816]		770	775	780
[2817]	Ala Leu Ala Ser Asn Ala Ile His Leu Leu Trp Lys Trp Gln Arg Asn			
[2818]	785	790	795	800
[2819]	Glu Arg Asn Ala Thr Gly Lys Ala Thr Ala Ser Leu Pro Pro Gln Gln			
[2820]		805	810	815
[2821]	Trp Gln Pro Ala Ser Gly Ile Leu Met Thr Asn Asp Val Ala Glu Thr			
[2822]		820	825	830
[2823]	Asn Pro Glu Glu Ala Val Pro Cys Phe Ala Leu Ser Lys Asn Asp Ser			
[2824]		835	840	845
[2825]	Tyr Val Met Ser Ala Ser Gly Gly Lys Ile Ser Leu Phe Asn Met Met			
[2826]		850	855	860
[2827]	Thr Phe Lys Thr Met Ala Thr Phe Met Pro Pro Pro Ala Ala Thr			
[2828]	865	870	875	880
[2829]	Phe Leu Ala Phe His Pro Gln Asp Asn Asn Ile Ile Ala Ile Gly Met			
[2830]		885	890	895
[2831]	Asp Asp Ser Thr Ile Gln Ile Tyr Asn Val Arg Val Asp Glu Val Lys			
[2832]		900	905	910
[2833]	Ser Lys Leu Lys Gly His Ser Lys Arg Ile Thr Gly Leu Ala Phe Ser			
[2834]		915	920	925
[2835]	Asn Val Leu Asn Val Leu Val Ser Ser Gly Ala Asp Ala Gln Leu Cys			
[2836]		930	935	940
[2837]	Val Trp Asn Thr Asp Gly Trp Glu Lys Gln Arg Ser Lys Val Leu Pro			
[2838]	945	950	955	960
[2839]	Leu Pro Gln Gly Arg Pro Asn Ser Ala Pro Ser Asp Thr Arg Val Gln			
[2840]		965	970	975
[2841]	Phe His Gln Asp Gln Ala His Phe Leu Val Val His Glu Thr Gln Leu			
[2842]		980	985	990
[2843]	Ala Ile Tyr Glu Thr Thr Lys Leu Glu Cys Met Lys Gln Trp Ala Val			
[2844]		995	1000	1005
[2845]	Arg Glu Ser Leu Ala Pro Ile Thr His Ala Thr Phe Ser Cys Asp			
[2846]		1010	1015	1020

[2847]	Ser Gln Leu Val Tyr Ala Ser Phe Met Asp Ala Thr Val Cys Val		
[2848]	1025	1030	1035
[2849]	Phe Ser Ser Ala Asn Leu Arg Leu Arg Cys Arg Val Asn Pro Ser		
[2850]	1040	1045	1050
[2851]	Ala Tyr Leu Pro Ala Ser Leu Ser Asn Ser Asn Val His Pro Leu		
[2852]	1055	1060	1065
[2853]	Val Ile Ala Ala His Pro Gln Glu Pro Asn Met Phe Ala Val Gly		
[2854]	1070	1075	1080
[2855]	Leu Ser Asp Gly Gly Val His Ile Phe Glu Pro Leu Glu Ser Glu		
[2856]	1085	1090	1095
[2857]	Gly Lys Trp Gly Val Ala Pro Pro Ala Glu Asn Gly Ser Ala Ser		
[2858]	1100	1105	1110
[2859]	Gly Ala Pro Thr Ala Pro Ser Val Gly Ala Ser Ala Ser Asp Gln		
[2860]	1115	1120	1125
[2861]	Pro Gln Arg		
[2862]	1130		
[2863]	<210> 64		
[2864]	<211> 1292		
[2865]	<212> PRT		
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[2867]	<220>		
[2868]	<223> Consensus sequence		
[2869]	<400> 64		
[2870]	Met Lys Leu Leu Ser Ser Ile Glu Glu Ala Cys Asn Ile Cys Arg Leu		
[2871]	1	5	10
[2872]	Lys Lys Leu Lys Cys Ser Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu		
[2873]	20	25	30
[2874]	Lys Asn Asn Trp Glu Cys Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro		
[2875]	35	40	45
[2876]	Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu		
[2877]	50	55	60
[2878]	Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu Asn Leu Asn Met Ile		
[2879]	65	70	75
[2880]	Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu		
[2881]	85	90	95
[2882]	Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala		
[2883]	100	105	110
[2884]	Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser		
[2885]	115	120	125

[2886]	Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu
[2887]	130 135 140
[2888]	Thr Val Ser Ser Arg Ser Asn Gln Thr Ser Leu Tyr Lys Lys Ala Gly
[2889]	145 150 155 160
[2890]	Ser Met Ser Ser Leu Ser Arg Glu Leu Val Phe Leu Ile Leu Gln Phe
[2891]	165 170 175
[2892]	Leu Asp Glu Glu Lys Phe Lys Glu Thr Val His Lys Leu Glu Gln Glu
[2893]	180 185 190
[2894]	Ser Gly Phe Phe Phe Asn Met Lys Tyr Phe Glu Asp Glu Val His Asn
[2895]	195 200 205
[2896]	Gly Asn Trp Asp Glu Val Glu Lys Tyr Leu Ser Gly Phe Thr Lys Val
[2897]	210 215 220
[2898]	Asp Asp Asn Arg Tyr Ser Met Lys Ile Phe Phe Glu Ile Arg Lys Gln
[2899]	225 230 235 240
[2900]	Lys Tyr Leu Glu Ala Leu Asp Lys His Asp Arg Pro Lys Ala Val Asp
[2901]	245 250 255
[2902]	Ile Leu Val Lys Asp Leu Lys Val Phe Ser Thr Phe Asn Glu Glu Leu
[2903]	260 265 270
[2904]	Phe Lys Glu Ile Thr Gln Leu Leu Thr Leu Glu Asn Phe Arg Glu Asn
[2905]	275 280 285
[2906]	Glu Gln Leu Ser Lys Tyr Gly Asp Thr Lys Ser Ala Arg Ala Ile Met
[2907]	290 295 300
[2908]	Leu Val Glu Leu Lys Lys Leu Ile Glu Ala Asn Pro Leu Phe Arg Asp
[2909]	305 310 315 320
[2910]	Lys Leu Gln Phe Pro Thr Leu Arg Asn Ser Arg Leu Arg Thr Leu Ile
[2911]	325 330 335
[2912]	Asn Gln Ser Leu Asn Trp Gln His Gln Leu Cys Lys Asn Pro Arg Pro
[2913]	340 345 350
[2914]	Asn Pro Asp Ile Lys Thr Leu Phe Val Asp His Ser Cys Gly Pro Pro
[2915]	355 360 365
[2916]	Asn Gly Ala Arg Ala Pro Ser Pro Val Asn Asn Pro Leu Leu Gly Gly
[2917]	370 375 380
[2918]	Ile Pro Lys Ala Gly Gly Phe Pro Pro Leu Gly Ala His Gly Pro Phe
[2919]	385 390 395 400
[2920]	Gln Pro Thr Ala Ser Pro Val Pro Thr Pro Leu Ala Gly Trp Met Ser
[2921]	405 410 415
[2922]	Ser Pro Ser Ser Val Pro His Pro Ala Val Ser Ala Gly Ala Ile Ala
[2923]	420 425 430
[2924]	Leu Gly Gly Pro Ser Ile Pro Ala Ala Leu Lys His Pro Arg Thr Pro

[2925]	435	440	445
[2926]	Pro Thr Asn Ala Ser Leu Asp Tyr Pro Ser Ala Asp Ser Glu His Val		
[2927]	450	455	460
[2928]	Ser Lys Arg Thr Arg Pro Met Gly Ile Ser Asp Glu Val Asn Leu Gly		
[2929]	465	470	475
[2930]	Val Asn Met Leu Pro Met Ser Phe Ser Gly Gln Ala His Gly His Ser		
[2931]	485	490	495
[2932]	Pro Ala Phe Lys Ala Pro Asp Asp Leu Pro Lys Thr Val Ala Arg Thr		
[2933]	500	505	510
[2934]	Leu Ser Gln Gly Ser Ser Pro Met Ser Met Asp Phe His Pro Ile Lys		
[2935]	515	520	525
[2936]	Gln Thr Leu Leu Leu Val Gly Thr Asn Val Gly Asp Ile Gly Leu Trp		
[2937]	530	535	540
[2938]	Glu Val Gly Ser Arg Glu Arg Leu Val Gln Lys Thr Phe Lys Val Trp		
[2939]	545	550	555
[2940]	Asp Leu Ser Lys Cys Ser Met Pro Leu Gln Ala Ala Leu Val Lys Glu		
[2941]	565	570	575
[2942]	Pro Val Val Ser Val Asn Arg Val Ile Trp Ser Pro Asp Gly Ser Leu		
[2943]	580	585	590
[2944]	Phe Gly Val Ala Tyr Ser Arg His Ile Val Gln Leu Tyr Ser Tyr His		
[2945]	595	600	605
[2946]	Gly Gly Glu Asp Met Arg Gln His Leu Glu Ile Asp Ala His Val Gly		
[2947]	610	615	620
[2948]	Gly Val Asn Asp Ile Ser Phe Ser Thr Pro Asn Lys Gln Leu Cys Val		
[2949]	625	630	635
[2950]	Ile Thr Cys Gly Asp Asp Lys Thr Ile Lys Val Trp Asp Ala Ala Thr		
[2951]	645	650	655
[2952]	Gly Val Lys Arg His Thr Phe Glu Gly His Glu Ala Pro Val Tyr Ser		
[2953]	660	665	670
[2954]	Val Cys Pro His Tyr Lys Glu Asn Ile Gln Phe Ile Phe Ser Thr Ala		
[2955]	675	680	685
[2956]	Leu Asp Gly Lys Ile Lys Ala Trp Leu Tyr Asp Asn Met Gly Ser Arg		
[2957]	690	695	700
[2958]	Val Asp Tyr Asp Ala Pro Gly Arg Trp Cys Thr Thr Met Ala Tyr Ser		
[2959]	705	710	715
[2960]	Ala Asp Gly Thr Arg Leu Phe Ser Cys Gly Thr Ser Lys Asp Gly Glu		
[2961]	725	730	735
[2962]	Ser Phe Ile Val Glu Trp Asn Glu Ser Glu Gly Ala Val Lys Arg Thr		
[2963]	740	745	750

[2964]	Tyr	Gln	Gly	Phe	His	Lys	Arg	Ser	Leu	Gly	Val	Val	Gln	Phe	Asp	Thr
[2965]			755				760						765			
[2966]	Thr	Lys	Asn	Arg	Tyr	Leu	Ala	Ala	Gly	Asp	Asp	Phe	Ser	Ile	Lys	Phe
[2967]			770				775						780			
[2968]	Trp	Asp	Met	Asp	Ala	Val	Gln	Leu	Leu	Thr	Ala	Ile	Asp	Gly	Asp	Gly
[2969]	785						790					795				800
[2970]	Gly	Leu	Gln	Ala	Ser	Pro	Arg	Ile	Arg	Phe	Asn	Lys	Glu	Gly	Ser	Leu
[2971]					805					810					815	
[2972]	Leu	Ala	Val	Ser	Gly	Asn	Glu	Asn	Val	Ile	Lys	Ile	Met	Ala	Asn	Ser
[2973]				820					825					830		
[2974]	Asp	Gly	Leu	Arg	Leu	Leu	His	Thr	Phe	Glu	Asn	Ile	Ser	Ser	Glu	Ser
[2975]			835					840						845		
[2976]	Ser	Lys	Pro	Ala	Ile	Asn	Ser	Ile	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala
[2977]			850				855							860		
[2978]	Thr	Ser	Ala	Gly	His	Ala	Asp	Arg	Ser	Ala	Asn	Val	Val	Ser	Ile	Gln
[2979]	865					870					875					880
[2980]	Gly	Met	Asn	Gly	Asp	Ser	Arg	Asn	Met	Val	Asp	Val	Lys	Pro	Val	Ile
[2981]				885					890							895
[2982]	Thr	Glu	Glu	Ser	Asn	Asp	Lys	Ser	Lys	Ile	Trp	Lys	Leu	Thr	Glu	Val
[2983]				900					905						910	
[2984]	Ser	Glu	Pro	Ser	Gln	Cys	Arg	Ser	Leu	Arg	Leu	Pro	Glu	Asn	Leu	Arg
[2985]			915					920						925		
[2986]	Val	Ala	Lys	Ile	Ser	Arg	Leu	Ile	Phe	Thr	Asn	Ser	Gly	Asn	Ala	Ile
[2987]			930					935						940		
[2988]	Leu	Ala	Leu	Ala	Ser	Asn	Ala	Ile	His	Leu	Leu	Trp	Lys	Trp	Gln	Arg
[2989]	945					950					955					960
[2990]	Asn	Glu	Arg	Asn	Ala	Thr	Gly	Lys	Ala	Thr	Ala	Ser	Leu	Pro	Pro	Gln
[2991]				965					970							975
[2992]	Gln	Trp	Gln	Pro	Ala	Ser	Gly	Ile	Leu	Met	Thr	Asn	Asp	Val	Ala	Glu
[2993]				980					985					990		
[2994]	Thr	Asn	Pro	Glu	Glu	Ala	Val	Pro	Cys	Phe	Ala	Leu	Ser	Lys	Asn	Asp
[2995]			995					1000						1005		
[2996]	Ser	Tyr	Val	Met	Ser	Ala	Ser	Gly	Gly	Lys	Ile	Ser	Leu	Phe	Asn	
[2997]			1010					1015						1020		
[2998]	Met	Met	Thr	Phe	Lys	Thr	Met	Ala	Thr	Phe	Met	Pro	Pro	Pro	Pro	
[2999]			1025					1030						1035		
[3000]	Ala	Ala	Thr	Phe	Leu	Ala	Phe	His	Pro	Gln	Asp	Asn	Asn	Ile	Ile	
[3001]			1040					1045						1050		
[3002]	Ala	Ile	Gly	Met	Asp	Asp	Ser	Thr	Ile	Gln	Ile	Tyr	Asn	Val	Arg	

[3003]	1055	1060	1065
[3004]	Val Asp Glu Val Lys Ser	Lys Leu Lys Gly His	Ser Lys Arg Ile
[3005]	1070	1075	1080
[3006]	Thr Gly Leu Ala Phe Ser	Asn Val Leu Asn Val	Leu Val Ser Ser
[3007]	1085	1090	1095
[3008]	Gly Ala Asp Ala Gln Leu	Cys Val Trp Asn Thr	Asp Gly Trp Glu
[3009]	1100	1105	1110
[3010]	Lys Gln Arg Ser Lys Val	Leu Pro Leu Pro Gln	Gly Arg Pro Asn
[3011]	1115	1120	1125
[3012]	Ser Ala Pro Ser Asp Thr	Arg Val Gln Phe His	Gln Asp Gln Ala
[3013]	1130	1135	1140
[3014]	His Phe Leu Val Val His	Glu Thr Gln Leu Ala	Ile Tyr Glu Thr
[3015]	1145	1150	1155
[3016]	Thr Lys Leu Glu Cys Met	Lys Gln Trp Ala Val	Arg Glu Ser Leu
[3017]	1160	1165	1170
[3018]	Ala Pro Ile Thr His Ala	Thr Phe Ser Cys Asp	Ser Gln Leu Val
[3019]	1175	1180	1185
[3020]	Tyr Ala Ser Phe Met Asp	Ala Thr Val Cys Val	Phe Ser Ser Ala
[3021]	1190	1195	1200
[3022]	Asn Leu Arg Leu Arg Cys	Arg Val Asn Pro Ser	Ala Tyr Leu Pro
[3023]	1205	1210	1215
[3024]	Ala Ser Leu Ser Asn Ser	Asn Val His Pro Leu	Val Ile Ala Ala
[3025]	1220	1225	1230
[3026]	His Pro Gln Glu Pro Asn	Met Phe Ala Val Gly	Leu Ser Asp Gly
[3027]	1235	1240	1245
[3028]	Gly Val His Ile Phe Glu	Pro Leu Glu Ser Glu	Gly Lys Trp Gly
[3029]	1250	1255	1260
[3030]	Val Ala Pro Pro Ala Glu	Asn Gly Ser Ala Ser	Gly Ala Pro Thr
[3031]	1265	1270	1275
[3032]	Ala Pro Ser Val Gly Ala	Ser Ala Ser Asp Gln	Pro Gln Arg
[3033]	1280	1285	1290
[3034]	<210> 65		
[3035]	<211> 425		
[3036]	<212> PRT		
[3037]	<213> Artificial Sequence		
[3038]	<220>		
[3039]	<223> Consensus sequence		
[3040]	<400> 65		
[3041]	Met Asp Asp Asp Asn Gly	Leu Glu Leu Ser Leu	Gly Leu Ser Cys Gly

[3042]	1	5	10	15
[3043]	Gly Ser Thr Gly Lys Ala Lys Gly Asn Asn Asn Asn Asn Ala Gly Ser			
[3044]	20	25	30	
[3045]	Ser Ser Glu Asn Tyr Arg Ala Glu Gly Gly Asp Arg Ser Ala Lys Val			
[3046]	35	40	45	
[3047]	Ile Asp Asp Phe Lys Asn Phe Leu His Pro Thr Ser Gln Arg Pro Ala			
[3048]	50	55	60	
[3049]	Glu Pro Ser Ser Gly Ser Gln Arg Ser Asp Ser Gly Gln Gln Pro Pro			
[3050]	65	70	75	80
[3051]	Gln Asn Phe Phe Asn Asp Leu Ser Lys Ala Pro Thr Thr Glu Ala Glu			
[3052]	85	90	95	
[3053]	Ala Ser Thr Lys Pro Leu Trp Val Glu Asp Glu Ser Arg Lys Glu Ala			
[3054]	100	105	110	
[3055]	Gly Asn Lys Arg Lys Phe Gly Phe Pro Gly Met Asn Asp Asp Lys Lys			
[3056]	115	120	125	
[3057]	Lys Glu Lys Asp Ser Ser His Val Asp Met His Glu Lys Lys Thr Lys			
[3058]	130	135	140	
[3059]	Ala Ser His Val Ser Thr Ala Thr Asp Glu Gly Ser Thr Ala Glu Asn			
[3060]	145	150	155	160
[3061]	Glu Asp Val Ala Glu Ser Glu Val Gly Gly Gly Ser Ser Ser Asn His			
[3062]	165	170	175	
[3063]	Ala Lys Glu Val Val Arg Pro Pro Thr Asp Thr Asn Ile Val Asp Asn			
[3064]	180	185	190	
[3065]	Leu Thr Gly Gln Arg Arg Ser Asn His Gly Gly Ser Gly Thr Glu Glu			
[3066]	195	200	205	
[3067]	Phe Thr Met Arg Asn Met Ser Tyr Thr Val Pro Phe Thr Val His Pro			
[3068]	210	215	220	
[3069]	Gln Asn Val Val Thr Ser Met Pro Tyr Ser Leu Pro Thr Lys Glu Ser			
[3070]	225	230	235	240
[3071]	Gly Gln His Ala Ala Ala Thr Ser Leu Leu Gln Pro Asn Ala Asn Ala			
[3072]	245	250	255	
[3073]	Gly Asn Leu Pro Ile Met Phe Gly Tyr Ser Pro Val Gln Leu Pro Met			
[3074]	260	265	270	
[3075]	Leu Asp Lys Asp Gly Ser Gly Gly Ile Val Ala Leu Ser Gln Ser Pro			
[3076]	275	280	285	
[3077]	Phe Ala Gly Arg Val Pro Ser Asn Ser Ala Thr Ala Lys Gly Glu Gly			
[3078]	290	295	300	
[3079]	Lys Gln Pro Val Ala Glu Glu Gly Ser Ser Glu Asp Ala Ser Glu Arg			
[3080]	305	310	315	320

[3081]	Pro Thr Gly Asp Asn Ser Asn Leu Asn Thr Ala Phe Ser Phe Asp Phe
[3082]	325 330 335
[3083]	Ser Ala Ile Lys Pro Gly Met Ala Ala Asp Val Lys Phe Gly Gly Ser
[3084]	340 345 350
[3085]	Gly Ala Arg Pro Asn Leu Pro Trp Val Ser Thr Thr Gly Ser Gly Pro
[3086]	355 360 365
[3087]	His Gly Arg Thr Ile Ser Gly Val Thr Tyr Arg Tyr Asn Ala Asn Gln
[3088]	370 375 380
[3089]	Ile Lys Ile Val Cys Ala Cys His Gly Ser His Met Ser Pro Glu Glu
[3090]	385 390 395 400
[3091]	Phe Val Arg His Ala Ser Glu Glu Tyr Val Ser Pro Glu Ser Ser Met
[3092]	405 410 415
[3093]	Gly Met Thr Ala Ala Ser Ala His Thr
[3094]	420 425
[3095]	<210> 66
[3096]	<211> 564
[3097]	<212> PRT
[3098]	<213> Artificial Sequence
[3099]	<220>
[3100]	<223> Consensus sequence
[3101]	<400> 66
[3102]	Met Pro Lys Lys Lys Arg Lys Val Ser Ser Gly Ala Asn Phe Asn Gln
[3103]	1 5 10 15
[3104]	Ser Gly Asn Ile Ala Asp Ser Ser Leu Ser Phe Thr Phe Thr Asn Ser
[3105]	20 25 30
[3106]	Ser Asn Gly Pro Asn Leu Ile Thr Thr Gln Thr Asn Ser Gln Ala Leu
[3107]	35 40 45
[3108]	Ser Gln Pro Ile Ala Ser Ser Asn Val His Asp Asn Phe Met Asn Asn
[3109]	50 55 60
[3110]	Glu Ile Thr Ala Ser Lys Ile Asp Asp Gly Asn Asn Ser Lys Pro Leu
[3111]	65 70 75 80
[3112]	Ser Pro Gly Trp Thr Asp Gln Thr Ala Tyr Asn Ala Phe Gly Ile Thr
[3113]	85 90 95
[3114]	Thr Gly Met Phe Asn Thr Thr Thr Met Asp Asp Val Tyr Asn Tyr Leu
[3115]	100 105 110
[3116]	Phe Asp Asp Glu Asp Thr Pro Pro Asn Pro Lys Lys Glu Gly Gly Ser
[3117]	115 120 125
[3118]	Asn Gln Thr Ser Leu Tyr Lys Lys Ala Gly Ser Met Asp Asp Asp Asn
[3119]	130 135 140

[3120]	Gly	Leu	Glu	Leu	Ser	Leu	Gly	Leu	Ser	Cys	Gly	Gly	Ser	Thr	Gly	Lys
[3121]	145					150				155						160
[3122]	Ala	Lys	Gly	Asn	Asn	Asn	Asn	Asn	Ala	Gly	Ser	Ser	Ser	Glu	Asn	Tyr
[3123]					165					170						175
[3124]	Arg	Ala	Glu	Gly	Gly	Asp	Arg	Ser	Ala	Lys	Val	Ile	Asp	Asp	Phe	Lys
[3125]					180					185						190
[3126]	Asn	Phe	Leu	His	Pro	Thr	Ser	Gln	Arg	Pro	Ala	Glu	Pro	Ser	Ser	Gly
[3127]					195					200						205
[3128]	Ser	Gln	Arg	Ser	Asp	Ser	Gly	Gln	Gln	Pro	Pro	Gln	Asn	Phe	Phe	Asn
[3129]					210					215						220
[3130]	Asp	Leu	Ser	Lys	Ala	Pro	Thr	Thr	Glu	Ala	Glu	Ala	Ser	Thr	Lys	Pro
[3131]	225					230					235					240
[3132]	Leu	Trp	Val	Glu	Asp	Glu	Ser	Arg	Lys	Glu	Ala	Gly	Asn	Lys	Arg	Lys
[3133]					245						250					255
[3134]	Phe	Gly	Phe	Pro	Gly	Met	Asn	Asp	Asp	Lys	Lys	Lys	Glu	Lys	Asp	Ser
[3135]					260					265						270
[3136]	Ser	His	Val	Asp	Met	His	Glu	Lys	Lys	Thr	Lys	Ala	Ser	His	Val	Ser
[3137]					275					280						285
[3138]	Thr	Ala	Thr	Asp	Glu	Gly	Ser	Thr	Ala	Glu	Asn	Glu	Asp	Val	Ala	Glu
[3139]					290						295					300
[3140]	Ser	Glu	Val	Gly	Gly	Gly	Ser	Ser	Ser	Asn	His	Ala	Lys	Glu	Val	Val
[3141]	305					310					315					320
[3142]	Arg	Pro	Pro	Thr	Asp	Thr	Asn	Ile	Val	Asp	Asn	Leu	Thr	Gly	Gln	Arg
[3143]					325						330					335
[3144]	Arg	Ser	Asn	His	Gly	Gly	Ser	Gly	Thr	Glu	Glu	Phe	Thr	Met	Arg	Asn
[3145]					340						345					350
[3146]	Met	Ser	Tyr	Thr	Val	Pro	Phe	Thr	Val	His	Pro	Gln	Asn	Val	Val	Thr
[3147]					355						360					365
[3148]	Ser	Met	Pro	Tyr	Ser	Leu	Pro	Thr	Lys	Glu	Ser	Gly	Gln	His	Ala	Ala
[3149]					370							375				380
[3150]	Ala	Thr	Ser	Leu	Leu	Gln	Pro	Asn	Ala	Asn	Ala	Gly	Asn	Leu	Pro	Ile
[3151]	385					390						395				400
[3152]	Met	Phe	Gly	Tyr	Ser	Pro	Val	Gln	Leu	Pro	Met	Leu	Asp	Lys	Asp	Gly
[3153]					405						410					415
[3154]	Ser	Gly	Gly	Ile	Val	Ala	Leu	Ser	Gln	Ser	Pro	Phe	Ala	Gly	Arg	Val
[3155]					420						425					430
[3156]	Pro	Ser	Asn	Ser	Ala	Thr	Ala	Lys	Gly	Glu	Gly	Lys	Gln	Pro	Val	Ala
[3157]					435						440					445
[3158]	Glu	Glu	Gly	Ser	Ser	Glu	Asp	Ala	Ser	Glu	Arg	Pro	Thr	Gly	Asp	Asn

[3159]	450	455	460
[3160]	Ser Asn Leu Asn Thr Ala Phe Ser Phe Asp Phe Ser Ala Ile Lys Pro		
[3161]	465	470	475 480
[3162]	Gly Met Ala Ala Asp Val Lys Phe Gly Gly Ser Gly Ala Arg Pro Asn		
[3163]	485	490	495
[3164]	Leu Pro Trp Val Ser Thr Thr Gly Ser Gly Pro His Gly Arg Thr Ile		
[3165]	500	505	510
[3166]	Ser Gly Val Thr Tyr Arg Tyr Asn Ala Asn Gln Ile Lys Ile Val Cys		
[3167]	515	520	525
[3168]	Ala Cys His Gly Ser His Met Ser Pro Glu Glu Phe Val Arg His Ala		
[3169]	530	535	540
[3170]	Ser Glu Glu Tyr Val Ser Pro Glu Ser Ser Met Gly Met Thr Ala Ala		
[3171]	545	550	555 560
[3172]	Ser Ala His Thr		
[3173]	<210> 67		
[3174]	<211> 475		
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[3180]	Met Pro Lys Lys Lys Arg Lys Val Ser Ser Gly Ala Asn Phe Asn Gln		
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[3182]	Ser Gly Asn Ile Ala Asp Ser Ser Leu Ser Phe Thr Phe Thr Asn Ser		
[3183]	20 25 30		
[3184]	Ser Asn Gly Pro Asn Leu Ile Thr Thr Gln Thr Asn Ser Gln Ala Leu		
[3185]	35 40 45		
[3186]	Ser Gln Pro Ile Ala Ser Ser Asn Val His Asp Asn Phe Met Asn Asn		
[3187]	50 55 60		
[3188]	Glu Ile Thr Ala Ser Lys Ile Asp Asp Gly Asn Asn Ser Lys Pro Leu		
[3189]	65 70 75 80		
[3190]	Ser Pro Gly Trp Thr Asp Gln Thr Ala Tyr Asn Ala Phe Gly Ile Thr		
[3191]	85 90 95		
[3192]	Thr Gly Met Phe Asn Thr Thr Thr Met Asp Asp Val Tyr Asn Tyr Leu		
[3193]	100 105 110		
[3194]	Phe Asp Asp Glu Asp Thr Pro Pro Asn Pro Lys Lys Glu Gly Gly Ser		
[3195]	115 120 125		
[3196]	Asn Gln Thr Ser Leu Tyr Lys Lys Ala Gly Ser Met Thr Ser Asp Gly		
[3197]	130 135 140		

[3198]	Ala Thr Ser Thr Ser Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala
[3199]	145 150 155 160
[3200]	Arg Arg Lys Pro Ser Trp Arg Glu Arg Glu Asn Asn Arg Arg Arg Glu
[3201]	165 170 175
[3202]	Arg Arg Arg Arg Ala Val Ala Ala Lys Ile Tyr Thr Gly Leu Arg Ala
[3203]	180 185 190
[3204]	Gln Gly Asp Tyr Asn Leu Pro Lys His Cys Asp Asn Asn Glu Val Leu
[3205]	195 200 205
[3206]	Lys Ala Leu Cys Val Glu Ala Gly Trp Val Val Glu Glu Asp Gly Thr
[3207]	210 215 220
[3208]	Thr Tyr Arg Lys Gly Cys Lys Pro Leu Pro Gly Glu Ile Ala Gly Thr
[3209]	225 230 235 240
[3210]	Ser Ser Arg Val Thr Pro Tyr Ser Ser Gln Asn Gln Ser Pro Leu Ser
[3211]	245 250 255
[3212]	Ser Ala Phe Gln Ser Pro Ile Pro Ser Tyr Gln Val Ser Pro Ser Ser
[3213]	260 265 270
[3214]	Ser Ser Phe Pro Ser Pro Ser Arg Gly Glu Pro Asn Asn Asn Met Ser
[3215]	275 280 285
[3216]	Ser Thr Phe Phe Pro Phe Leu Arg Asn Gly Gly Ile Pro Ser Ser Leu
[3217]	290 295 300
[3218]	Pro Ser Leu Arg Ile Ser Asn Ser Cys Pro Val Thr Pro Pro Val Ser
[3219]	305 310 315 320
[3220]	Ser Pro Thr Ser Lys Asn Pro Lys Pro Leu Pro Asn Trp Glu Ser Ile
[3221]	325 330 335
[3222]	Ala Lys Gln Ser Met Ala Ile Ala Lys Gln Ser Met Ala Ser Phe Asn
[3223]	340 345 350
[3224]	Tyr Pro Phe Tyr Ala Val Ser Ala Pro Ala Ser Pro Thr His Arg His
[3225]	355 360 365
[3226]	Gln Phe His Thr Pro Ala Thr Ile Pro Glu Cys Asp Glu Ser Asp Ser
[3227]	370 375 380
[3228]	Ser Thr Val Asp Ser Gly His Trp Ile Ser Phe Gln Lys Phe Ala Gln
[3229]	385 390 395 400
[3230]	Gln Gln Pro Phe Ser Ala Ser Met Val Pro Thr Ser Pro Thr Phe Asn
[3231]	405 410 415
[3232]	Leu Val Lys Pro Ala Pro Gln Gln Met Ser Pro Asn Thr Ala Ala Phe
[3233]	420 425 430
[3234]	Gln Glu Ile Gly Gln Ser Ser Glu Phe Lys Phe Glu Asn Ser Gln Val
[3235]	435 440 445
[3236]	Lys Pro Trp Glu Gly Glu Arg Ile His Asp Val Gly Met Glu Asp Leu

[3237]	450	455	460
[3238]	Glu Leu Thr Leu Gly Asn Gly Lys Ala Arg Gly		
[3239]	465	470	475
[3240]	<210> 68		
[3241]	<211> 587		
[3242]	<212> PRT		
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[3244]	<220>		
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[3247]	Met Lys Arg Asp His His Gln Phe Gln Gly Arg Leu Ser Asn His Gly		
[3248]	1	5	10
[3249]	Thr Ser Ser Ser Ser Ser Ser Ile Ser Lys Asp Lys Met Met Met Val		
[3250]	20	25	30
[3251]	Lys Lys Glu Glu Asp Gly Gly Gly Asn Met Asp Asp Glu Leu Leu Ala		
[3252]	35	40	45
[3253]	Val Leu Gly Tyr Lys Val Arg Ser Ser Glu Met Ala Glu Val Ala Leu		
[3254]	50	55	60
[3255]	Lys Leu Glu Gln Leu Glu Thr Met Met Ser Asn Val Gln Glu Asp Gly		
[3256]	65	70	75
[3257]	Leu Ser His Leu Ala Thr Asp Thr Val His Tyr Asn Pro Ser Glu Leu		
[3258]	85	90	95
[3259]	Tyr Ser Trp Leu Asp Asn Met Leu Ser Glu Leu Asn Pro Pro Pro Leu		
[3260]	100	105	110
[3261]	Pro Ala Ser Ser Asn Gly Leu Asp Pro Val Leu Pro Ser Pro Glu Ile		
[3262]	115	120	125
[3263]	Cys Gly Phe Pro Ala Ser Asp Tyr Asp Leu Lys Val Ile Pro Gly Asn		
[3264]	130	135	140
[3265]	Ala Ile Tyr Gln Phe Pro Ala Ile Asp Ser Ser Ser Ser Ser Asn Asn		
[3266]	145	150	155
[3267]	Gln Asn Lys Arg Leu Lys Ser Cys Ser Ser Pro Asp Ser Met Val Thr		
[3268]	165	170	175
[3269]	Ser Thr Ser Thr Gly Thr Gln Ile Gly Gly Val Ile Gly Thr Thr Val		
[3270]	180	185	190
[3271]	Thr Thr Thr Thr Thr Thr Thr Thr Ala Ala Gly Glu Ser Thr Arg Ser		
[3272]	195	200	205
[3273]	Val Ile Leu Val Asp Ser Gln Glu Asn Gly Val Arg Leu Val His Ala		
[3274]	210	215	220
[3275]	Leu Met Ala Cys Ala Glu Ala Ile Gln Gln Asn Asn Leu Thr Leu Ala		

[3276]	225	230	235	240
[3277]	Glu Ala Leu Val Lys Gln Ile Gly Cys Leu Ala Val Ser Gln Ala Gly			
[3278]		245	250	255
[3279]	Ala Met Arg Lys Val Ala Thr Tyr Phe Ala Glu Ala Leu Ala Arg Arg			
[3280]		260	265	270
[3281]	Ile Tyr Arg Leu Ser Pro Pro Gln Asn Gln Ile Asp His Cys Leu Ser			
[3282]		275	280	285
[3283]	Asp Thr Leu Gln Met His Phe Tyr Glu Thr Cys Pro Tyr Leu Lys Phe			
[3284]		290	295	300
[3285]	Ala His Phe Thr Ala Asn Gln Ala Ile Leu Glu Ala Phe Glu Gly Lys			
[3286]	305	310	315	320
[3287]	Lys Arg Val His Val Ile Asp Phe Ser Met Asn Gln Gly Leu Gln Trp			
[3288]		325	330	335
[3289]	Pro Ala Leu Met Gln Ala Leu Ala Leu Arg Glu Gly Gly Pro Pro Thr			
[3290]		340	345	350
[3291]	Phe Arg Leu Thr Gly Ile Gly Pro Pro Ala Pro Asp Asn Ser Asp His			
[3292]		355	360	365
[3293]	Leu His Glu Val Gly Cys Lys Leu Ala Gln Leu Ala Glu Ala Ile His			
[3294]		370	375	380
[3295]	Val Glu Phe Glu Tyr Arg Gly Phe Val Ala Asn Ser Leu Ala Asp Leu			
[3296]	385	390	395	400
[3297]	Asp Ala Ser Met Leu Glu Leu Arg Pro Ser Asp Thr Glu Ala Val Ala			
[3298]		405	410	415
[3299]	Val Asn Ser Val Phe Glu Leu His Lys Leu Leu Gly Arg Pro Gly Gly			
[3300]		420	425	430
[3301]	Ile Glu Lys Val Leu Gly Val Val Lys Gln Ile Lys Pro Val Ile Phe			
[3302]		435	440	445
[3303]	Thr Val Val Glu Gln Glu Ser Asn His Asn Gly Pro Val Phe Leu Asp			
[3304]		450	455	460
[3305]	Arg Phe Thr Glu Ser Leu His Tyr Tyr Ser Thr Leu Phe Asp Ser Leu			
[3306]	465	470	475	480
[3307]	Glu Gly Val Pro Asn Ser Gln Asp Lys Val Met Ser Glu Val Tyr Leu			
[3308]		485	490	495
[3309]	Gly Lys Gln Ile Cys Asn Leu Val Ala Cys Glu Gly Pro Asp Arg Val			
[3310]		500	505	510
[3311]	Glu Arg His Glu Thr Leu Ser Gln Trp Gly Asn Arg Phe Gly Ser Ser			
[3312]		515	520	525
[3313]	Gly Leu Ala Pro Ala His Leu Gly Ser Asn Ala Phe Lys Gln Ala Ser			
[3314]		530	535	540

[3315]	Met	Leu	Leu	Ser	Val	Phe	Asn	Ser	Gly	Gln	Gly	Tyr	Arg	Val	Glu	Glu
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[3317]	Ser	Asn	Gly	Cys	Leu	Met	Leu	Gly	Trp	His	Thr	Arg	Pro	Leu	Ile	Thr
[3318]							565				570					575
[3319]	Thr	Ser	Ala	Trp	Lys	Leu	Ser	Thr	Ala	Ala	Tyr					
[3320]							580				585					
[3321]	<210>	69														
[3322]	<211>	726														
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[3324]	<213>	Artificial Sequence														
[3325]	<220>															
[3326]	<223>	Consensus sequence														
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[3328]	Met	Pro	Lys	Lys	Lys	Arg	Lys	Val	Ser	Ser	Gly	Ala	Asn	Phe	Asn	Gln
[3329]	1				5					10					15	
[3330]	Ser	Gly	Asn	Ile	Ala	Asp	Ser	Ser	Leu	Ser	Phe	Thr	Phe	Thr	Asn	Ser
[3331]					20					25					30	
[3332]	Ser	Asn	Gly	Pro	Asn	Leu	Ile	Thr	Thr	Gln	Thr	Asn	Ser	Gln	Ala	Leu
[3333]					35					40				45		
[3334]	Ser	Gln	Pro	Ile	Ala	Ser	Ser	Asn	Val	His	Asp	Asn	Phe	Met	Asn	Asn
[3335]		50						55					60			
[3336]	Glu	Ile	Thr	Ala	Ser	Lys	Ile	Asp	Asp	Gly	Asn	Asn	Ser	Lys	Pro	Leu
[3337]	65						70					75				80
[3338]	Ser	Pro	Gly	Trp	Thr	Asp	Gln	Thr	Ala	Tyr	Asn	Ala	Phe	Gly	Ile	Thr
[3339]						85				90					95	
[3340]	Thr	Gly	Met	Phe	Asn	Thr	Thr	Thr	Met	Asp	Asp	Val	Tyr	Asn	Tyr	Leu
[3341]					100					105					110	
[3342]	Phe	Asp	Asp	Glu	Asp	Thr	Pro	Pro	Asn	Pro	Lys	Lys	Glu	Gly	Gly	Ser
[3343]					115					120					125	
[3344]	Asn	Gln	Thr	Ser	Leu	Tyr	Lys	Lys	Ala	Gly	Ser	Met	Lys	Arg	Asp	His
[3345]		130							135					140		
[3346]	His	Gln	Phe	Gln	Gly	Arg	Leu	Ser	Asn	His	Gly	Thr	Ser	Ser	Ser	Ser
[3347]	145						150					155				160
[3348]	Ser	Ser	Ile	Ser	Lys	Asp	Lys	Met	Met	Met	Val	Lys	Lys	Glu	Glu	Asp
[3349]						165					170					175
[3350]	Gly	Gly	Gly	Asn	Met	Asp	Asp	Glu	Leu	Leu	Ala	Val	Leu	Gly	Tyr	Lys
[3351]						180									190	
[3352]	Val	Arg	Ser	Ser	Glu	Met	Ala	Glu	Val	Ala	Leu	Lys	Leu	Glu	Gln	Leu
[3353]							195								205	

[3354]	Glu Thr Met Met Ser Asn Val Gln Glu Asp Gly Leu Ser His Leu Ala
[3355]	210 215 220
[3356]	Thr Asp Thr Val His Tyr Asn Pro Ser Glu Leu Tyr Ser Trp Leu Asp
[3357]	225 230 235 240
[3358]	Asn Met Leu Ser Glu Leu Asn Pro Pro Pro Leu Pro Ala Ser Ser Asn
[3359]	245 250 255
[3360]	Gly Leu Asp Pro Val Leu Pro Ser Pro Glu Ile Cys Gly Phe Pro Ala
[3361]	260 265 270
[3362]	Ser Asp Tyr Asp Leu Lys Val Ile Pro Gly Asn Ala Ile Tyr Gln Phe
[3363]	275 280 285
[3364]	Pro Ala Ile Asp Ser Ser Ser Ser Ser Asn Asn Gln Asn Lys Arg Leu
[3365]	290 295 300
[3366]	Lys Ser Cys Ser Ser Pro Asp Ser Met Val Thr Ser Thr Ser Thr Gly
[3367]	305 310 315 320
[3368]	Thr Gln Ile Gly Gly Val Ile Gly Thr Thr Val Thr Thr Thr Thr Thr
[3369]	325 330 335
[3370]	Thr Thr Thr Ala Ala Gly Glu Ser Thr Arg Ser Val Ile Leu Val Asp
[3371]	340 345 350
[3372]	Ser Gln Glu Asn Gly Val Arg Leu Val His Ala Leu Met Ala Cys Ala
[3373]	355 360 365
[3374]	Glu Ala Ile Gln Gln Asn Asn Leu Thr Leu Ala Glu Ala Leu Val Lys
[3375]	370 375 380
[3376]	Gln Ile Gly Cys Leu Ala Val Ser Gln Ala Gly Ala Met Arg Lys Val
[3377]	385 390 395 400
[3378]	Ala Thr Tyr Phe Ala Glu Ala Leu Ala Arg Arg Ile Tyr Arg Leu Ser
[3379]	405 410 415
[3380]	Pro Pro Gln Asn Gln Ile Asp His Cys Leu Ser Asp Thr Leu Gln Met
[3381]	420 425 430
[3382]	His Phe Tyr Glu Thr Cys Pro Tyr Leu Lys Phe Ala His Phe Thr Ala
[3383]	435 440 445
[3384]	Asn Gln Ala Ile Leu Glu Ala Phe Glu Gly Lys Lys Arg Val His Val
[3385]	450 455 460
[3386]	Ile Asp Phe Ser Met Asn Gln Gly Leu Gln Trp Pro Ala Leu Met Gln
[3387]	465 470 475 480
[3388]	Ala Leu Ala Leu Arg Glu Gly Gly Pro Pro Thr Phe Arg Leu Thr Gly
[3389]	485 490 495
[3390]	Ile Gly Pro Pro Ala Pro Asp Asn Ser Asp His Leu His Glu Val Gly
[3391]	500 505 510
[3392]	Cys Lys Leu Ala Gln Leu Ala Glu Ala Ile His Val Glu Phe Glu Tyr

[3393]	515	520	525
[3394]	Arg Gly Phe Val Ala Asn Ser Leu Ala Asp Leu Asp Ala Ser Met Leu		
[3395]	530	535	540
[3396]	Glu Leu Arg Pro Ser Asp Thr Glu Ala Val Ala Val Asn Ser Val Phe		
[3397]	545	550	555
[3398]	Glu Leu His Lys Leu Leu Gly Arg Pro Gly Gly Ile Glu Lys Val Leu		
[3399]	565	570	575
[3400]	Gly Val Val Lys Gln Ile Lys Pro Val Ile Phe Thr Val Val Glu Gln		
[3401]	580	585	590
[3402]	Glu Ser Asn His Asn Gly Pro Val Phe Leu Asp Arg Phe Thr Glu Ser		
[3403]	595	600	605
[3404]	Leu His Tyr Tyr Ser Thr Leu Phe Asp Ser Leu Glu Gly Val Pro Asn		
[3405]	610	615	620
[3406]	Ser Gln Asp Lys Val Met Ser Glu Val Tyr Leu Gly Lys Gln Ile Cys		
[3407]	625	630	635
[3408]	Asn Leu Val Ala Cys Glu Gly Pro Asp Arg Val Glu Arg His Glu Thr		
[3409]	645	650	655
[3410]	Leu Ser Gln Trp Gly Asn Arg Phe Gly Ser Ser Gly Leu Ala Pro Ala		
[3411]	660	665	670
[3412]	His Leu Gly Ser Asn Ala Phe Lys Gln Ala Ser Met Leu Leu Ser Val		
[3413]	675	680	685
[3414]	Phe Asn Ser Gly Gln Gly Tyr Arg Val Glu Glu Ser Asn Gly Cys Leu		
[3415]	690	695	700
[3416]	Met Leu Gly Trp His Thr Arg Pro Leu Ile Thr Thr Ser Ala Trp Lys		
[3417]	705	710	715
[3418]	Leu Ser Thr Ala Ala Tyr		720
[3419]	725		
[3420]	<210> 70		
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[3422]	<212> PRT		
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[3425]	<223> Consensus sequence		
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[3427]	Met Lys Leu Leu Ser Ser Ile Glu Glu Ala Cys Asn Ile Cys Arg Leu		
[3428]	1	5	10
[3429]	Lys Lys Leu Lys Cys Ser Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu		
[3430]	20	25	30
[3431]	Lys Asn Asn Trp Glu Cys Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro		

[3432]	35	40	45
[3433]	Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu		
[3434]	50	55	60
[3435]	Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu Asn Leu Asn Met Ile		
[3436]	65	70	75
[3437]	Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu		
[3438]	85	90	95
[3439]	Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala		
[3440]	100	105	110
[3441]	Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser		
[3442]	115	120	125
[3443]	Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu		
[3444]	130	135	140
[3445]	Thr Val Ser Ser Arg Ser Asn Gln Thr Ser Leu Tyr Lys Lys Ala Gly		
[3446]	145	150	155
[3447]	Ser Tyr Glu Pro Gly Asp Asp Ser Gly Ala Gly Ile Phe Arg Lys Ile		
[3448]	165	170	175
[3449]	Leu Val Ser Gln Pro Val Asn Pro Pro Arg Val Thr Thr Thr Leu Ile		
[3450]	180	185	190
[3451]	Glu Pro Ser Asn Glu Leu Glu Ala Cys Gly Arg Val Ser Tyr Pro Glu		
[3452]	195	200	205
[3453]	Asp Asn Gly Ala Cys His Arg Met Asp Ser Pro Arg Ser Ala Glu Phe		
[3454]	210	215	220
[3455]	Ser Gly Gly Ser Gly His Phe Val Ser Glu Lys Asp Gly His Lys Thr		
[3456]	225	230	235
[3457]	Thr Ile Ser Pro Arg Ser Pro Ala Glu Thr Ser Glu Leu Val Gly Gln		
[3458]	245	250	255
[3459]	Met Thr Ile Phe Tyr Ser Gly Lys Val Asn Val Tyr Asp Gly Ile Pro		
[3460]	260	265	270
[3461]	Pro Glu Lys Ala Arg Ser Ile Met His Phe Ala Ala Asn Pro Ile Asp		
[3462]	275	280	285
[3463]	Leu Pro Glu Asn Gly Ile Phe Ala Ser Ser Arg Met Ile Ser Lys Leu		
[3464]	290	295	300
[3465]	Ile Ser Lys Glu Lys Met Met Glu Leu Pro Gln Lys Gly Leu Glu Lys		
[3466]	305	310	315
[3467]	Ala Asn Ser Ser Arg Asp Ser Gly Met Glu Gly Gln Ala Asn Arg Lys		
[3468]	325	330	335
[3469]	Val Ser Leu Gln Arg Tyr Arg Glu Lys Arg Lys Asp Arg Lys Phe Ser		
[3470]	340	345	350

[3471]	Lys	Ala	Lys	Lys	Cys	Pro	Gly	Val	Ala	Ser	Ser	Ser	Leu	Glu	Met	Phe
[3472]				355				360					365			
[3473]	Leu	Asn	Cys	Gln	Pro	Arg	Met	Lys	Ala	Ala	Tyr	Ser	Gln	Asn	Leu	Gly
[3474]			370					375					380			
[3475]	Cys	Thr	Gly	Ser	Pro	Leu	His	Ser	Gln	Ser	Pro	Glu	Ser	Gln	Thr	Lys
[3476]	385					390					395					400
[3477]	Ser	Pro	Asn	Leu	Ser	Val	Asp	Leu	Asn	Ser	Glu	Gly	Ile			
[3478]				405						410						
[3479]	<210>	71														
[3480]	<211>	449														
[3481]	<212>	PRT														
[3482]	<213>	Artificial Sequence														
[3483]	<220>															
[3484]	<223>	Consensus sequence														
[3485]	<400>	71														
[3486]	Met	Lys	Leu	Leu	Ser	Ser	Ile	Glu	Glu	Ala	Cys	Asn	Ile	Cys	Arg	Leu
[3487]	1			5						10				15		
[3488]	Lys	Lys	Leu	Lys	Cys	Ser	Lys	Glu	Lys	Pro	Lys	Cys	Ala	Lys	Cys	Leu
[3489]				20					25					30		
[3490]	Lys	Asn	Asn	Trp	Glu	Cys	Arg	Tyr	Ser	Pro	Lys	Thr	Lys	Arg	Ser	Pro
[3491]			35					40					45			
[3492]	Leu	Thr	Arg	Ala	His	Leu	Thr	Glu	Val	Glu	Ser	Arg	Leu	Glu	Arg	Leu
[3493]		50					55					60				
[3494]	Glu	Gln	Leu	Phe	Leu	Leu	Ile	Phe	Pro	Arg	Glu	Asn	Leu	Asn	Met	Ile
[3495]	65					70					75				80	
[3496]	Leu	Lys	Met	Asp	Ser	Leu	Gln	Asp	Ile	Lys	Ala	Leu	Leu	Thr	Gly	Leu
[3497]				85						90					95	
[3498]	Phe	Val	Gln	Asp	Asn	Val	Asn	Lys	Asp	Ala	Val	Thr	Asp	Arg	Leu	Ala
[3499]				100						105					110	
[3500]	Ser	Val	Glu	Thr	Asp	Met	Pro	Leu	Thr	Leu	Arg	Gln	His	Arg	Ile	Ser
[3501]				115						120					125	
[3502]	Ala	Thr	Ser	Ser	Ser	Glu	Glu	Ser	Ser	Asn	Lys	Gly	Gln	Arg	Gln	Leu
[3503]			130							135					140	
[3504]	Thr	Val	Ser	Ser	Arg	Ser	Asn	Gln	Thr	Ser	Leu	Tyr	Lys	Lys	Ala	Gly
[3505]	145					150					155					160
[3506]	Ser	Met	Asp	Val	Gly	Val	Ser	Pro	Ala	Lys	Ser	Ile	Leu	Ala	Lys	Pro
[3507]					165						170					175
[3508]	Leu	Lys	Leu	Leu	Thr	Glu	Glu	Asp	Ile	Ser	Gln	Leu	Thr	Arg	Glu	Asp
[3509]				180							185					190

[3510]	Cys Arg Lys Phe Leu Lys Asp Lys Gly Met Arg Arg Pro Ser Trp Asn
[3511]	195 200 205
[3512]	Lys Ser Gln Ala Ile Gln Gln Val Leu Ser Leu Lys Ala Leu Tyr Glu
[3513]	210 215 220
[3514]	Pro Gly Asp Asp Ser Gly Ala Gly Ile Phe Arg Lys Ile Leu Val Ser
[3515]	225 230 235 240
[3516]	Gln Pro Val Asn Pro Pro Arg Val Thr Thr Thr Leu Ile Glu Pro Ser
[3517]	245 250 255
[3518]	Asn Glu Leu Glu Ala Cys Gly Arg Val Ser Tyr Pro Glu Asp Asn Gly
[3519]	260 265 270
[3520]	Ala Cys His Arg Met Asp Ser Pro Arg Ser Ala Glu Phe Ser Gly Gly
[3521]	275 280 285
[3522]	Ser Gly His Phe Val Ser Glu Lys Asp Gly His Lys Thr Thr Ile Ser
[3523]	290 295 300
[3524]	Pro Arg Ser Pro Ala Glu Thr Ser Glu Leu Ile Met His Phe Ala Ala
[3525]	305 310 315 320
[3526]	Asn Pro Ile Asp Leu Pro Glu Asn Gly Ile Phe Ala Ser Ser Arg Met
[3527]	325 330 335
[3528]	Ile Ser Lys Leu Ile Ser Lys Glu Lys Met Met Glu Leu Pro Gln Lys
[3529]	340 345 350
[3530]	Gly Leu Glu Lys Ala Asn Ser Ser Arg Asp Ser Gly Met Glu Gly Gln
[3531]	355 360 365
[3532]	Ala Asn Arg Lys Val Ser Leu Gln Arg Tyr Arg Glu Lys Arg Lys Asp
[3533]	370 375 380
[3534]	Arg Lys Phe Ser Lys Ala Lys Lys Cys Pro Gly Val Ala Ser Ser Ser
[3535]	385 390 395 400
[3536]	Leu Glu Met Phe Leu Asn Cys Gln Pro Arg Met Lys Ala Ala Tyr Ser
[3537]	405 410 415
[3538]	Gln Asn Leu Gly Cys Thr Gly Ser Pro Leu His Ser Gln Ser Pro Glu
[3539]	420 425 430
[3540]	Ser Gln Thr Lys Ser Pro Asn Leu Ser Val Asp Leu Asn Ser Glu Gly
[3541]	435 440 445
[3542]	Ile
[3543]	<210> 72
[3544]	<211> 390
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[3546]	<213> Artificial Sequence
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[3549]	<400> 72
[3550]	Met Lys Leu Leu Ser Ser Ile Glu Glu Ala Cys Asn Ile Cys Arg Leu
[3551]	1 5 10 15
[3552]	Lys Lys Leu Lys Cys Ser Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu
[3553]	20 25 30
[3554]	Lys Asn Asn Trp Glu Cys Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro
[3555]	35 40 45
[3556]	Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu
[3557]	50 55 60
[3558]	Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu Asn Leu Asn Met Ile
[3559]	65 70 75 80
[3560]	Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu
[3561]	85 90 95
[3562]	Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala
[3563]	100 105 110
[3564]	Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser
[3565]	115 120 125
[3566]	Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu
[3567]	130 135 140
[3568]	Thr Val Ser Ser Arg Ser Asn Gln Thr Ser Leu Tyr Lys Lys Ala Gly
[3569]	145 150 155 160
[3570]	Ser Met Asp Val Gly Val Ser Pro Ala Lys Ser Ile Leu Ala Lys Pro
[3571]	165 170 175
[3572]	Leu Lys Leu Leu Thr Glu Glu Asp Ile Ser Gln Leu Thr Arg Glu Asp
[3573]	180 185 190
[3574]	Cys Arg Lys Phe Leu Lys Asp Lys Gly Met Arg Arg Pro Ser Trp Asn
[3575]	195 200 205
[3576]	Lys Ser Gln Ala Ile Gln Gln Val Leu Ser Leu Lys Ala Leu Tyr Glu
[3577]	210 215 220
[3578]	Pro Gly Asp Asp Ser Gly Ala Gly Ile Phe Arg Lys Ile Leu Val Ser
[3579]	225 230 235 240
[3580]	Gln Pro Val Asn Pro Pro Arg Val Thr Thr Thr Leu Ile Glu Pro Ser
[3581]	245 250 255
[3582]	Asn Glu Leu Glu Ala Cys Gly Arg Val Ser Tyr Pro Glu Asp Asn Gly
[3583]	260 265 270
[3584]	Ala Cys His Arg Met Asp Ser Pro Arg Ser Ala Glu Phe Ser Gly Gly
[3585]	275 280 285
[3586]	Ser Gly His Phe Val Ser Glu Lys Asp Gly His Lys Thr Thr Ile Ser
[3587]	290 295 300

[3588]	Pro Arg Ser Pro Ala Glu Thr Ser Glu Leu Val Gly Gln Met Thr Ile
[3589]	305 310 315 320
[3590]	Phe Tyr Ser Gly Lys Val Asn Val Tyr Asp Gly Ile Pro Pro Glu Lys
[3591]	325 330 335
[3592]	Ala Arg Ser Ile Met His Phe Ala Ala Asn Pro Ile Asp Leu Pro Glu
[3593]	340 345 350
[3594]	Asn Gly Ile Phe Ala Ser Ser Arg Met Ile Ser Lys Leu Ile Ser Lys
[3595]	355 360 365
[3596]	Glu Lys Met Met Glu Leu Pro Gln Lys Gly Leu Glu Lys Ala Asn Ser
[3597]	370 375 380
[3598]	Ser Arg Asp Ser Gly Met
[3599]	385 390
[3600]	<210> 73
[3601]	<211> 142
[3602]	<212> PRT
[3603]	<213> Artificial Sequence
[3604]	<220>
[3605]	<223> Consensus sequence
[3606]	<400> 73
[3607]	Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
[3608]	1 5 10 15
[3609]	Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
[3610]	20 25 30
[3611]	Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Asp Asp Gly Asn
[3612]	35 40 45
[3613]	Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn
[3614]	50 55 60
[3615]	Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu
[3616]	65 70 75 80
[3617]	Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr
[3618]	85 90 95
[3619]	Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His
[3620]	100 105 110
[3621]	Asn Ile Glu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Gly Pro Arg
[3622]	115 120 125
[3623]	Pro Thr Ser Gly Ser Val Asp Leu Glu Gly Thr Ala Pro Gly
[3624]	130 135 140
[3625]	<210> 74
[3626]	<211> 111

[3627]	<212>	PRT
[3628]	<213>	Artificial Sequence
[3629]	<220>	
[3630]	<223>	Consensus sequence
[3631]	<400>	74
[3632]	Met Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His	
[3633]	1 5 10 15	
[3634]	Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Asp His Tyr Gln Gln Asn	
[3635]	20 25 30	
[3636]	Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu	
[3637]	35 40 45	
[3638]	Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His	
[3639]	50 55 60	
[3640]	Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met	
[3641]	65 70 75 80	
[3642]	Asp Glu Leu Tyr Lys Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Pro	
[3643]	85 90 95	
[3644]	Arg Pro Thr Ser Gly Ser Val Asp Leu Glu Gly Thr Ala Pro Gly	
[3645]	100 105 110	
[3646]	<210>	75
[3647]	<211>	568
[3648]	<212>	PRT
[3649]	<213>	Artificial Sequence
[3650]	<220>	
[3651]	<223>	Consensus sequence
[3652]	<400>	75
[3653]	Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu	
[3654]	1 5 10 15	
[3655]	Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly	
[3656]	20 25 30	
[3657]	Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Asp Asp Gly Asn	
[3658]	35 40 45	
[3659]	Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn	
[3660]	50 55 60	
[3661]	Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu	
[3662]	65 70 75 80	
[3663]	Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Thr	
[3664]	85 90 95	
[3665]	Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His	

[3666]	100	105	110
[3667]	Asn Ile Glu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Gly Pro Arg		
[3668]	115	120	125
[3669]	Pro Thr Ser Gly Ser Val Asp Leu Glu Gly Thr Ala Pro Gly Ser Met		
[3670]	130	135	140
[3671]	Asp Asp Asp Asn Gly Leu Glu Leu Ser Leu Gly Leu Ser Cys Gly Gly		
[3672]	145	150	155
[3673]	Ser Thr Gly Lys Ala Lys Gly Asn Asn Asn Asn Asn Ala Gly Ser Ser		
[3674]	165	170	175
[3675]	Ser Glu Asn Tyr Arg Ala Glu Gly Gly Asp Arg Ser Ala Lys Val Ile		
[3676]	180	185	190
[3677]	Asp Asp Phe Lys Asn Phe Leu His Pro Thr Ser Gln Arg Pro Ala Glu		
[3678]	195	200	205
[3679]	Pro Ser Ser Gly Ser Gln Arg Ser Asp Ser Gly Gln Gln Pro Pro Gln		
[3680]	210	215	220
[3681]	Asn Phe Phe Asn Asp Leu Ser Lys Ala Pro Thr Thr Glu Ala Glu Ala		
[3682]	225	230	235
[3683]	Ser Thr Lys Pro Leu Trp Val Glu Asp Glu Ser Arg Lys Glu Ala Gly		
[3684]	245	250	255
[3685]	Asn Lys Arg Lys Phe Gly Phe Pro Gly Met Asn Asp Asp Lys Lys Lys		
[3686]	260	265	270
[3687]	Glu Lys Asp Ser Ser His Val Asp Met His Glu Lys Lys Thr Lys Ala		
[3688]	275	280	285
[3689]	Ser His Val Ser Thr Ala Thr Asp Glu Gly Ser Thr Ala Glu Asn Glu		
[3690]	290	295	300
[3691]	Asp Val Ala Glu Ser Glu Val Gly Gly Gly Ser Ser Ser Asn His Ala		
[3692]	305	310	315
[3693]	Lys Glu Val Val Arg Pro Pro Thr Asp Thr Asn Ile Val Asp Asn Leu		
[3694]	325	330	335
[3695]	Thr Gly Gln Arg Arg Ser Asn His Gly Gly Ser Gly Thr Glu Glu Phe		
[3696]	340	345	350
[3697]	Thr Met Arg Asn Met Ser Tyr Thr Val Pro Phe Thr Val His Pro Gln		
[3698]	355	360	365
[3699]	Asn Val Val Thr Ser Met Pro Tyr Ser Leu Pro Thr Lys Glu Ser Gly		
[3700]	370	375	380
[3701]	Gln His Ala Ala Ala Thr Ser Leu Leu Gln Pro Asn Ala Asn Ala Gly		
[3702]	385	390	395
[3703]	Asn Leu Pro Ile Met Phe Gly Tyr Ser Pro Val Gln Leu Pro Met Leu		
[3704]	405	410	415

[3705]	Asp	Lys	Asp	Gly	Ser	Gly	Gly	Ile	Val	Ala	Leu	Ser	Gln	Ser	Pro	Phe
[3706]				420				425					430			
[3707]	Ala	Gly	Arg	Val	Pro	Ser	Asn	Ser	Ala	Thr	Ala	Lys	Gly	Glu	Gly	Lys
[3708]				435				440					445			
[3709]	Gln	Pro	Val	Ala	Glu	Glu	Gly	Ser	Ser	Glu	Asp	Ala	Ser	Glu	Arg	Pro
[3710]				450				455					460			
[3711]	Thr	Gly	Asp	Asn	Ser	Asn	Leu	Asn	Thr	Ala	Phe	Ser	Phe	Asp	Phe	Ser
[3712]	465						470				475					480
[3713]	Ala	Ile	Lys	Pro	Gly	Met	Ala	Ala	Asp	Val	Lys	Phe	Gly	Gly	Ser	Gly
[3714]					485					490					495	
[3715]	Ala	Arg	Pro	Asn	Leu	Pro	Trp	Val	Ser	Thr	Thr	Gly	Ser	Gly	Pro	His
[3716]				500					505					510		
[3717]	Gly	Arg	Thr	Ile	Ser	Gly	Val	Thr	Tyr	Arg	Tyr	Asn	Ala	Asn	Gln	Ile
[3718]				515					520					525		
[3719]	Lys	Ile	Val	Cys	Ala	Cys	His	Gly	Ser	His	Met	Ser	Pro	Glu	Glu	Phe
[3720]				530				535					540			
[3721]	Val	Arg	His	Ala	Ser	Glu	Glu	Tyr	Val	Ser	Pro	Glu	Ser	Ser	Met	Gly
[3722]	545					550					555					560
[3723]	Met	Thr	Ala	Ala	Ser	Ala	His	Thr								
[3724]				565												
[3725]	<210> 76															
[3726]	<211> 479															
[3727]	<212> PRT															
[3728]	<213> Artificial Sequence															
[3729]	<220>															
[3730]	<223> Consensus sequence															
[3731]	<400> 76															
[3732]	Met	Val	Ser	Lys	Gly	Glu	Glu	Leu	Phe	Thr	Gly	Val	Val	Pro	Ile	Leu
[3733]	1			5					10					15		
[3734]	Val	Glu	Leu	Asp	Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly
[3735]				20					25					30		
[3736]	Glu	Gly	Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Asp	Asp	Gly	Asn
[3737]				35					40					45		
[3738]	Tyr	Lys	Thr	Arg	Ala	Glu	Val	Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn
[3739]				50				55					60			
[3740]	Arg	Ile	Glu	Leu	Lys	Gly	Ile	Asp	Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu
[3741]	65					70					75					80
[3742]	Gly	His	Lys	Leu	Glu	Tyr	Asn	Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile	Thr
[3743]				85						90						95

[3744]	Ala Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His
[3745]	100 105 110
[3746]	Asn Ile Glu Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Gly Pro Arg
[3747]	115 120 125
[3748]	Pro Thr Ser Gly Ser Val Asp Leu Glu Gly Thr Ala Pro Gly Ser Met
[3749]	130 135 140
[3750]	Thr Ser Asp Gly Ala Thr Ser Thr Ser Ala Ala Ala Ala Ala Ala
[3751]	145 150 155 160
[3752]	Ala Ala Ala Ala Arg Arg Lys Pro Ser Trp Arg Glu Arg Glu Asn Asn
[3753]	165 170 175
[3754]	Arg Arg Arg Glu Arg Arg Arg Arg Ala Val Ala Ala Lys Ile Tyr Thr
[3755]	180 185 190
[3756]	Gly Leu Arg Ala Gln Gly Asp Tyr Asn Leu Pro Lys His Cys Asp Asn
[3757]	195 200 205
[3758]	Asn Glu Val Leu Lys Ala Leu Cys Val Glu Ala Gly Trp Val Val Glu
[3759]	210 215 220
[3760]	Glu Asp Gly Thr Thr Tyr Arg Lys Gly Cys Lys Pro Leu Pro Gly Glu
[3761]	225 230 235 240
[3762]	Ile Ala Gly Thr Ser Ser Arg Val Thr Pro Tyr Ser Ser Gln Asn Gln
[3763]	245 250 255
[3764]	Ser Pro Leu Ser Ser Ala Phe Gln Ser Pro Ile Pro Ser Tyr Gln Val
[3765]	260 265 270
[3766]	Ser Pro Ser Ser Ser Ser Phe Pro Ser Pro Ser Arg Gly Glu Pro Asn
[3767]	275 280 285
[3768]	Asn Asn Met Ser Ser Thr Phe Phe Pro Phe Leu Arg Asn Gly Gly Ile
[3769]	290 295 300
[3770]	Pro Ser Ser Leu Pro Ser Leu Arg Ile Ser Asn Ser Cys Pro Val Thr
[3771]	305 310 315 320
[3772]	Pro Pro Val Ser Ser Pro Thr Ser Lys Asn Pro Lys Pro Leu Pro Asn
[3773]	325 330 335
[3774]	Trp Glu Ser Ile Ala Lys Gln Ser Met Ala Ile Ala Lys Gln Ser Met
[3775]	340 345 350
[3776]	Ala Ser Phe Asn Tyr Pro Phe Tyr Ala Val Ser Ala Pro Ala Ser Pro
[3777]	355 360 365
[3778]	Thr His Arg His Gln Phe His Thr Pro Ala Thr Ile Pro Glu Cys Asp
[3779]	370 375 380
[3780]	Glu Ser Asp Ser Ser Thr Val Asp Ser Gly His Trp Ile Ser Phe Gln
[3781]	385 390 395 400
[3782]	Lys Phe Ala Gln Gln Gln Pro Phe Ser Ala Ser Met Val Pro Thr Ser

[3783]		405		410		415
[3784]	Pro Thr Phe Asn Leu Val Lys Pro Ala Pro Gln Gln Met Ser Pro Asn					
[3785]		420		425		430
[3786]	Thr Ala Ala Phe Gln Glu Ile Gly Gln Ser Ser Glu Phe Lys Phe Glu					
[3787]		435		440		445
[3788]	Asn Ser Gln Val Lys Pro Trp Glu Gly Glu Arg Ile His Asp Val Gly					
[3789]		450		455		460
[3790]	Met Glu Asp Leu Glu Leu Thr Leu Gly Asn Gly Lys Ala Arg Gly					
[3791]	465		470		475	
[3792]	<210> 77					
[3793]	<211> 425					
[3794]	<212> PRT					
[3795]	<213> Artificial Sequence					
[3796]	<220>					
[3797]	<223> Consensus sequence					
[3798]	<400> 77					
[3799]	Met Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His					
[3800]	1	5		10		15
[3801]	Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Asp His Tyr Gln Gln Asn					
[3802]		20		25		30
[3803]	Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu					
[3804]		35		40		45
[3805]	Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His					
[3806]		50		55		60
[3807]	Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met					
[3808]	65		70		75	80
[3809]	Asp Glu Leu Tyr Lys Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Pro					
[3810]		85		90		95
[3811]	Arg Pro Thr Ser Gly Ser Val Asp Leu Glu Gly Thr Ala Pro Gly Ser					
[3812]		100		105		110
[3813]	Met Asp Val Gly Val Ser Pro Ala Lys Ser Ile Leu Ala Lys Pro Leu					
[3814]		115		120		125
[3815]	Lys Leu Leu Thr Glu Glu Asp Ile Ser Gln Leu Thr Arg Glu Asp Cys					
[3816]		130		135		140
[3817]	Arg Lys Phe Leu Lys Asp Lys Gly Met Arg Arg Pro Ser Trp Asn Lys					
[3818]	145		150		155	160
[3819]	Ser Gln Ala Ile Gln Gln Val Leu Ser Leu Lys Ala Leu Tyr Glu Pro					
[3820]		165		170		175
[3821]	Gly Asp Asp Ser Gly Ala Gly Ile Phe Arg Lys Ile Leu Val Ser Gln					

[3822]	180	185	190
[3823]	Pro Val Asn Pro Pro Arg Val Thr Thr Thr Leu Ile Glu Pro Ser Asn		
[3824]	195	200	205
[3825]	Glu Leu Glu Ala Cys Gly Arg Val Ser Tyr Pro Glu Asp Asn Gly Ala		
[3826]	210	215	220
[3827]	Cys His Arg Met Asp Ser Pro Arg Ser Ala Glu Phe Ser Gly Gly Ser		
[3828]	225	230	235
[3829]	Gly His Phe Val Ser Glu Lys Asp Gly His Lys Thr Thr Ile Ser Pro		
[3830]	245	250	255
[3831]	Arg Ser Pro Ala Glu Thr Ser Glu Leu Val Gly Gln Met Thr Ile Phe		
[3832]	260	265	270
[3833]	Tyr Ser Gly Lys Val Asn Val Tyr Asp Gly Ile Pro Pro Glu Lys Ala		
[3834]	275	280	285
[3835]	Arg Ser Ile Met His Phe Ala Ala Asn Pro Ile Asp Leu Pro Glu Asn		
[3836]	290	295	300
[3837]	Gly Ile Phe Ala Ser Ser Arg Met Ile Ser Lys Leu Ile Ser Lys Glu		
[3838]	305	310	315
[3839]	Lys Met Met Glu Leu Pro Gln Lys Gly Leu Glu Lys Ala Asn Ser Ser		
[3840]	325	330	335
[3841]	Arg Asp Ser Gly Met Glu Gly Gln Ala Asn Arg Lys Val Ser Leu Gln		
[3842]	340	345	350
[3843]	Arg Tyr Arg Glu Lys Arg Lys Asp Arg Lys Phe Ser Lys Ala Lys Lys		
[3844]	355	360	365
[3845]	Cys Pro Gly Val Ala Ser Ser Ser Leu Glu Met Phe Leu Asn Cys Gln		
[3846]	370	375	380
[3847]	Pro Arg Met Lys Ala Ala Tyr Ser Gln Asn Leu Gly Cys Thr Gly Ser		
[3848]	385	390	395
[3849]	Pro Leu His Ser Gln Ser Pro Glu Ser Gln Thr Lys Ser Pro Asn Leu		
[3850]	405	410	415
[3851]	Ser Val Asp Leu Asn Ser Glu Gly Ile		
[3852]	420	425	
[3853]	<210> 78		
[3854]	<211> 537		
[3855]	<212> PRT		
[3856]	<213> Artificial Sequence		
[3857]	<220>		
[3858]	<223> Consensus sequence		
[3859]	<400> 78		
[3860]	Met Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His		

[3861]	1	5	10	15
[3862]	Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Asp His Tyr Gln Gln Asn			
[3863]	20	25	30	
[3864]	Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu			
[3865]	35	40	45	
[3866]	Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His			
[3867]	50	55	60	
[3868]	Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met			
[3869]	65	70	75	80
[3870]	Asp Glu Leu Tyr Lys Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Pro			
[3871]	85	90	95	
[3872]	Arg Pro Thr Ser Gly Ser Val Asp Leu Glu Gly Thr Ala Pro Gly Ser			
[3873]	100	105	110	
[3874]	Met Asp Asp Asp Asn Gly Leu Glu Leu Ser Leu Gly Leu Ser Cys Gly			
[3875]	115	120	125	
[3876]	Gly Ser Thr Gly Lys Ala Lys Gly Asn Asn Asn Asn Asn Ala Gly Ser			
[3877]	130	135	140	
[3878]	Ser Ser Glu Asn Tyr Arg Ala Glu Gly Gly Asp Arg Ser Ala Lys Val			
[3879]	145	150	155	160
[3880]	Ile Asp Asp Phe Lys Asn Phe Leu His Pro Thr Ser Gln Arg Pro Ala			
[3881]	165	170	175	
[3882]	Glu Pro Ser Ser Gly Ser Gln Arg Ser Asp Ser Gly Gln Gln Pro Pro			
[3883]	180	185	190	
[3884]	Gln Asn Phe Phe Asn Asp Leu Ser Lys Ala Pro Thr Thr Glu Ala Glu			
[3885]	195	200	205	
[3886]	Ala Ser Thr Lys Pro Leu Trp Val Glu Asp Glu Ser Arg Lys Glu Ala			
[3887]	210	215	220	
[3888]	Gly Asn Lys Arg Lys Phe Gly Phe Pro Gly Met Asn Asp Asp Lys Lys			
[3889]	225	230	235	240
[3890]	Lys Glu Lys Asp Ser Ser His Val Asp Met His Glu Lys Lys Thr Lys			
[3891]	245	250	255	
[3892]	Ala Ser His Val Ser Thr Ala Thr Asp Glu Gly Ser Thr Ala Glu Asn			
[3893]	260	265	270	
[3894]	Glu Asp Val Ala Glu Ser Glu Val Gly Gly Gly Ser Ser Ser Asn His			
[3895]	275	280	285	
[3896]	Ala Lys Glu Val Val Arg Pro Pro Thr Asp Thr Asn Ile Val Asp Asn			
[3897]	290	295	300	
[3898]	Leu Thr Gly Gln Arg Arg Ser Asn His Gly Gly Ser Gly Thr Glu Glu			
[3899]	305	310	315	320

[3900]	Phe Thr Met Arg Asn Met Ser Tyr Thr Val Pro Phe Thr Val His Pro
[3901]	325 330 335
[3902]	Gln Asn Val Val Thr Ser Met Pro Tyr Ser Leu Pro Thr Lys Glu Ser
[3903]	340 345 350
[3904]	Gly Gln His Ala Ala Ala Thr Ser Leu Leu Gln Pro Asn Ala Asn Ala
[3905]	355 360 365
[3906]	Gly Asn Leu Pro Ile Met Phe Gly Tyr Ser Pro Val Gln Leu Pro Met
[3907]	370 375 380
[3908]	Leu Asp Lys Asp Gly Ser Gly Gly Ile Val Ala Leu Ser Gln Ser Pro
[3909]	385 390 395 400
[3910]	Phe Ala Gly Arg Val Pro Ser Asn Ser Ala Thr Ala Lys Gly Glu Gly
[3911]	405 410 415
[3912]	Lys Gln Pro Val Ala Glu Glu Gly Ser Ser Glu Asp Ala Ser Glu Arg
[3913]	420 425 430
[3914]	Pro Thr Gly Asp Asn Ser Asn Leu Asn Thr Ala Phe Ser Phe Asp Phe
[3915]	435 440 445
[3916]	Ser Ala Ile Lys Pro Gly Met Ala Ala Asp Val Lys Phe Gly Gly Ser
[3917]	450 455 460
[3918]	Gly Ala Arg Pro Asn Leu Pro Trp Val Ser Thr Thr Gly Ser Gly Pro
[3919]	465 470 475 480
[3920]	His Gly Arg Thr Ile Ser Gly Val Thr Tyr Arg Tyr Asn Ala Asn Gln
[3921]	485 490 495
[3922]	Ile Lys Ile Val Cys Ala Cys His Gly Ser His Met Ser Pro Glu Glu
[3923]	500 505 510
[3924]	Phe Val Arg His Ala Ser Glu Glu Tyr Val Ser Pro Glu Ser Ser Met
[3925]	515 520 525
[3926]	Gly Met Thr Ala Ala Ser Ala His Thr
[3927]	530 535
[3928]	<210> 79
[3929]	<211> 448
[3930]	<212> PRT
[3931]	<213> Artificial Sequence
[3932]	<220>
[3933]	<223> Consensus sequence
[3934]	<400> 79
[3935]	Met Asp Lys Gln Lys Asn Gly Ile Lys Ala Asn Phe Lys Ile Arg His
[3936]	1 5 10 15
[3937]	Asn Ile Glu Asp Gly Gly Val Gln Leu Ala Asp His Tyr Gln Gln Asn
[3938]	20 25 30

[3939]	Thr	Pro	Ile	Gly	Asp	Gly	Pro	Val	Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu
[3940]			35					40					45			
[3941]	Ser	Tyr	Gln	Ser	Ala	Leu	Ser	Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp	His
[3942]			50					55				60				
[3943]	Met	Val	Leu	Leu	Glu	Phe	Val	Thr	Ala	Ala	Gly	Ile	Thr	Leu	Gly	Met
[3944]	65					70					75				80	
[3945]	Asp	Glu	Leu	Tyr	Lys	Tyr	Pro	Tyr	Asp	Val	Pro	Asp	Tyr	Ala	Gly	Pro
[3946]					85					90					95	
[3947]	Arg	Pro	Thr	Ser	Gly	Ser	Val	Asp	Leu	Glu	Gly	Thr	Ala	Pro	Gly	Ser
[3948]				100					105					110		
[3949]	Met	Thr	Ser	Asp	Gly	Ala	Thr	Ser	Thr	Ser	Ala	Ala	Ala	Ala	Ala	Ala
[3950]				115					120					125		
[3951]	Ala	Ala	Ala	Ala	Ala	Arg	Arg	Lys	Pro	Ser	Trp	Arg	Glu	Arg	Glu	Asn
[3952]			130					135				140				
[3953]	Asn	Arg	Arg	Arg	Glu	Arg	Arg	Arg	Arg	Ala	Val	Ala	Ala	Lys	Ile	Tyr
[3954]	145					150					155					160
[3955]	Thr	Gly	Leu	Arg	Ala	Gln	Gly	Asp	Tyr	Asn	Leu	Pro	Lys	His	Cys	Asp
[3956]					165					170					175	
[3957]	Asn	Asn	Glu	Val	Leu	Lys	Ala	Leu	Cys	Val	Glu	Ala	Gly	Trp	Val	Val
[3958]				180						185					190	
[3959]	Glu	Glu	Asp	Gly	Thr	Thr	Tyr	Arg	Lys	Gly	Cys	Lys	Pro	Leu	Pro	Gly
[3960]			195					200					205			
[3961]	Glu	Ile	Ala	Gly	Thr	Ser	Ser	Arg	Val	Thr	Pro	Tyr	Ser	Ser	Gln	Asn
[3962]			210					215					220			
[3963]	Gln	Ser	Pro	Leu	Ser	Ser	Ala	Phe	Gln	Ser	Pro	Ile	Pro	Ser	Tyr	Gln
[3964]	225					230					235					240
[3965]	Val	Ser	Pro	Ser	Ser	Ser	Ser	Phe	Pro	Ser	Pro	Ser	Arg	Gly	Glu	Pro
[3966]					245					250					255	
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[4008]	Asp Glu Leu Tyr Lys Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Pro		
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[4010]	Arg Pro Thr Ser Gly Ser Val Asp Leu Glu Gly Thr Ala Pro Gly Ser		
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[4030]	Ser Lys Glu Lys Met Met Glu Leu Pro Gln Lys Gly Leu Glu Lys Ala			
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[4032]	Asn Ser Ser Arg Asp Ser Gly Met Glu Gly Gln Ala Asn Arg Lys Val			
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[4034]	Ser Leu Gln Arg Tyr Arg Glu Lys Arg Lys Asp Arg Lys Phe Ser Lys			
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[4067]	Lys Leu Leu Thr Glu Glu Asp Ile Ser Gln Leu Thr Arg Glu Asp Cys		
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[4069]	Arg Lys Phe Leu Lys Asp Lys Gly Met Arg Arg Pro Ser Trp Asn Lys		
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[4073]	Gly Asp Asp Ser Gly Ala Gly Ile Phe Arg Lys Ile Leu Val Ser Gln		
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[4075]	Pro Val Asn Pro Pro Arg Val Thr Thr Thr Leu Ile Glu Pro Ser Asn		
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[4079]	Cys His Arg Met Asp Ser Pro Arg Ser Ala Glu Phe Ser Gly Gly Ser		
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[4081]	Gly His Phe Val Ser Glu Lys Asp Gly His Lys Thr Thr Ile Ser Pro		
[4082]	245	250	255
[4083]	Arg Ser Pro Ala Glu Thr Ser Glu Leu Ile Met His Phe Ala Ala Asn		
[4084]	260	265	270
[4085]	Pro Ile Asp Leu Pro Glu Asn Gly Ile Phe Ala Ser Ser Arg Met Ile		
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[4087]	Ser Lys Leu Ile Ser Lys Glu Lys Met Met Glu Leu Pro Gln Lys Gly		
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[4089]	Leu Glu Lys Ala Asn Ser Ser Arg Asp Ser Gly Met Glu Gly Gln Ala		
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[4091]	Asn Arg Lys Val Ser Leu Gln Arg Tyr Arg Glu Lys Arg Lys Asp Arg		
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[4093]	Lys Phe Ser Lys Ala Lys Lys Cys Pro Gly Val Ala Ser Ser Ser Leu		
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[4095]	Glu Met Phe Leu Asn Cys Gln Pro Arg Met Lys Ala Ala Tyr Ser Gln
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[4114]	Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His
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[4143]	275	280 285
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[4146]	Gly Ile Phe Ala Ser Ser Arg Met Ile Ser Lys Leu Ile Ser Lys Glu	
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挪威云杉	LEDLSHEDIMQLTREDCRRYLIEKGMRRPSWNKSQAIQQVLSLKKL
北美云杉	LEELTEEDIFQLTREDCRRYLKEKGMRRPSWNKSQAIQQVLSLKSL
紫萍	LSELTEEDIAQVLTREDCRRFLKEKGMRRPSWNKSQAVQQVISLKAL
江南卷柏 PPD1	LEELTELDIRQLTREDCRRYLKEKGMRRPSWNKAQAIQQVLSLRSL
江南卷柏 PPD2	LEELTEEDVMQLTREDCRRYLKEKGMRRPSWNKAQAVQQLSLKSL
芭蕉属物种	LAELTEEDIAQLTREDCRRFLKAKGMRRPSWNKSQAIQQVISLKAL
海棠	LSLLTEEDIAQLTREDCRRFLKEKGMRRPSWNKSQAIQQVISLKAL
白蝴蝶兰	LNLLTEDDIAQITREDCRRFLKDRGMRRPSWNKSQAIQQVISLKAL
烟草	LHLLTDDDISQLTREDCRRYLKEKGMRRPSWNKSQAIQQVISLKAL
番茄	LYLLTDDDISQLTREDCRRFLKAKGMRRPSWNKSQAIQQVISLKAL
马铃薯	LNQLTDDDISQLTREDCRRYLKQKGMRRPSWNKSQAIQQVISLKAL
洛矶山楼斗菜	LFQLTDEDISQLTREDCRKFLRDKGMRRPSWNKSQAIQQVISLKT
毛果杨	LHQLTEDDISQVLTREDCRRYLKEKGMRRPSWNKSQAIQQVISLKT
大豆	LNQLTEDDISQLTREDCRRFLKEKGMRRPSWNKSQAIQQVISLKAL
蕨类状苜蓿	LWQLTEDDISQLTREDCRRFLKDKGMRRPSWNKSQAIQQVISLKAL
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蓖麻	LTQLTEEDISQLTREDCRKYLKEKGMRRPSWNKSQAIQQVISLKAL
葡萄	LHELTEEDISQLTREDCRKYLKEKGMRRPSWNKSQAIQQVISLKSL
相同的氨基酸	* * * * *

图1

无油樟	TREDCRRYLKEKGMRRPSWNKYQAIQQ
挪威云杉	TREDCRRYLKEKGMRRPSWNKSQAIQQ
北美云杉	TREDCRRYLKEKGMRRPSWNKSQAIQQ
紫萍	TREDCRRFLKEKGMRRPSWNKSQAVQQ
江南卷柏 PPD1	TREDCRRYLKERGMRRPSWNKAQAIQQ
江南卷柏 PPD2	TREDCRRYLKEKGMRRPSWNKAQAVQQ
芭蕉属物种	TREDCRRFLKAKGMRRPSWNKSQAIQQ
海枣	TREDCRRFLKEKGMRRPSWNKSQAIQQ
白蝴蝶兰	TREDCRRFLKDRGMRRPSWNKSQAIQQ
烟草	TREDCRRYLKEKGMRRPSWNKSQAIQQ
番茄	TREDCRRFLKAKGMRKPSWNKSQAIQQ
马铃薯	TREDCRRYLKQKGMRKPSWNKSQAIQQ
洛矶山楼斗菜	TREDCRKFLRDKGMRRPSWNKSQAIEQ
毛果杨	TREDCRRYLKEKGMRRPSWNKSQAIQQ
大豆	TREDCRRFLKEKGMRRPSWNKSQAIQQ
蒺藜状苜蓿	TREDCRRFLKDKGMRRPSWNKSQAIQQ
白三叶草	TREDCRRFLKDKGMRRPSWNKSQAIQQ
拟南芥 PPD1	TREDCRKFLKDKGMRRPSWNKSQAIQQ
拟南芥 PPD2	TREDCRKFLKEKGMRRPSWNKSQAIQQ
雷蒙德氏棉	TREDCRKFLKEKGMRRPSWNKSQAIQQ
可可	TREDCRKFLKEKGMRRPSWNKSQAIQQ
克莱门氏柑桔	TREDCRKFLKEKGMRRPSWNKSQAIQQ
川桑	TREDCRKYLKEKGMRRPSWNKSQAIQQ
蓖麻	TREDCRKYLKEKGMRRPSWNKSQAIQQ
葡萄	TREDCRKYLKEKGMRRPSWNKSQAIQQ
相同的氨基酸	*** ** * *** ** ** *

图2

无油樟	TIFYAG
挪威云杉	TIFYAG
北美云杉	TIFYSG
紫萍	TIFYDG
江南卷柏 PPD1	TIFYSG
江南卷柏 PPD2	TMFYDG
芭蕉属物种	TIFYDG
海枣	TIFYDG
白蝴蝶兰	TIFYGG
烟草	TIFYCG
番茄	TIFYCG
马铃薯	TIFYRG
洛矶山楼斗菜	TIFYCG
毛果杨	TIFYCG
大豆	TIFYCG
蒺藜状苜蓿	TIFYCG
白三叶草	TIFYCG
拟南芥 PPD1	TIFYSG
拟南芥 PPD2	TIFYSG
雷蒙德氏棉	TIFYCG
可可	TIFYCG
克莱门氏柑桔	TIFYCG
川桑	TIFYCG
蓖麻	TIFYCG
葡萄	TIFYCG
相同的氨基酸	* * *

图3

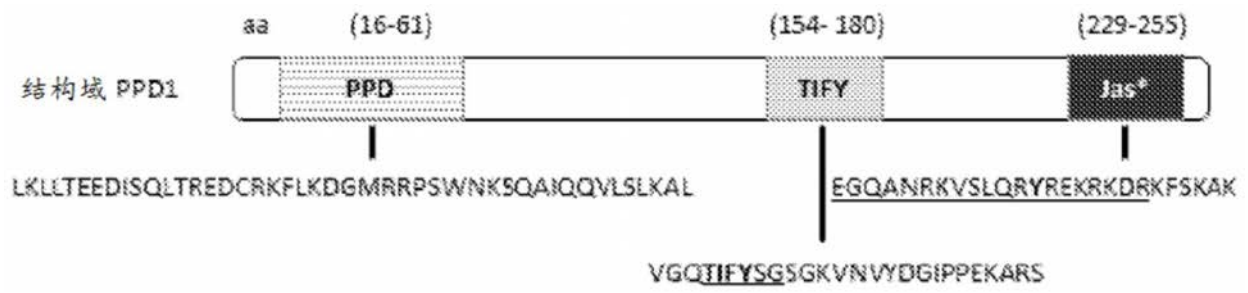


图4

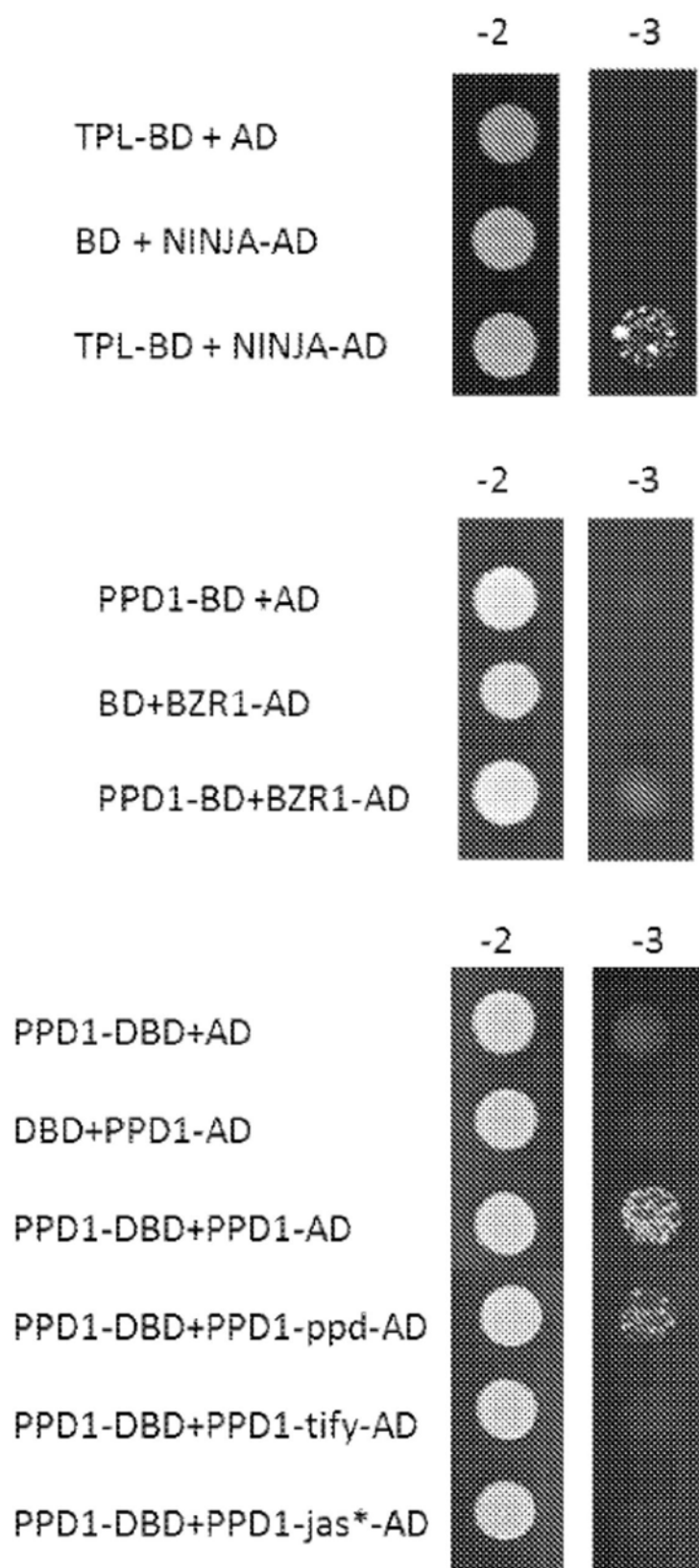


图5

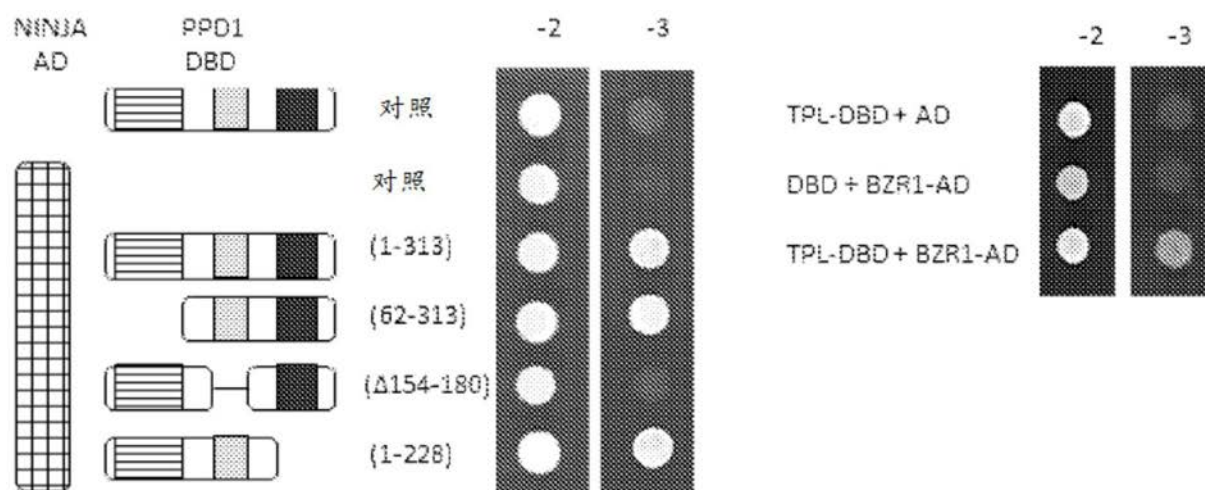


图6

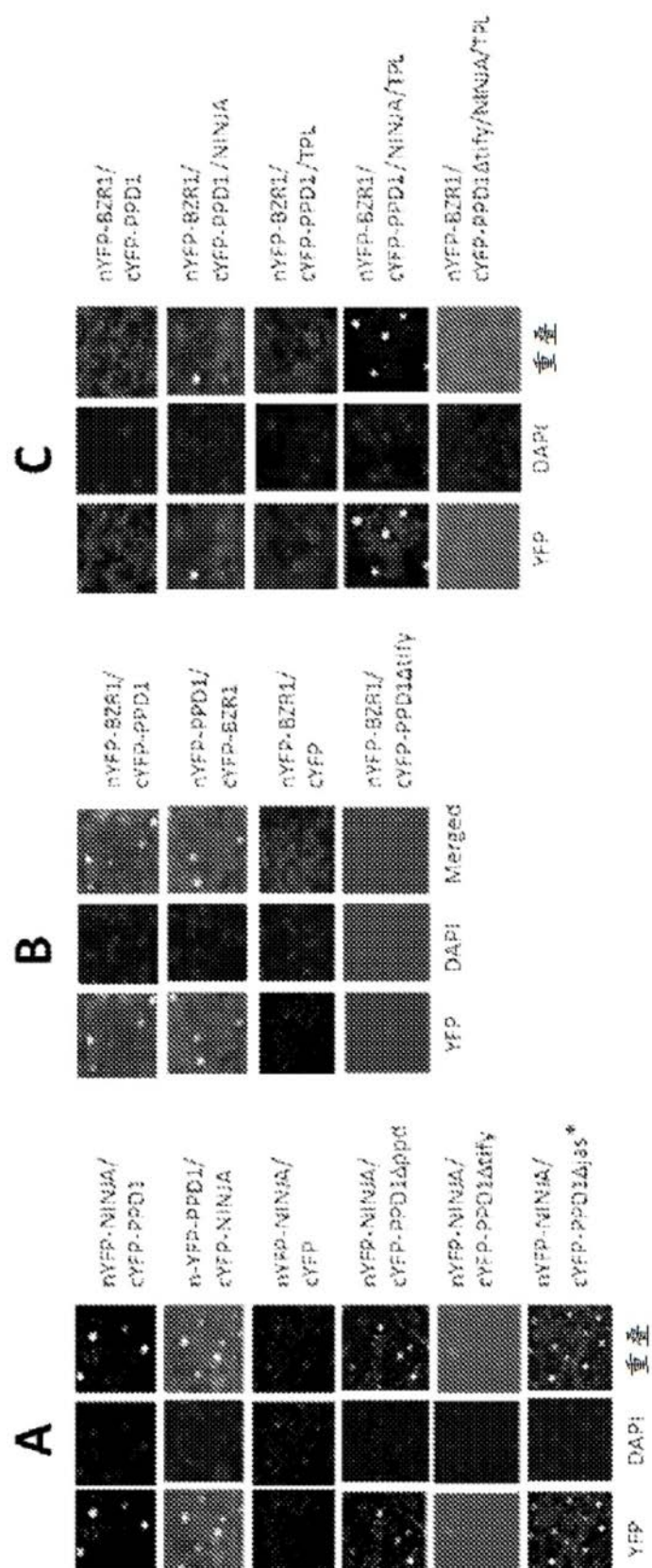


图7

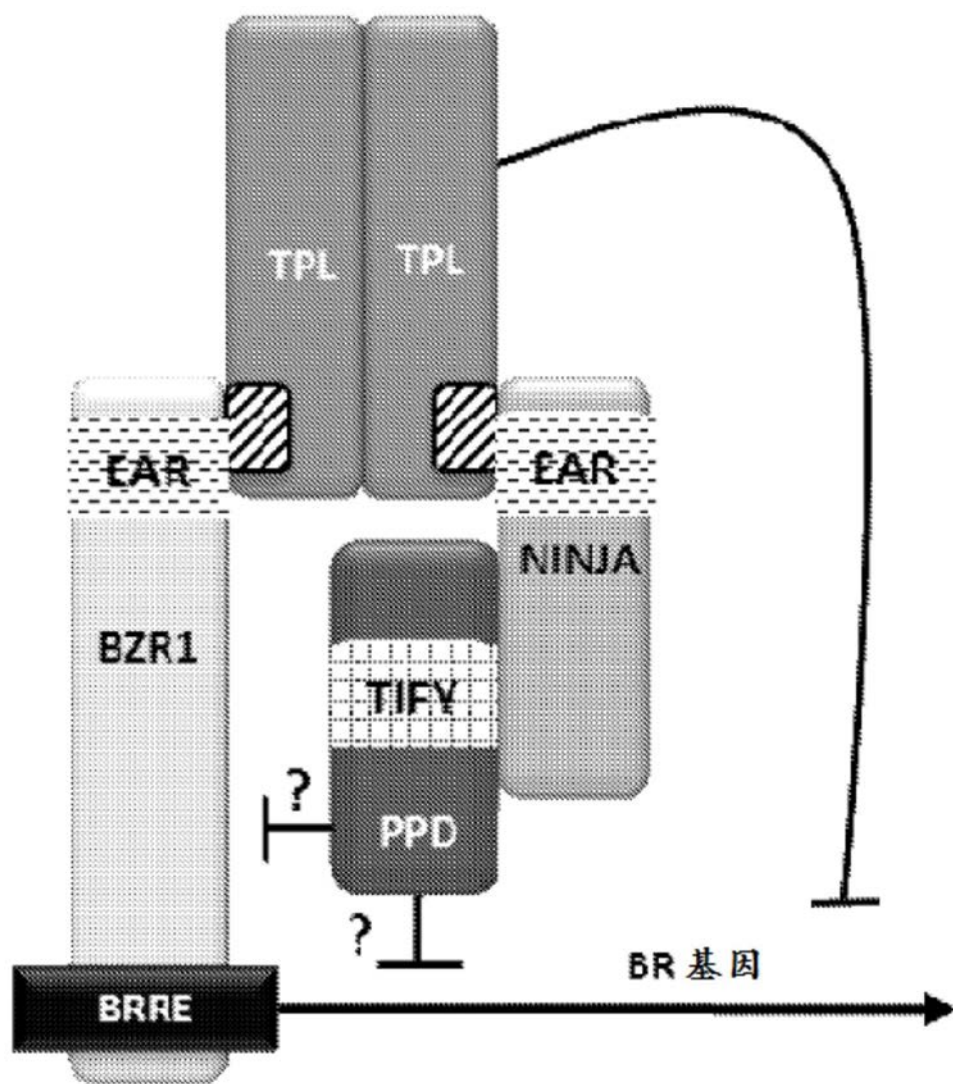


图8

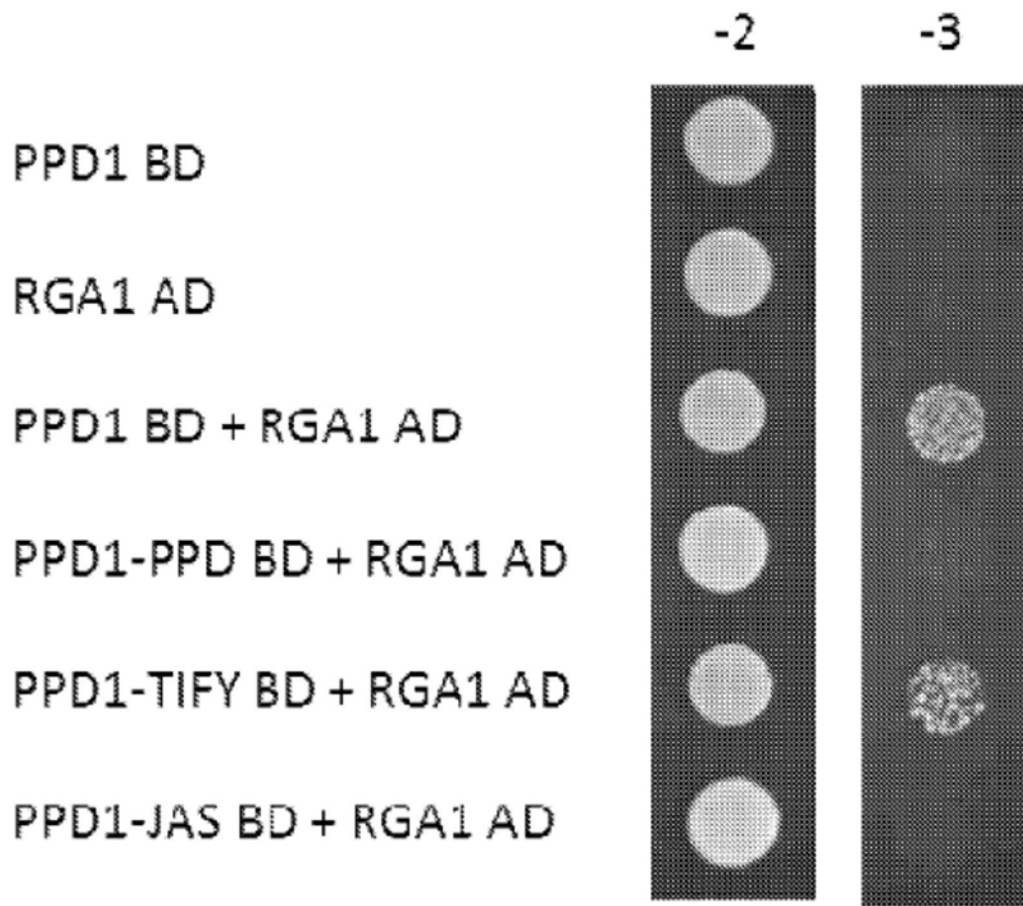


图9

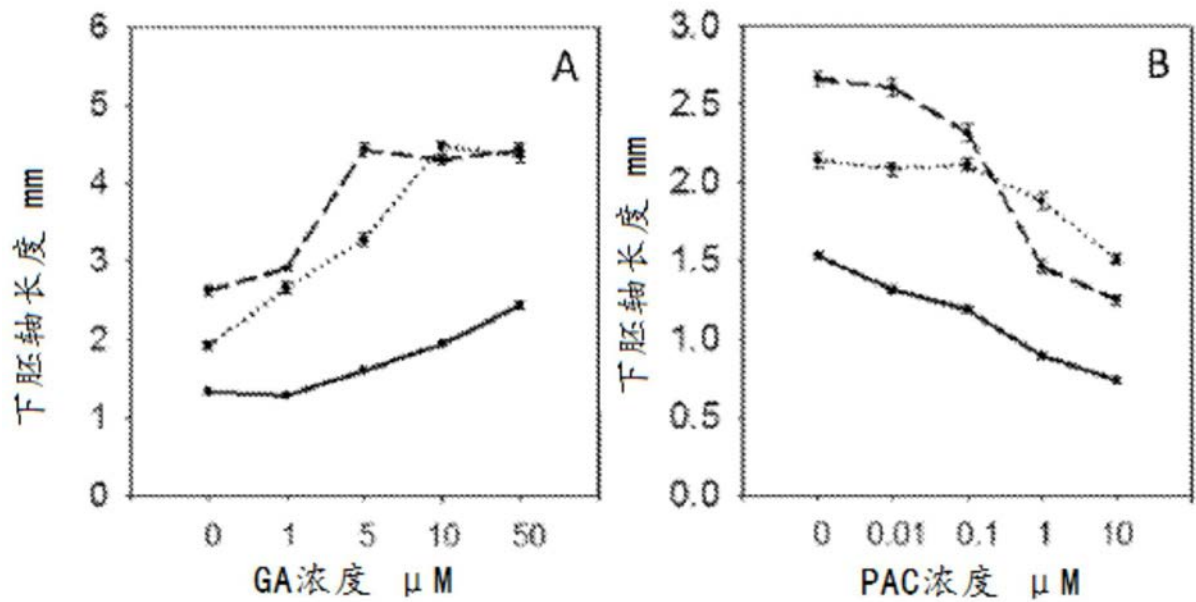


图10

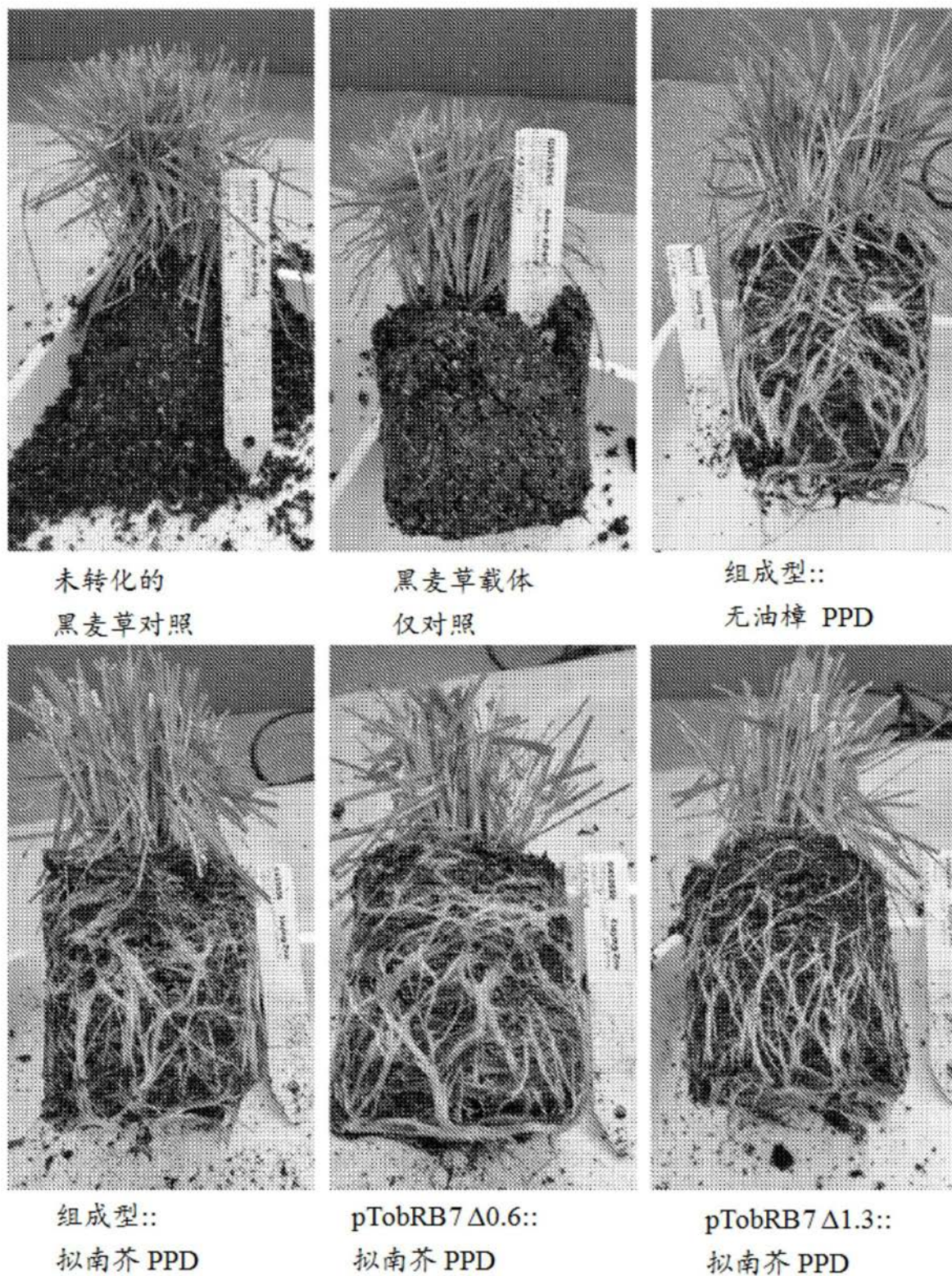


图11

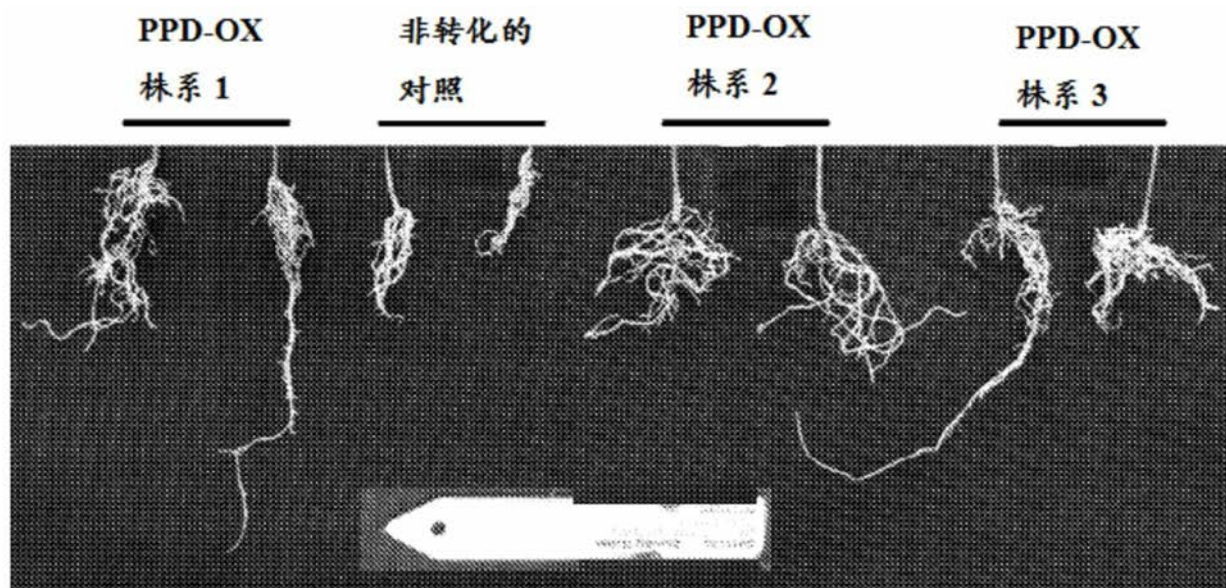


图12

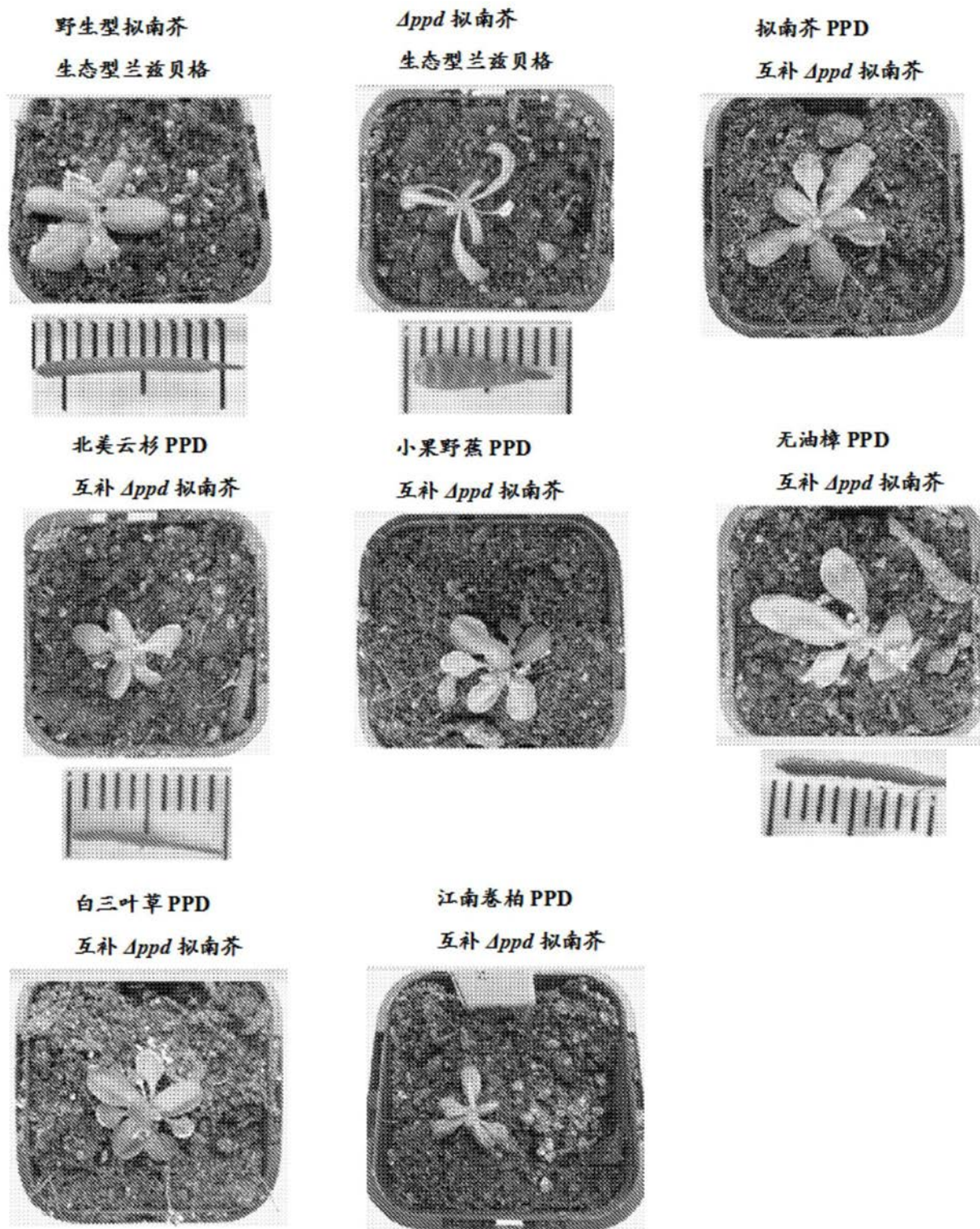


图13