耐非生物胁迫转基因水稻的培育——现在和未来
Transgenic Rice Breeding for Abiotic Stress Tolerance—Present and Future

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摘 要 环境胁迫严重降低了作物产量,日益减少的耕地和膨胀的人口对世界粮食安全造成了威胁。长期以来,改善作物的抗逆性一直是农业生产的主要目标。水稻是重要的粮食作物之一,培育具有抗逆性的水稻品种对全球的粮食生产将产生重要影响。在改善水稻的抗逆性方面,转基因比传统方法更有发展潜力。近年来,已有许多抗逆相关基因转入水稻并获得了一些提高抗逆性的转基因植株,文章重点讨论了耐非生物胁迫转基因水稻的研究进展。

关键词 耐非生物胁迫,培育,转基因水稻
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Abstract Environmental stresses and the continuing deterioration of arable land, along with an explosive increase in world population, pose serious threats to global agricultural production and food security. Improving the tolerance of the major crop plants to abiotic stresses has been a main goal in agriculture for a long time. As rice is considered one of the major crops, the development of new cultivars with enhanced abiotic stress-tolerance will undoubtedly have an important effect on global food production. The transgenic approach offers an attractive alternative to conventional techniques for the genetic improvement of rice cultivars. In recent years, an array of stress-related genes has already been transferred to rice to improve its resistance against abiotic stresses. Many transgenic rice plants with enhanced abiotic stress-tolerance have been obtained. This article focuses on the progress in the study of abiotic stress tolerance in transgenic rice breeding.

Key words abiotic stress tolerance, breeding, transgenic rice

环境胁迫如干旱、土壤盐渍化和极端温度等严重影响了作物的生长和发育,降低了产量。为了保障粮食安全,改善和提高作物对非生物胁迫的抗性一直是农业生产的主要目标之一。水稻是世界上近一半人口的主要粮食资源,而水稻生产不能满足消费需求,在未来 50 年,水稻生产将会面临更大的挑战[1],所以培育具有抗逆性的新品种,对全球的粮食生产将产生重要影响。然而,由于植物抗逆反应是

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一个极为复杂的过程,过去,利用传统的育种方法进行耐逆品种的培育进展缓慢。近年来,随着植物分子生物学的发展,发掘了一些与抗逆相关的新基因,对这些基因的表达方式及其在抗逆反应中的作用已逐步得到了解,这为耐逆转基因作物品种的培育开辟了新的途径。在改善水稻的抗逆性方面,转基因技术比传统方法更有发展潜力,因为将一个或多个基因转入其基因组中不会影响水稻的遗传背景[2]且能够实现遗传性状的定向改变。近 10 年来,已有许多抗逆相关基因转入水稻,并获得了一些提高抗逆性的转基因植株,其主要进展概况见(表 1)。

表 1 携带非生物胁迫相关基因的转基因水稻

Table 1 Transgenic rice carrying abiotic stress – related genes

Gene	Enzyme/protein encoded	Phenotypic expression
*ABF*3	Transcription factor	Increased tolerance to drought stress[3]
ADC	Arginine decarboxylase	Increased salt and drought tolerance[4-5]
*AgNHX*1	Vacuolar Na^+/H^+ antiporter	Enhanced salt tolerance[6]
*Aldh*2*a*	Aldehyde dehydrogenase	Submergence tolerance[7]
Calcineurin	A Ca^{2+}-and calmodulin-dependent serine/threonine phosphatase	Improved salt stress tolerance[8]
CAT + *GST*	Catalase + Glutathione S-transferase	Improved salt, paraquat[9] and chilling stress resistance[10]
*CBF*3	C-repeat/dehydration-responsive element binding factor 3	Elevated tolerance to drought and high salinity[3]
cbnA	Chlorocatechol dioxygenase	Degrade chloroaromatic compounds[11]
codA	Choline oxidase (glycine betaine synthesis)	Higher tolerance to salinity and cold[12-13]
codA	Choline oxidase (glycine betaine synthesis)	Higher tolerance to salinity[13]
*CYP*2*B*6	A cytochrome P450 monooxygenase	Detoxified various classes of herbicides[14]
ferritin	A protein that store large amouints of iron	Higher iron level[15-16]
GPAT	Glycerol-3-phosphate acyltransferase	Improved chilling stress resistance[17]
*GS*2	Chloroplastic glutamine synthetase	Enhanced salinity resistance and chilling tolerance[18]
GS	Glutamine synthetase	Tolerant to nitrogen deficiency[19]
GST	Glutathione S-transferase	Improved abiotic stress resistance[20]
*hsp*101	Heat shock protein 101	Improved high temperature tolerance[21]
*HVA*1	Group 3 LEA protein	Higher tolerance to drought and salinity stresses[22]
MnSOD	Manganese superoxide dismutase	Improved drought tolerance[23]
naat	Nicotianamine aminotransferase	Increased Fe efficiency, phytosiderophores secretion and grain yield[24]
*OsCDPK*7	Regulatory factor	Improved cold, salinity and drought tolerance[25-26]
*OsMAPK*5	Mitogen-activated protein kinase	Higher tolerance to drought, salt and cold stresses[27]
*OsNHX*1	Vacuolar Na^+/H^+ antiporter	Enhanced salt tolerance[28]
*OsPTF*1	Transcription factor	Tolerance to phosphate starvation[29]
OtsA OtsB	Trehalose-6-phosphate synthase, trehalose-6-phosphate phosphatase	Higher level tolerance to abiotic stresses[30]
PEPC	Phosphoenolpyruvate carboxylase	Enhanced stress tolerance, photosynthetic capacity and yield under photoinhibitory and photooxidative conditions[31-32]
PHYA	Phytochrome	Altered plant architecture and increased grain yield[33]
PPDK	Pyruvate orthophosphate dikinase	Enhanced stress tolerance, photosynthetic capacity and yield under photoinhibitory and photooxidative conditions[32]
Protox	Protoporphyrinogen oxidase	Higher resistance to herbicide[34-35]
samdc	S – adenosylmethionine decarboxylase	Higher polyamines level and salt tolerance[36]
*SOD*2	Plasma membrane Na^+/H^+ antiporter	Increased salinity tolerance[37]
*SsNHX*1	Vacuolar Na^+/H^+ antiporter	Enhanced salt tolerance[38]
*SsNHX*1 + *AVP*1	Vacuolar Na^+/H^+ antiporter + vacuolar H^+-PPase	Enhanced salt tolerance[39]
TPSP	Bifunctional fusion of trehalose-6-phosphate (T-6-P) synthase (TPS) and T-6-P phosphatase (TPP)	Increased trehalose accumulation and abiotic stress tolerance without stunting growth under stress conditions[40]

1 增强对高盐和干旱的耐性

土壤盐渍化是影响作物产量的最主要的环境胁迫因子之一。越来越多的证据表明,Na^+/H^+ 逆向转运蛋白(Na^+/H^+ antiporter)在植物抗盐中起重要作用。近来研究发现,携带 Na^+/H^+ 逆向转运蛋白的

转基因水稻增强了对盐胁迫的耐性，如过量表达 *OsNHX*1（*NHX*1 from *Oryza sativa* L.）和 *AgNHX*1（*NHX*1 from *Atriplex gmelini* C. A. Mey）[28,6] 的转基因水稻均增加了对盐胁迫的抗性，本实验室将 *SOD*2（a plasma membrane Na^+/H^+ antiporter from *Schizosaccharomyces pombe*）[37] 和 *SsNHX*1（*NHX*1 from *Suaeda salsa* L.）分别导入水稻也得到类似的结果[38]。实验结果显示，*SOD*2 转基因水稻根部的 P-ATPase 和 SsNHX1 转基因水稻地上部的 V-ATPase 的水解活性在盐胁迫下明显高于非转基因对照，说明维持 Na^+/H^+ 逆向转运蛋白的活性需要跨膜质子电化学梯度提供驱动力。因此，预测，将 Na^+/H^+ 逆向转运蛋白和质子泵共表达可能会比其中之一单独表达赋予转基因植物更强的耐盐能力。本实验室研究表明，无论在室外含盐（300mmol/L NaCl）土壤中和还是在室内含盐（150mmol/L NaCl）培养基上共表达 *SsNHX*1 和 *AVP*1（*Arabidopsis* vacuolar H^+-PPase）的转化苗的抗盐水平都比单一表达 *SsNHX*1 转化苗的高[39]，为上述预测提供了初步的实验证据。

高盐不仅破坏离子平衡而且还导致渗透胁迫。研究发现，通过积累渗透保护物质调节渗透平衡是植物在极端环境中生存的基本策略[41]。大量研究表明，将合成此类物质的基因转入不能合成渗透保护物质的植物中，是从遗传上提高植物抗渗透胁迫能力的有效途径。Sakamoto 等[12] 发现，将 *Arthrobacter globiformis* 的 *codA*（choline oxidase）分别定向转入水稻叶绿体（ChlCOD plants）和细胞质（CytCOD plants）得到的转基因植株在盐和低温胁迫下对光抑制的耐性不同，其中 ChlCOD 植株比 CytCOD 植株对光抑制具有更大的耐受性，说明转基因植物甜菜碱生物合成的亚细胞定位是有效增强抗逆性的关键因素。Mohanty 等[13] 也报道，携带同样基因的转基因水稻增加了对盐胁迫（150mmol/L NaCl）的抗性。

多胺类物质如亚精胺和精胺在生理浓度下对植物耐盐起重要作用。表达燕麦 ADC（Arginine decarboxylase）cDNA 的转基因水稻在盐胁迫下 ADC 活性增加，积累聚氨，与对照相比，生物量也增加[4]，Capell 等[5] 也报道了类似结果。Thu-Hang 等[36] 证明，在水稻中过量表达 *samdc*（Datura stramonium S-adenosylmethionine decarboxylase）cDNA 能在叶中充分积累亚精胺并在种子内积累亚精胺和精胺。他们的研究暗示，多胺合成下游途径 2 个组分中任何一个酶活性的增加都会引起高水平多胺的积累，从而增强转基因水稻对非生物胁迫的抗性。

海藻糖广泛分布于植物界，在水胁迫条件下，能稳定脱水酶、蛋白和脂膜并保护生物大分子结构的完整性[42]。过量表达大肠杆菌（*Escherichia coli*）海藻糖生物合成基因（*otsA* 和 *otsB*）的转基因水稻在盐、干旱和低温下，海藻糖积累增加，能够持续生长，减少了光氧化伤害并较好的维持矿质营养平衡[30]。Jang 等[40] 也研究了大肠杆菌海藻糖合成基因在转基因水稻中的作用，他们把编码双功能融合蛋白的基因 *TPSP*（bifunctional fusion of the *TPS* and *TPP*）转入水稻发现，由于海藻糖的积累使转基因水稻提高了对干旱、盐和寒冷胁迫的抗性。

尽管耐盐转基因水稻的培育取得了很大进步，但迄今为止，绝大多数研究结果来自实验室、温室或人为控制盐浓度的条件下，在盐渍化土壤中生长的转基因水稻既没有增加产量也没有实现商业化生产。主要的原因可能是因为植物很少经历单一胁迫，在田间条件下往往是多种胁迫相互作用，所以，目前得到的转基因水稻不能很好地在盐渍化土壤中生长。此外，耐盐转基因水稻的培育没有取得令人满意的结果还与植物对盐胁迫应答的遗传复杂性有很大关系。

2 改善光合作用

环境胁迫降低了光合效率，导致作物产量下降。十几年来，研究者尝试通过将 C4 作物光合循环中的关键酶导入 C3 作物来改善其光合效率并提高产量。Ku 等[31] 发现，将玉米 *PEPC*（phosphoenolpyruvate carboxylase）转入水稻，降低了转基因水稻光合作用的氧抑制，提高了光合效率。他们还发现，表达玉米 *PEPC* 或 *PPDK*（pyruvate，orthophosphate dikinase）的转基因水稻光合能力提高，同时高水平表达这 2 种酶的转基因植株也具有高的光合能力。携带 *PEPC* 的转基因水稻能够保持高的光合效率，PSⅡ产生的光量子产量高且在光抑制和光氧化条件下具有消散过剩光化学和非光化学能量的能力。此外，表达 *PEPC* 和 *PPDK* 的转基因水稻产量也高于非转基因对照。这些结果表明，将 C4 植物的光合酶转入水稻，对增强其抗逆性、提高光合能力和产量具有较大潜力[32]。

3 提高对极端温度的耐性

极端温度抑制了水稻的生长和发育。研究表明，将耐受低温/高温的相关基因导入水稻能提高转

基因水稻对极端温度的耐性。Ariizumi 等[17]发现,在水稻中过量表达 *GPAT*(glycerol-3-phosphate acyltransferase)增加了叶内磷脂酰甘油中的不饱和脂肪酸的量,提高了转基因植株在低温下的光合效率,增强了生长能力。表达拟南芥 *hsp*101(*Athsp*101)cDNA 的转基因水稻在不同水平的高温胁迫后,恢复生长情况明显好于对照[21]。

4 调节胁迫相关基因的表达

许多研究表明,控制信号传导的调节因子是改善植物耐逆水平的富有前途的方法。水稻 *OsCDPK*7(Ca^{2+}-dependent protein kinase)是与耐寒冷和盐/干旱有关的正调节因子(主要在维管组织表达)。Saijo 等[26]发现,过量表达 *OsCDPK*7 的转基因水稻增强了胁迫信号在遗传作用区(维管组织)的传导,改善了耐逆性。他们还报道,在水稻中过量表达 *OsCDPK*7,促进了对干旱/盐而不是寒冷应答的某些胁迫应答基因的诱导[25],暗示引起寒冷和盐/干旱耐性的下游途径不同。这些结果显示,在改善植物的抗逆方面,单一控制 *CDPK* 的活性就有很大潜力。然而,过量表达 *OsCDPK*2(*CDPK* isoform)发现,在有光条件下,转基因水稻的绿色叶内没有 *OsCDPK*2 的过量表达,且其种子的形成和发育在早期就受到严重抑制,只有 3% ~7% 的花产生种子,这说明不同的 *CDPK* 同工型在不同条件下具有复杂的调控机制[43]。*CBF*3 和 *ABF*3 分别是拟南芥中不依赖于 ABA 和依赖于 ABA 胁迫应答途径的调节基因。Oh 等[3]发现,表达 *CBF*3 的转基因水稻提高了对干旱和高盐的耐性,但对低温的耐受水平较低,此结果与 *CBF*3 在拟南芥中的作用相反 - 主要增强耐寒性;而 *ABF*3 转基因水稻只增加了对干旱胁迫的耐性。由此推测,不耐寒冷的水稻与适应寒冷的拟南芥可能具有不同的进化途经。

5 改良营养状况

全世界有近 30% 的耕地由于土壤 pH 过高降低了 Fe 的有效性,影响了作物的生长和发育,造成减产。水稻是对 Fe 缺乏特别敏感的作物之一。表达大麦 *naat*(nicotianamine aminotransferase)的转基因水稻在缺 Fe 条件下表现出高的该酶活性并能分泌大量的植物含 Fe 细胞,增强了对低 Fe 的耐性且在碱性土壤中其粮食产量比未转基因对照多 4.1 倍[24]。Goto 等[16]将编码大豆 *ferritin* 的全长序列导入水稻发现,该基因的表达使转化体 T1 代种子内 Fe 的含量比非转化体的增加了 3 倍。Drakakaki 等[15]也研究了含 *ferritin* 的转基因小麦和水稻,他们发现这 2 种植物的营养组织中 *ferritin* 的 mRNA 水平相似,但其蛋白水平在水稻中较高。利用 ICAP(inductively coupled argon plasma)光谱测定法进一步分析显示,Fe 水平只在转基因植物的营养组织内提高,种子中并没有改变,说明该基因的表达具有器官特异性且受转录后调控。

6 减少氧化胁迫

已知 *Bacillus subtilis Protox*(Protoporphyrinogen oxidase)是一种抗除草剂的酶。Lee 等[34]将 *B. subtilis Protox* 分别定位在水稻的细胞质和质体内发现,转基因水稻增加了对除草剂(oxyfluorfen)的抗性,减少了细胞质渗漏和叶绿体损伤,降低了脂质过氧化。他们还发现,转基因水稻对除草剂的抗性水平与 *Protox* 的亚细胞定位密切相关,其中 *Protox* 定位在质体内的比其定位在细胞质内的转化体呈现出更高的水平。Shimizu 等[11]研究表明,表达 *Ralstonia eutropha NH*9 *cbnA*(chlorocatechol dioxygenase)的转基因水稻,能有效的将有毒化合物(3-chlorocatechol)转化为无毒化合物(2-chloromucote),而转化体的生长和形态与对照无明显差异。他们的结果说明,利用含此类基因的转基因水稻分解土壤和水体表面的氯化芳香化合物(chloroaromatic compounds)是可能的。

抗氧化剂基因对促进人们了解胁迫条件下特定抗氧化剂的防御作用有重要意义。本实验室从盐地碱蓬(*Suaeda salsa* L.)cDNA 文库中获得谷胱甘肽转移酶基因(Glutathione S-transferase, EC 2.5.1.18, *GST*)和过氧化氢酶基因(Catalase, EC 1.11.1.6, *CAT*1)[44-45],在水稻中表达 *GST* 和 *GST* + *CAT*1 减轻了盐、除草剂[9,20]和低温胁迫下的氧化损伤[10]。在应用的氧化胁迫下,转基因水稻特别是 *GST* + *CAT*1 共转化体细胞膜透性、H_2O_2 的产生和 MDA(malon dialdehyde)的积累均低于非转基因对照。这些结果说明,*GST* 和 *CAT* 在植物防御和抗氧化胁迫中起重要作用。

总之,不同基因对转基因水稻耐逆性的贡献不同。虽然由于外源基因的作用机制、整合位点及转录后调控等原因很难比较不同基因转化体的耐逆效果,但从本实验室的研究结果来看,*SsNHX*1 和 *AVP*1 共表达比 *SsNHX*1 单一表达赋予转基因水稻更高的耐盐水平;表达单一抗氧化酶(如 *GST*)的转化苗不如表达单一 Na^+/H^+ 逆向转运蛋白(如 *SsNHX*1)的

转化苗耐盐效果好;共表达双抗氧化酶(如 *GST* + *CAT1*)的转基因水稻比表达单一抗氧化酶(如 *GST*)的转化体抗逆性强。

7 现在和未来

尽管耐逆转基因水稻的培育取得了显著进步,但目前的成绩并不令人满意。主要原因包括(1)大多数转基因水稻的耐逆水平有限,不足以在环境胁迫条件实现正常生长,更不用说增加产量和商品化生产;(2) 植物耐逆是一个复杂的性状,受基因网络中各个基因的协调和差异表达的影响,而目前人们对此机制还缺乏全面了解,只有获得功能基因组内关于耐逆主效基因和重要目标基因的更多信息,才能有效控制抗逆基因的表达;(3) 外源基因在水稻基因组中的整合率低,仅在活泼转录区(即基因丰富区)发生整合[46],导致转化率低,获得的转化体少;(4) 由于基因沉默等原因使转入的基因不能正常表达,或不能在后代中稳定遗传,或导致植物不育[47-48];(5) 转基因水稻中使用的标记基因可能会影响环境和食品的安全性,因而限制了其在农业生产中的推广和应用。尽管如此,随着对植物功能基因组的研究,人们会获得关于植物感受和传递胁迫信号,启动适应反应机制的更多信息,因此,把新的目标表达调控系统与有用基因结合起来,将为耐逆转基因水稻和其它作物的培育提供更为有效合理的新策略。此外,随着转基因技术的不断改进,必将产生无标记基因的抗逆转基因水稻[49-50]。

简而言之,抗逆转基因研究的主要目的是增加水稻和其他作物的产量。水稻耐逆是一个复杂的性状,牵涉许多生理和生化机制及无数基因,因此,要从遗传上改善水稻的耐逆水平,就必须将不同的策略进行实验尝试。最终将这些不同策略和具有特定功能的基因融合起来以大幅度增强水稻的耐逆能力。更重要的是若把转基因技术和传统育种方法结合起来,将是培育耐逆水稻品种的有效途径。

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