Online system: http://www.jabiotech.org
农 业 生 物 技 术 学 报
Journal of Agricultural Biotechnology
2022, 30(7): 1394~1411
DOI: 10.3969/j.issn.1674-7968.2022.07.016

植物几丁质酶和β-1,3-葡聚糖酶及其协同抗病性研究

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摘 要 植物几丁质酶(chitinase)和β-1,3-葡聚糖酶(β-1,3-glucanase)能够水解病原菌细胞壁的主成分几丁质、β-1,3-葡聚糖和肽聚糖，有效抑制病原菌的生长，是植物防御系统中的两个重要的防卫因子。此外，两者还具有协同抑菌作用，被广泛应用于植物抗病基因工程。本文通过阐述植物几丁质酶和β-1,3-葡聚糖酶的结构、分类、生物学特性、表达调控机制及两者之间的协同抗病作用研究进展，为系统了解植物几丁质酶和β-1,3-葡聚糖酶的作用模式以及植物抗病遗传改良提供参考资料。

关键词 几丁质酶；β-1,3-葡聚糖酶；结构特点；功能特性；协同抗病作用

中图分类号 S184; S435.661

Plant Chitinase and β-1, 3-glucanase and Their Synergistic Function in Disease Resistance

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Abstract Plant chitinase and β-1,3-glucanase, two important defensive factors in the plant defense system, can hydrolyze the main components of pathogenic cell wall chitin, β-1, 3-glucan and peptidoglycan, thus effectively inhibit the growth of pathogens. In addition, they also have synergistic antimicrobial effects and are widely used in plant disease resistance genetic engineering. In this paper, the research progress on the structure, classification, biological characteristics, expression regulation mechanism and the synergistic disease resistance of plant chitinase and β-1,3-glucanase were reviewed. This study will provide references for systematically understand the action mode of plant chitinase and β-1, 3-glucanase, and for the genetic improvement of plant disease resistance.

Keywords Chitinase; β-1, 3-Glucanase; Structural features; Functional characteristics; Synergistic disease resistance

植物在生长阶段易遭受细菌、真菌和病毒感染，为避免受病原体侵害，植物会激发防御应答反应，产生或积累多种防御物质，进而实现自我保护(Grover, 2012)。几丁质酶(EC 3.2.1.14, chitinase)和

基金项目：国家重点研发计划项目(2018YFD1000503; 2019YFD1000500)；福建省自然科学基金项目(2020J01591)；财政部和农业农村部国家现代农业产业技术体系资助项目(CARS-17)

收稿日期：2021-09-01 接受日期：2021-12-17

β-1,3-葡聚糖酶(EC 3.2.1.39, β-1,3-glucanase)作为水解酶，具有降解真菌细胞壁的主要成分几丁质和β-1,3-葡聚糖以及细菌细胞壁的肽聚糖的作用，是植物防御系统中的两个重要防卫因子(van Loon, 1985; Zhang et al., 2016)。研究显示，几丁质酶和β-1,3-葡聚糖酶基因联合作用时的抑菌效果优于单个基因，表明二者在抑制病原菌生长的过程中发挥协同作用(Mauch et al., 1988; Sela-Buurlage, 1993)。近年来，植物几丁质酶和β-1,3-葡聚糖酶基因陆续在许多物种中被克隆和鉴定，如水稻(Oryza sativa) (Karmakar et al., 2016)、玉米(*Zea mays*) (Xie et al., 2015)、大豆(*Glycine max*)(Cheong et al., 2000)、甘蔗(*Saccharum* spp.) (Su et al., 2016)、烟草(*Nicotiana tabacum*) (Sundaresha et al., 2010)、辣椒(*Capsicum annuum*) (Ali et al., 2019)和大麦(*Hordeum vulgare*) (Jayaraj, Punja, 2007)等。由于几丁质酶和β-1,3-葡聚糖酶在防御植物病虫害方面具有广谱抗性，能够降低环境污染和化学农药的使用，二者在植物抗病基因工程被广泛应用(Kumar et al., 2018; Langner, Göhre, 2016)。本文通过系统阐述植物几丁质酶和β-1,3-葡聚糖酶的生物学特性、结构、分类、表达调控机制及其近年来两者协同抗病性的研究进展，为植物抗病分子育种提供有益参考。

1 植物几丁质酶

1.1 植物几丁质酶的生物学特性

几丁质酶是一种能够水解几丁质中的β-1,4-糖苷键而产生几丁质单糖或几丁寡糖的糖苷酶(Grover, 2012)，主要存在于甲壳类、昆虫和真菌中(Malik, Preety, 2019)。尽管植物本身不含几丁质，但能通过真菌、细菌、病毒、植物激素、昆虫咬食等诱导因子诱发产生几丁质酶(Cletus et al., 2013; Pusztahelyi, 2018)，且该酶类普遍存在于植物不同组织器官中(Beerhues, Kombrink, 1994)。植物几丁质酶属于糖苷水解酶第18 (GH18)和第19 (GH19)家族，通常以单体形式存在，分布在病程相关蛋白(pathogenesis-related protein, PR protein)的PR-3、-4、-8和-11家族中(van Loon, 1985; Neuhaus et al., 1996)。该酶类的蛋白分子量约为22~35 kD，等电点为3~10。除了具有几丁质酶活性外，特殊的几丁质酶还含有溶菌酶双重活性以及参与催化转糖基反应等(Kuo et al., 2008)。

1.2 植物几丁质酶的结构与分类

植物几丁质酶在结构上一般含有一个N端信号肽区、富含半胱氨酸的几丁质结合区(chitin-binding domain, CBD)、一个可变交连区(hinge)以及一个C端高度保守的主要功能区域—催化区(catalysis)(Neuhaus et al., 1996)。根据蛋白结构特点和氨基酸序列同源性，植物几丁质酶可划分为七类(Singh et al., 2007)，即Class Ⅰ~Ⅶ (图1)。Class Ⅰ几丁质酶大部分存在于液泡中，具有较强的抗真菌活性，其N端CBD约含40个氨基酸(富含8个半胱氨酸)，可以特异性结合几丁质与氨基葡萄糖；C端由约300个氨基酸组成的催化区；二者经富含甘氨酸和脯氨酸的可变交连区相连接(Araki, Torikata, 2014)。Class Ⅱ几丁质酶主要分布于细胞间隙内，其只含催化区，无CBD和交连区(Araki, Torikata, 1995)。Class Ⅲ几丁质酶与Class Ⅰ和Ⅱ成员在氨基酸序列上无同源性，存在于细胞间隙，无CBD，但含有催化区，具有几丁质酶和溶菌酶双重活性(Kuo et al., 2008; Patil et al., 2009)。Class Ⅳ几丁质酶与Class Ⅰ成员的结构相似，但其CBD和催化区存在少量氨基酸缺失，且C端为不完整的延长区(Mitsunaga et al., 2004)。Class Ⅴ几丁质酶与Class Ⅰ类似，含有两个重复的CBD (Chaudet et al., 2014; Taira et al., 2009)。Class Ⅵ具有催化区与C-端延长区，与前五类的同源性不高，但与细菌几丁质酶具有同源性(Tyler et al., 2010)。Class Ⅶ几丁质酶与Class Ⅳ成员高度相似，缺少CBD，且催化区有少量氨基酸缺失(Kolosova et al., 2014)。

1.3 植物几丁质酶的抗病功能

在1986年，Schlumbaum等(1986)首次报道了提纯的菜豆(*Macphophomina phaseolina*)几丁质酶对绿色木霉菌(*Trichoderma viridea*)具有体外抑制作用。同年，Broglie等(1986)从菜豆中鉴定到第一个植物几丁质酶基因。目前，研究者已从水稻、棉花(*Gossypium hirsutum*)(Zhong et al., 2021)、油菜(*Brassica oleracea*) (Zhu et al., 2021)、辣椒(Ali et al., 2020)、葡萄(*Vitis vinifera* (Zheng et al., 2020)和甘蔗(Su et al., 2015; Su et al., 2014)等植物中分离获得几丁质酶基因，并将其广泛应用于植物基因工程中，通过调节几丁质酶的活性或将外源几丁质酶基因转化到目标植物的方式来增强植物的抗病性(表1)。

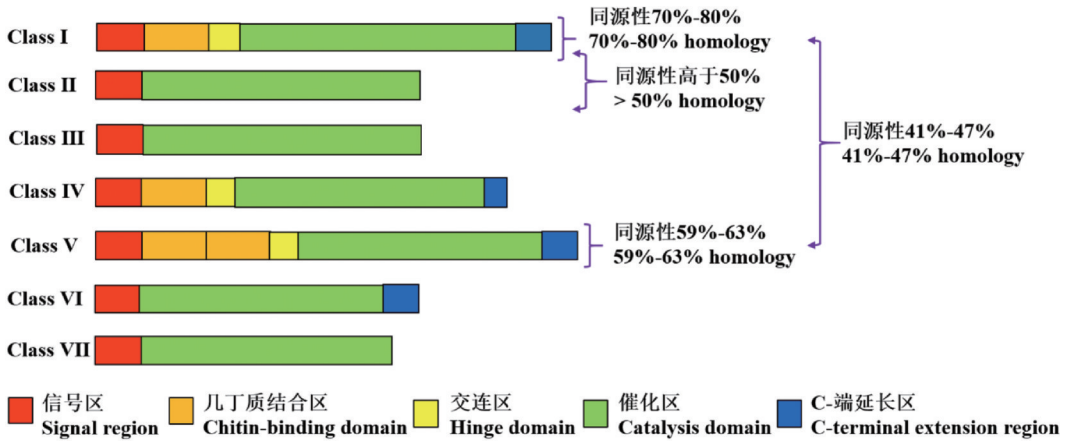

图1 不同类型的植物几丁质酶的蛋白结构

Figure 1 Protein structures of different types of plant chitinases

真菌 植物病原真菌通过菌丝的大量生长来完成对寄主的侵染，而几丁质酶是一类降解真菌细胞壁主成分—几丁质的关键酶，其水解产物几丁寡糖能够作为激发子诱导植物产生防御反应，进而抑制病原菌的生长(Kumar et al., 2018; Pusztahelyi, 2018)。例如，Zhang 等(2019a)将苦瓜(*Momordica charantia*)几丁质酶基因 *McCHIT1* 过表达到水稻中，经立枯丝核菌(*Rhizoctonia solani*)处理后，转基因水稻的病情指数远低于野生型。Xiao 等(2007)发现苦瓜几丁质酶基因 *Mcchit1* 可以提高棉花对黄萎病的抗性。Durechova 等(2019)将从圆叶茅膏菜(*Drosera rotundifolia*)中分离到的几丁质酶基因 DrChit 导入烟草，发现转基因植株的蛋白粗提物对绿色木霉的菌丝生长具有明显的抑制作用。辣椒几丁质酶基因 *ChiIV3* 和 *CaChiIV1* 均可以增强辣椒对疫霉菌(*Phytophthora capsici*)的抗性；*ChiIV3* 能通过病毒诱导的基因沉默和辣椒植株的瞬时表达，触发防御信号，上调 *PR* 基因的表达；*CaChiIV1* 沉默植株中的丙二醛含量和相对电解质渗漏较高，但抗氧化酶活性、叶绿素含量、根系活性和脯氨酸含量较低(Liu et al., 2017; Ali et al., 2019)。在两个不同的水稻绿色组织特异性启动子的控制下，通过共表达水稻几丁质酶基因 *OsCHI11* 和草酸氧化酶 4 基因(oxalate oxidase 4, *OsOXO4*)，获得了具有纹枯病抗性的转基因水稻植株，其 PR 基因的表达显著增加，H_2O_2 水平升高、活性氧清除酶活性显著变化，膜损伤减少(Karmakar et al., 2016)。此外，有研究表明水稻几丁质酶基因对百合(*Lilium brownii*)和黄瓜(*Cucumis sativus*)的灰霉病菌(*Botrytis cinerea*)、香蕉(*Musa nana*)斐济假尾孢(*Cercospora fijiensis*)、花生叶斑病病菌(*Cladosporium personatatum*)有一定的抗性作用(Kishimoto et al., 2002; Kim et al., 2003; Iqbal et al., 2012; Kovács et al., 2013; Núñez De Cáceres González et al., 2015)。Jayaraj 和 Punja (2007)通过农杆菌转化法将大麦几丁质酶基因 *chi-2* 和小麦(*Triticum aestivum*)脂质转移蛋白基因 *ltp* (lipid-transfer-protein gene)导入胡萝卜(*Daucus carota*)植株并接种灰霉病菌和根链格孢菌(*Alternaria radicicola*)，发现与单基因相比，两个基因的共表达使胡萝卜植株对两种病原菌均表现出较高的抗性。小麦几丁质酶基因 *Wch2* 增强了拟南芥(*Arabidopsis thaliana*)对尖孢镰刀菌(*Fusarium oxysporum*)的抗性(李和平等, 2005)。芥菜(*Brassica juncea*)几丁质酶基因 *BjCHI1* 能够抑制灰霉病菌的生长(Gao et al., 2014)。番茄(*Lycopersicon esculentum*)几丁质酶基因 pcht28 提高了草莓(*Fragaria ananassa*)对大丽轮枝菌(*Verticillium dahliae*)的抗性(Chalavi et al., 2003)。

细菌 植物几丁质酶不仅能水解细胞壁中的几丁质，而且 Class Ⅲ几丁质酶的溶菌酶活性有助于降解细菌细胞壁上的肽聚糖，从而抑制细菌的增殖(Kuo et al., 2008)。Hong 和 Hwang (2006)将辣椒几丁质酶基因 *CAChi2* 过表达到拟南芥中，发现 *CAChi2* 基因的启动子激活了 β-葡萄糖醛酸苷酶(β-glucuronidase, *GUS*)报告基因在丁香假单胞菌(*Pseudomonas syringae*)侵染和渗透胁迫下的表达，转基因材料对细菌侵染和渗透胁迫的抗性增强。Sytwala 等(2015)在大戟科(Euphorbiaceae)植物的乳胶中发现具有溶菌酶功能的几丁质酶，其在防御

表1　植物几丁质酶在抗病基因工程中的应用研究

Table 1　Application of plant chitinase in disease resistance genetic engineering

基因来源 Gene source	基因名称 Gene name	受体植物 *Receptor plant*	研究方法 Research method	病原菌 Pathogen	抗病效果 Disease resistance effect	参考文献 Reference
真菌胁迫 Fungi pathogen stress						
圆叶茅膏菜 *Drosera rotundifolia*	*DrChit*	烟草 *Nicotiana tabacum*	过表达 Overexpression	绿色木霉 *Trichoderma viride*	抑制绿色木霉的生长 Inhibited the growth of T. iride	Durechova et al., 2019
苦瓜 *Momordica charantia*	*McCHIT1*	水稻 *Oryza sativa*	过表达 Overexpression	立枯丝核菌 *Rhizoctonia solani*	提高对水稻纹枯病的抗性，且转基因水稻抗纹枯病与几丁质酶活性之间有显著相关性 Improved the resistance to rice sheath blight. The correlation between the rice resistance to sheath blight and chitinase activity in transgenic plants was significant	Zhang et al., 2019a
苦瓜 *M. charantia*	*Mcchit1*	本氏烟, 棉花 *Nicotiana benthamiana, Gossypium hirsutum*	过表达 Overexpression	大丽轮枝菌, 疫霉病菌 *Verticillium dahlia, Phytophthora nicotianae*	显著增强转基因本氏烟对烟草疫霉菌的抗性，以及转基因棉花对黄萎病的抗性 Significantly enhanced resistance to the P. nicotianae in transgenic *N. benthamiana* and against *Verticillium* wilt in transgenic cottons	Xiao et al., 2007
辣椒 *Capsicum annuum*	*CaChiIII7*	辣椒 *C. annuum*	基因沉默, 瞬时表达 Knockdown, transient expression	辣椒炭疽病菌 *Colletotrichum acutatum*	影响防御反应基因的表达以及过氧化氢等含量，敲除后增加了对辣椒炭疽病菌的敏感性，但瞬时表达后抗病性增强 Affected the expression of defense response genes and the content of hydrogen peroxide. Knockout increased the sensitivity to C. acutatum, but the disease resistance increased after transient expression	Ali et al., 2020
辣椒 *C. annuum*	*CaChiIV1*	辣椒 *C. annuum*	基因沉默 Knockdown	辣椒疫霉菌 *Phytophthora capsici*	增加了对辣椒疫霉菌的敏感性，沉默植株的丙二醛含量和相对电解质渗漏较高，但抗氧化酶活性、叶绿素含量、根系活性和脯氨酸含量较低 Increased susceptibility to P. capsicum. The silenced plants had higher malondialdehyde con-	Ali et al., 2019

					tent and relative electrolyte leakage, but lower antioxidant enzyme activity, chlorophyll content, root activity and proline content	
辣椒 *C. annuum*	*ChiIV3*	辣椒 *C. annuum*	病毒介导的基因沉默；瞬时过表达 virus-induced gene silencing; Transient overexpression	辣椒疫霉菌 *P. capsici*	ChiIV3 作为植物细胞凋亡的调节器，并触发防御信号和病程相关蛋白基因的上调，增强对辣椒疫霉菌的抗性 ChiIV3 acted as a positive regulator of plant cell death and in triggering defense signaling and upregulation of pathogenesis related genes, and enhanced the resistance to P. capsici	Liu et al., 2017
水稻 *O. sativa*	*OsOXO4, OsCHI11*	水稻 *O. sativa*	共表达 Co-expression	立枯丝核菌 *R. solani*	提高水稻对纹枯病的抗性，PR 基因的表达增加，过氧化氢水平升高、活性氧清除酶活性显著变化，膜损伤减少 Improved the resistance of rice to sheath blight. Increased PR gene expression and hydrogen peroxide level, significantly changed reactive oxygen species scavenging enzyme activity, and reduced membrane damage	Karmakar et al., 2016
水稻 *O. sativa*	*RCH10*	百合 *Lilium brownii*	过表达 Overexpression	灰葡萄孢 *Botrytis cinerea*	提高对灰霉病的抗性 Improved the resistance to gray mold	Núñez De Cáceres González et al., 2015
水稻 *O. sativa*	*Rcc2; rcg3*	香蕉 *Musa nana*	过表达 Overexpression	斐济假尾孢 *Cercospora fijiensis*	增强对黑叶条斑病的抗性，坏死叶面积减少了 73%~94% Enhanced the resistance to black leaf streak, the necrotic leaf area decreased by 73%~94 %	Kovács et al., 2013
水稻 *O. sativa*	*Oschit*	花生 *Arachim subgaea*	过表达 Overexpression	花生叶斑病病菌 *Cladosporium personatatum*	提高对叶斑病的抗性 Improved the resistance to leaf spot	Iqbal et al., 2012
水稻 *O. sativa*	*CHT−2; Rcc2*	黑麦草 *Lolium multiflorum*	过表达 Overexpression	禾冠柄锈菌 *Puccinia coronata*	增强对冠锈病的抗性 Enhanced the resistance to crown rust	Takahashi et al., 2005
水稻 *O. sativa*	*RCH10, MOD1*	水稻 *O. sativa*	共表达 Co-expression	水稻纹枯病菌 *Rhizoctonia solani*	提高对纹枯病的抗性 Improved the resistance to sheath blight	Kim et al., 2003

水稻 *O. sativa*	*RCC2*	黄瓜 *Cucumis sativus*	过表达 Overexpression	灰霉病菌 *Botrytis cinerea*	提高对灰霉病抗病性 Improved the resistance to gray mold	Kishimoto et al., 2002
大麦 *Hordeum vulgare*	class II chitinase, *RIP*	芥菜 *Brassica juncea*	共表达 Co-expression	油菜交链孢菌 *Alternaria brassicola*	提高对叶斑病的抗性 Improved the resistance to leaf spot	Chhikara et al., 2012
大麦 *H. vulgare*	*chi-2*, *ltp*	胡萝卜 *Daucus carota*	共表达 Co-expression	根链格孢菌, 灰霉病菌 *Alternaria radicicola*, *B. cinerea*	增强对根链格孢菌和灰霉病菌的抗性，且优于单基因转化 Enhanced the resistance to A. radicicola and B. cinerea, and better than single-gene transformations	Jayaraj, Punja, 2007
小麦 *Triticum aestivum*	*Wch2*	拟南芥 *Arabidopsis thaliana*	过表达 Overexpression	尖孢镰刀菌 *Fusarium oxysporum*	显著增强对尖孢镰刀菌的抗性 Significantly enhanced the resistance to F. oxysporum	李和平等, 2005；Li et al., 2005
芥菜 *Brassica juncea*	*chit42*	芥菜 *B. juncea*	过表达 Overexpression	芸苔链格孢菌 *Alternaria brassicae*	提高对链格孢叶枯病的抵抗力 Improved the resistance against Alternaria leaf blight	Ojaghian et al., 2020
芥菜 *B. juncea*	*BjCHI1*	拟南芥 *A. thaliana*	过表达 Overexpression	灰霉病菌 *B. Cinerea*	显著提高对灰霉病菌的抗性 Significantly improved the resistance to B. cinerea	Gao et al., 2014
葡萄 *Vitis vinifera*	*VvChi5*, *VvChi17*, *VvChi22*, *VvChi26*, *VvChi31*	草莓, 番茄 *Fragaria ananassa*, *Lycopersicon esculentum*	瞬时表达 Transient expression	灰霉病菌 *B. cinerea*	提高抗氧化酶活性，且不同VvChis基因过表达后的果实对灰霉病菌的抗性不同 Improved the activities of antioxidant enzymes, and the resistance to B. cinerea in different VvChis overexpression fruit was different	Zheng et al., 2020
番茄 *Lycopersicon esculentum*	*Pcht28*	草莓 *F. ananassa*	过表达 Overexpression	大丽轮枝菌 *V. dahliae*	提高对大丽轮枝菌的抗性Improved the resistance to V. dahliae	Chalavi et al., 2003
细菌胁迫 Bacterial pathogen stress						
辣椒 *C. annuum*	*CAChi2*	拟南芥 *A. thaliana*	过表达 Overexpression	丁香假单胞菌 *Pseudomonas syringae*	增强对丁香假单胞菌的抗病性 Enhanced the disease resistance to P. syringae	Hong, Hwang, 2006

病原体侵袭时具有一定的作用。

1.4 植物几丁质酶的表达调控

生长发育调节 几丁质酶的表达受植株生长发育阶段的调控。例如，马铃薯(*Solanum tuberosum*)几丁质酶的活性和mRNA的含量在老叶中普遍很高，在根中也普遍存在，在老叶和幼节间、叶片和叶柄中构成气孔复合体的维管组织和细胞中都有较高的含量(Büchter et al., 1997)，而在幼叶、块茎、根尖和所有其他花器官(花瓣、雄蕊、心皮)中的含量较低(Beerhues, Kombrink, 1994)。烟草几丁质酶在植株顶部附近的叶片中含量较低或者不存在，在下部叶片和根中的含量较高(Shinshi et al., 1987)。几丁质酶活性在健康转基因拟南芥植株的根、叶维管组织、水囊、保卫细胞和花药中均能检测到(Samac, Shah, 1991)。拟南芥几丁质酶基因*AtCTL2*主要在茎中表达，而*AtCTL1*转录本在大部分器官中都存在(Hossain et al., 2010)。甘蔗几丁质酶基因*ScChi*在蔗叶和茎表皮中高表达，其次是在根和茎髓中，在蔗芽中的表达水平较低，而*ScChiVII1*基因在芽中的表达显著高于根、茎、叶和茎表皮(Su et al., 2014)。

植物激素诱导调控 植物暴露在多变的环境(包括生物和非生物胁迫)下会做出快速和特异性的反应，产生植物激素如水杨酸(salicylic acid, SA)、茉莉酸(jasmonate, JA)、脱落酸(abscisic Acid, ABA)和乙烯(ethylene, ET)等，不同植物激素起着不同的作用，涉及植物生长和发育的各个方面以及植物对多种生物和非生物条件的适应性(Kasprzewska, 2003; Atamian, Harmer, 2016)。ET是植物激素中分子最小的，能够调节叶片发育、果实成熟、衰老等(Dubois et al., 2018)，其作为诱导植物几丁质酶的内在信号分子，在病原物侵染时能增加几丁质酶合成，诱导植物体内几丁质酶基因的表达(Taira et al., 2014)。拟南芥几丁质酶基因*CTL1*通过调节ET的生物合成来调控根系发育(Gu et al., 2019)。水稻幼苗的叶片、叶鞘与根组织中的几丁质酶被JA诱导积累(Rakwal et al., 2004)。马铃薯叶片被真菌激发子处理或晚疫病菌(*Phytophthora infestans*)侵染后，酸性几丁质酶mRNA受SA强烈诱导，碱性几丁质酶mRNA受ET或创伤诱导累积(Büchter et al., 1997)。甘蔗几丁质酶基因*ScChiI2*、*ScChiIII2*和*ScChiV1*受激素SA、ABA和茉莉酸甲酯(jasmonic acid, MeJA)诱导表达上调，而*ScChiII1*表达下调；此外，*ScChiVI1*被MeJA和ABA诱导表达下调，而被SA诱导表达上调；SA和MeJA上调*ScChiI3*的表达，而ABA抑制*ScChiI3*的表达；ABA处理下调*ScChiII1*的表达，而上调*ScChiVII*1的表达，表明甘蔗几丁质酶基因的转录受SA、MeJA和ABA诱导调控，且不同成员的应答反应模式存在差异(Su et al., 2015)。

顺式元件作用 启动子在植物不同器官和生育时期中表达，其含有多种重要的顺式作用元件，影响植物的生长发育和逆境响应(Kumar et al., 2018)。拟南芥酸性几丁质酶的嵌合基因与*GUS*编码区融合，转化拟南芥和番茄，结果显示在健康的转基因植物中，酸性几丁质酶启动子的活性仅限于根、叶维管组织、保卫细胞和花药中，而*GUS*在转基因拟南芥侵染病斑周围的叶肉细胞中被诱导表达；在转基因番茄植株中，*GUS*基因在番茄早疫病菌(*Alternaria solani*)和致病疫霉(*Phytophthora infestam*)引起的坏死病斑周围被诱导表达(Samac, Shah, 1991)。构建芥菜型油菜*BjCHI1*启动子(BjCHI1 promoter, BJC-P)与GUS报告基因融合的双元植物转化载体pBI121-GUSint，利用瞬时表达系统发现，转基因拟南芥对灰霉病菌的抗性显著增强(Gao et al., 2014)。水稻几丁质酶(*OsCHI11*)和草酸氧化酶4 (*OsOXO4*)基因在两个不同的绿色组织特异性启动子PD540-544和PEPC的控制下联合共表达，获得了对纹枯病具有增强保护作用的转基因水稻(Karmakar et al., 2016)。水稻*OsWRKY114*转录因子与病程相关蛋白基因*OsPR1a*和几丁质酶基因*Chitinase*的启动子结合，上调OsPR1a和Chitinase启动子的活性，获得的转基因植株增强了亚洲水稻对黄单胞菌(*Xanthomonas oryzae*)的免疫力(Son et al., 2020)。水稻几丁质酶基因*OsChia4a*中的E-box启动子被JA诱导上调表达，且OsChia4a蛋白对稻瘟病菌(*Magnaporthe oryzae*)的孢子萌发和菌丝生长具有抑制作用(Miyamoto et al., 2012)。

2 植物β−1,3−葡聚糖酶

2.1 植物β−1,3−葡聚糖酶的生物学特性

与几丁质酶相似，β-1,3-葡聚糖酶能水解真菌细胞壁的主成分β-1,3-葡聚糖，作用产物低聚糖也可以诱导产生其他抗病相关酶系，进而提高植物的抗病性(Wojtkowiak et al., 2013)。β-1,3-葡聚糖酶

普遍存在于细菌、真菌、藻类、部分动物和高等植物体内，其中真菌为主要酶源(Balasubramanian et al., 2012)。β-1,3-葡聚糖酶能够被病原菌侵染、昆虫咬食、ET与SA等多种生物和非生物因子诱导产生，参与植物的生长发育及多种逆境胁迫响应过程(Sels et al., 2008)。植物β-1,3-葡聚糖酶隶属于糖苷水解酶17家族成员，为PR-2蛋白，蛋白分子量一般在32~37 kD，存在酸性和碱性等电点(van Loon, 1985; Zavaleta, Eyzaguirre, 2016; Zhang et al., 2019b)。

2.2 植物β−1,3−葡聚糖酶的结构和分类

植物β-1,3-葡聚糖酶可分为3种类型，包括外切β-1, 3-葡聚糖酶(exo-β-l, 3-glucanase, EC3.2.1.58)、内切β-1,3-葡聚糖酶(endo-β-l,3-glucanase, EC3.2.1.39)以及β-1,3-糖基转移酶(β-l,3-glycosyltransferase, EC2.4.1.-)，它们的底物均为β-1,3-葡聚糖，不同的催化机制可将β-1,3-葡聚糖转化为不同的催化产物，3种酶共同组成了β-1,3-葡聚糖生物合成、降解及代谢系统(Mestre et al., 2017)。植物β-1,3-葡聚糖酶的结构特征主要在于拥有一个(β/α) 8TIM桶状结构，由8个α-螺旋和8个β-折叠环绕组合形成(Varghese et al., 1994)。根据等电点、亚细胞定位、基因表达模式以及氨基酸序列的同源性等特点，可将植物β-1,3-葡聚糖酶分为Ⅰ~Ⅳ类(表2)。

2.3 植物β−1,3−葡聚糖酶的抗病功能

β-1,3-葡聚糖同样是大多数真菌细胞壁的主要成分(Adams, 2004)，而β-1,3-葡聚糖酶能够在植物与病原菌相互作用中表达，通过破坏病原菌细胞壁的方式抑制其生长，提高植物的防御能力(Aimanianda et al., 2017)。当植物受到病原物或非生物胁迫时能上调β-1,3-葡聚糖酶的活性或产生新的β-1,3-葡聚糖酶同工酶，以提高植物的抗逆性。例如，Shi等(2006)证明草莓(*Fragaria ananassa*) β-1,3-葡聚糖酶基因*FaBG2-1*的表达水平在炭疽菌(*Colletotrichum fragariae*)侵染48 h后提高了116.9倍。Liu等(2010)发现小麦β-1,3-葡聚糖酶基因*TaGlu*的转录水平在接种条锈菌(*Puccinia striiformis*) 24 h后增加了35倍。Su等(2016)鉴定到的甘蔗β-1,3-葡聚糖酶基因*ScGluD2*在甘蔗抗病品种中的表达量经甘蔗黑穗病菌(*Sporisorium scitamineum*)处理1 d后上调了14.77倍，且在甘蔗抗病品种与黑穗病菌互

表2 不同类型的植物β−1,3−葡聚糖酶的等电点、亚细胞定位及其他特性

Table 2 Isoelectric point, subcellular localization and other properties of different types of plant β−1,3−glucanases

类型 Type	等电点 pI	定位 Location	其他特性 Other properties	参考文献 Reference
I类 Class I	碱性 Alkaline	液泡 Vacuole	含有一个N-末端信号肽、一个糖基化的C-末端序列和一个液泡定位的羧基末端多肽结构；在植物体外具有较高的抑菌活性 Contains an N-terminal signal peptide, a glycosylated C-terminal sequence, and a vacuolar-located carboxylterminal polypetide structure; has high antimicrobial activity in vitro	van Kan et al., 1992
II类 Class II	酸性 Acidic	细胞间隙 Intercellular	在植物体外没有抑菌活性；PR-2a、-2b和-2c是其特征成员 Has no antimicrobial activity in vitro; PR-2a, -2b and -2c are its main members	van Kan et al., 1992
III类 Class III	酸性 Acidic	胞外 Extracellular	属于诱导型β-1,3-葡聚糖酶；分子量约为35 kDa；与Class I氨基酸序列同源性介于50%~70%，与Class II的同源性在54%~59% An inducible β-1,3-glucanase; the molecular weight is about 35 kDa; the amino acid sequence homology with Class I is 50%~70%, and that with Class II is 54%~59%	Payne et al., 1990; van Kan et al., 1992
IV类 Class IV	酸性 Acidic	胞外 Extracellular	属于非诱导型β-1,3-葡聚糖酶；分子量约为25 kDa；与ClassII和Class III的氨基酸序列相似性较低 A non-inducible β-1,3-glucanase; the molecular weight is about 25 kDa; low amino acid sequence similarity with Class II and Class III members	Ward et al., 1991

作早期(1 d或3 d)的表达量比感病品种的高。大量研究证据显示，外源β-1,3-葡聚糖酶基因在植物中的表达可以有效抵抗病原物的侵袭(表3)。

真菌 植物β-1,3-葡聚糖酶作为一类典型的PR蛋白，在植物抗真菌基因工程的应用越来越多。如Zhang等(2019b)通过体外抑菌实验发现β-1,3-葡聚糖酶对小麦籽粒常见的真菌链格孢菌(*Alternaria alternata*)、黄链格孢(*Alternaria flavus*)和黑链格孢(*Alternaria nigra*)的孢子形成和菌丝形态有明显的抑制作用。Xie等(2015)克隆了玉米β-1,3-葡聚糖酶基因ZmGns，经体外抑菌实验证明其编码蛋白对黄曲霉(*Aspergillus flavus*)和丁香假单胞菌均有较强的抑制作用。Taif等(2020)从三七(*Panax notoginseng*)中分离出一个β-1,3-葡聚糖酶基因*PnGlu1*，将其过表达到烟草植株中并接种茄病镰刀菌(*Fusarium solani*)，结果表明转基因烟草中的JA生物合成基因和PRs基因的表达水平明显上调，表现出对茄病镰刀菌感染的强抗性。Su等(2016)发现甘蔗β-1,3-葡聚糖酶基因*ScGluD2*在烟草叶片中的瞬时过表达诱导了烟草的防御反应，并提高了烟草对青枯菌(*Pseudomonas solanacearum*)和灰霉病菌的抗性。Sundaresha等(2010)将烟草β-1,3-葡聚糖酶基因Glu在花生中过表达，随后对花生尾孢菌(*Cercospora arachidicola*)进行抗性筛选，发现转基因植株不仅斑点数量减少，发病时间延迟，并且对花生的另一重要病原菌黄曲霉也表现出抗性。Liu等(2013)将梨(*Pyrus pyrifolia*) β-1,3-葡聚糖酶基因*PpGlu*过表达到烟草中，体外实验发现转基因烟草品系的粗蛋白提取物在不同程度上抑制了拟茎点霉属(*Pseudomonas* sp.)、链格孢属(*Alternaria* sp.)和镰刀菌属(*Fusarium* sp.)菌丝的生长。苜蓿β-1,3-葡聚糖酶基因*AGLU1*可以增强高羊茅(*Festuca arundinacea*)对灰斑病与褐斑病的抗性(Dong et al., 2007)。马铃薯β-1,3-葡聚糖酶基因*gluB20-2*能够提高亚麻(*Linum usitatisimum*)对尖孢镰刀菌和黄瓜镰刀菌(*Fusarium culmorum*)的抗性(Wróbel-Kwiatkowska et al., 2004)。

细菌 Cheong等(2000)分离鉴定了一个编码碱性β-1,3-葡聚糖酶的大豆基因*SGN1*，将其在烟草中过表达，发现当接种丁香假单胞菌或用多种防御相关信号如H_2O_2、创伤、由疫霉(*Phytophthora* spp)制备的真菌激发子胁迫处理后均能诱导*SGN1*显著上调表达，表明*SGN1*基因可能在防御细菌性病害或其他逆境胁迫中发挥一定的作用。

2.4 植物β−1,3−葡聚糖酶的表达调控

生长发育调节 β-1,3-葡聚糖酶参与了植物小孢子的发生、韧皮部运输、胼胝质的运动、细胞壁生物合成、细胞分裂、谷类作物种子的萌发、花的发育、果实成熟以及植物衰老等生长发育过程(Balasubramanian et al., 2012)。β-1,3-葡聚糖酶基因*Glub*在番茄种子珠孔胚乳帽中表达(Wu, Bradford, 2003)。香蕉β-1,3-葡聚糖酶在果实成熟软化过程中具有一定的生理功能(Roy Choudhury et al., 2010)。在烟草中，Ⅰ类β-1,3-葡聚糖酶在萌发过程中被诱导表达，特别是在胚根萌发前的胚乳组织中，β-1,3-葡聚糖酶通过细胞壁水解削弱胚乳，促进胚根突起以完成萌发，表明β-1,3-葡聚糖酶对双子叶种子萌发、休眠释放和后熟有重要的调节作用(Leubner-Metzger, 2003)。水稻β-1,3-葡聚糖酶基因*Osg1*在整个植株中都有表达，其主要在花药减数分裂晚期以及小孢子发育早期和中期表达(高金玉等, 2017)，在转基因水稻中，通过RNA干扰沉默*Osg1*基因的表达，导致小孢子早期花药室胼胝质的降解中断，致使水稻雄性不育(Wan et al., 2011)。证实了β-1,3-葡聚糖酶与植物的多种生理和发育过程有关。

植物激素诱导调控 β-1,3-葡聚糖酶的表达受激素调节而分布于植物体内(Wojtkowiak et al., 2013)。在番茄中，几丁质酶基因*Chi9*和β-1,3-葡聚糖酶基因*Glub*的表达受激素和伤害的特异性调控，MeJA和伤害诱导了GIB-1种子低水平的*Glub*表达，两者通常在番茄种子萌发过程中同时表达(Wu, Bradford, 2003)。在烟草的培养细胞和组织中，β-1,3-葡聚糖酶受到ET的诱导，同时也受生长素和细胞分裂素组合诱导表达下调，在烟草髓细胞悬液和培养的叶片外植体中ABA诱导下调了β-1,3-葡聚糖酶基因的转录，增加了ABA对植物防御反应的影响(Rezzonico et al., 1998)。在香蕉果实成熟期，ET能够强烈诱导β-1,3-葡聚糖酶的转录本累积，而ABA只能诱导部分β-1,3-葡聚糖酶基因的表达，降低香蕉果肉的软化(Roy Choudhury et al., 2010)。

顺式元件作用 王合春等(2013)发现花生β-1,3-葡聚糖酶基因启动子Ah-Glu-P能够被SA诱导，

表3　植物β−1,3−葡聚糖酶在抗病基因工程中的应用研究

Table 3　Application of plant β−1,3−glucanase in disease resistance genetic engineering

基因来源 Gene source	基因名称 Gene name	受体植物 *Receptor plant*	研究方法 Research method	病原菌 Pathogen	抗病效果 Disease resistance effect	参考文献 Reference
真菌胁迫 Fungi pathogen stress						
三七 *Panax notoginseng*	*PnGlu1*	烟草 *Nicotiana tabacum*	过表达 Overexpression	茄镰刀菌 *Fusarium solani*	增强对茄病镰刀菌的抗性，茉莉酸生物合成基因和病程相关蛋白基因的表达水平显著上调 Enhanced the resistance to F. solani, the expression of jasmonic acid biosynthesis genes and PRs were significantly up-regulated	Taif et al., 2020
甘蔗 *Saccharum spp.*	*ScGluD2*	本氏烟 *N. benthamiana*	瞬时表达 Transient expression	青枯菌, 灰霉病菌 *Pseudomonas solanacearum, Botrytis cinerea*	提高对青枯菌和灰霉病菌侵染的抗性 Improved the resistance to P. solanacearum and B. cinerea	Su et al., 2016
烟草 *N. attenuate*	*Glu*	洋桔梗 *Eustoma grandiflorum*	过表达 Overexpression	灰霉病菌 *Botrytis cinerea*	提高对灰霉病菌的抗性 Improved the resistance to B. cinerea	付晓佳等, 2016;
烟草 *N. attenuate*	*Glu*	花生 *Arachim subgaea*	过表达 Overexpression	花生尾孢菌, 黄曲霉 *Cercospora arachidicola, Aspergillus flavus*	增强对尾孢霉和黄曲霉的抗性 Enhanced the resistance to C. arachidicola and A. flavus	Sundaresha et al., 2010
玉米 *Zea mays*	*ZmGns*	拟南芥 *Arabidopsis thaliana*	过表达 Overexpression	灰霉病菌 *B. cinerea*	增强对灰霉病的抗性 Enhanced the resistance to gray mold	Xie et al., 2015
梨 *Pyrus pyrifolia*	*PpGlu*	烟草 *N. attenuate*	过表达 Overexpression	假单胞菌属, 链格孢属, 镰刀菌属 *Phomopsis sp., Alternaria sp., Fusarium sp.*	抑制了病原菌菌丝的生长 The growth of pathogens hyphae was inhibited	Liu et al., 2013
杨树 *Populus*	*BG2*	杨树 *Populus*	过表达 Overexpression	杨树溃疡病菌 *Dothichiza populea*	提高对杨树溃疡病的抗性 Improved the resistance of poplar canker	牛庆霖等, 2013
紫花苜蓿 *Medicago sativa*	*AGLU1*	高羊茅 *Festuca arundinacea*	过表达 Overexpression	立枯丝核菌, 稻瘟病菌 *R. solani, M. grisea*	增强对灰斑病和褐斑病的抗性 Enhanced the resistance to gray spot and brown spot	Dong et al., 2007
马铃薯 *Solanum tuberosum*	*gluB20−2*	亚麻 *Linum usitatisimum*	过表达 Overexpression	尖孢镰刀菌, 黄瓜镰刀菌 *Fusarium oxysporum, Fusarium culmorum*	提高对尖孢镰刀菌和黄瓜镰刀菌的抗性 Improved the resistance to F. oxysporum and F. culmorum	Wróbel-Kwiatkowska et al., 2004
细菌胁迫 Bacterial pathogen stress						
大豆 *G. max*	*SGN1*	烟草 *N. attenuate*	过表达 Overexpression	丁香假单胞菌 *Pseudomonas syringae*	SGN1的表达受多种防御相关信号以及丁香假单胞菌侵染的强烈诱导 The expression of SGN1 was strongly induced by a variety of defense-related signals and P. syringae infection	Cheong et al., 2000

其所驱动的GUS活性是未经SA处理的1.45倍，且β-1,3葡聚糖酶基因的表达量提高了1.8倍。Vande Rhee等(1993)将碱性葡聚糖酶基因的启动子区域融合到*GUS*基因中，发现碱性葡聚糖酶启动子1476 bp的上游序列能够响应烟草花叶病毒(*Tobacco mosaic virus*, TMV)感染和ET处理的表达。Vögeli-Lange等(1994)研究了融合于烟草I类β-l,3-葡聚糖酶B基因(GLB)上游1.6 kb序列的嵌合报告基因GUS在转基因烟草植株中的表达，发现GLB启动子在幼苗的根中高度表达，在成熟烟草植株中GLB启动子主要在下部叶和根中表达，并且该启动子主要在叶片中响应ET和TMV处理。Li等(2005)发现在转基因水稻中，大麦β-1,3-葡聚糖酶的同功酶基因*GⅢ*的启动子能够被稻瘟病菌诱导表达，并且所驱动的GUS活性明显增强，提高了β-1,3葡聚糖酶基因在水稻植株中的表达。

上述研究结果表明，植物几丁质酶和β-1,3-葡聚糖酶均属于多基因家族蛋白，参与了植物生命周期的各个方面，存在时空表达特性，该表达特性取决于发育时期和环境条件，即几丁质酶和β-1,3-葡聚糖酶在植物不同发育阶段和不同组织器官中的表达模式存在差异，且受植物激素和逆境胁迫的诱导表达以及启动子顺式元件的调节作用。大量证据表明，植物几丁质酶和β-1,3-葡聚糖酶均能够抑制多种病原菌的生长增殖，其转基因植株显示出高抗菌能力，日益成为植物病害防治的有效途径。

3 植物几丁质酶和β−1,3−葡聚糖酶的协同抗病性研究

几丁质和β-1,3-葡聚糖均为病原真菌细胞壁的主要成分，大多数真菌细胞壁的外层覆盖有大量葡聚糖，β-1,3-葡聚糖酶可催化水解表层的葡聚糖，促使内层的几丁质暴露出来，以便几丁质酶发挥作用，因此，植物几丁质酶和β-1,3-葡聚糖酶的协同抑菌作用研究也成为人们关注的焦点。植物几丁质酶和β-1,3-葡聚糖酶均属于典型的PR蛋白，两者具有直接侵入和杀死病原菌的潜在能力，有效抑制病原的繁殖和生长，在植物抗病途径中存在密切的相关性(Wojtkowiak et al., 2013; Kumar et al., 2018)。当植物受到病原物、激素和机械损伤等诱导因子的诱导时，几丁质酶与β-1,3-葡聚糖酶能够协同出现(Vogeli et al., 1988)。在1988年，Mauch等(1988)最早将纯化的豌豆(*Pisum sativum*)几丁质酶和β-1,3-葡聚糖酶进行体外抑菌试验，发现添加单一的β-1,3-葡聚糖酶和几丁质酶对多数真菌的抑制效果不明显，而当二者共同作用时却对18种真菌的生长表现出抑制效果，证实了这两个酶具有协同抑菌作用(Mauch et al., 1988)。Zhu等(1994)研究显示，与单独表达β-1,3-葡聚糖酶或几丁质酶基因的植物相比，共表达β-1,3-葡聚糖酶和几丁质酶基因的转基因烟草对真菌病原体产生的抗性明显增强。Sela-Buurlage等(1993)发现I类几丁质酶和β-1,3-葡聚糖酶对茄病镰刀菌具有抑制作用，二者能够协同表达。

目前，几丁质酶和β-1,3-葡聚糖酶的协同抗病性已在甘蔗、烟草、甜瓜(*Cucumis melo*)、油菜(*Brassica napus*)等多种植物中被证实，并被广泛应用于植物抗病基因工程中(表4)。Moravčíková等(2007)将黄瓜Ⅲ类几丁质酶和烟草I类β-1,3-葡聚糖酶基因共同导入马铃薯中，发现从转基因微型薯中分离出的蛋白粗提物对立枯丝核菌的菌丝生长具有抑制作用。张志忠等(2005)将番茄几丁质酶基因(*Chi3*)和β-1,3-葡聚糖酶基因(*Glu-Ac*)遗传转化到西瓜(*Citrullus lanatus*)中育一号中，尖孢镰刀菌接种实验表明转基因植株对枯萎病的抵抗力提高。程红梅等(2005)构建了4个单价(pBCI、pBCE、pBGI、pBGE)和2个双价(GCI、pGCE)的植物表达载体，将菜豆几丁质酶基因(*Chi-E*/*Chi-I*)与橡胶(*Hevea brasiliensis*) β-1,3-葡聚糖酶基因(*Glu-E*/*Glu-I*)通过花粉管通道法导入棉花中，培育的棉花转基因株系对枯萎病和黄萎病的抗性增强。Wu等(2004)将竹节花(*Dianthus chinensis*)黄斑病毒启动子(CoYMV)启动的几丁质酶基因(*Chi*)和CaMV35S启动子启动的β-1,3-葡聚糖酶基因(*Glu*)转化到棉花基因组中，对T3代植株进行抗病性检测，发现有7个品系转基因植株对黄萎病菌(*Verticillium dahliea*)具有抗性或耐受性。顾丽红等(2008)构建了含有烟草Ⅰ类几丁质酶基因*Chi*和烟草I类β-1,3-葡聚糖酶基因*Glu*的双价表达载体，通过农杆菌转化到甘蔗(ROC10和ROC22)中，发现转基因植株的汁液粗提物对甘蔗黑穗病菌表现出不同程度的抑制作用。蓝海燕等(2000b)将菜豆几丁质酶基因*Chi*与烟草β-1,3-葡聚糖酶基因*glu*共转化到甘蓝型油菜中，接种油菜菌核病菌后，与未转化的对照相比，转基因植株对油菜菌核病的抗性明显增强。此外，菜豆几

表4　植物几丁质酶与β−1,3−葡聚糖酶基因的协同抗病性研究

Table 4　Studies on synergistic disease resistance of plant chitinase and β−1,3− glucanase genes

基因来源 Gene source	基因名称 Gene	受体植物 Receptor plant	研究方法 Research method	病原菌类型 Pathogen type	抗病效果 Disease resistance effect	参考文献 References
水稻, 苜蓿	*RCH10*	油棕	过表达	白腐菌	增强对白腐病的抗性	Hanin et al.,
Oryza sativa, Medicago sativa	*AGLU1*	*Elaeis guineensis*	Overexpression	*Ganoderma boninense*	Enhanced the resistance to white rot	2020
水稻, 苜蓿	*RCH10*	水稻	过表达	稻瘟病菌	提高对稻瘟病的抗性	Mao et al.,
O. sativa, M. sativa	*AGLU1*	*O. sativa*	Overexpression	*Magnorpathe oryzae*	Improved the resistance to rice blast	2014
橄榄绿链霉菌, 大麦	*Chit30*	豌豆	过表达	哈茨木霉	对哈茨木霉的孢子萌发有较高的抑制作用	Amian et al.,
Streptomyces olivaceoviridis, Hordeum vulgare	*gluc*	*Pisum sativum*	Overexpression	*Trichoderma harzianum*	Showed higher inhibition to the spore germination of T. harzianum	2011
小麦	*Chi83*	胡萝卜	过表达	灰霉病菌, 菌核病菌	增强对灰霉病菌和菌核病菌的抗性	Wally et al.,
Triticum aestivum	*Glu638*	*Daucus carota*	Overexpression	*Botrytis cinerea, Sclerotinia sclerotiorium*	Enhanced the resistance to B. cinerea and S. sclerotiorium	2009
小麦	*chitinase*	葡萄		霜霉病菌	增加了对霜霉病菌的抗性	Nookaraju,
T. aestivum	*β−1,3−glucanase*	*Vitis vinifera*		*Plasmopara viticola*	Enhanced the resistance to P. viticola	Agrawal, 2012
烟草	*Chi*	甘蔗	过表达	甘蔗鞭黑粉菌	对甘蔗黑穗病菌有抑制作用	顾丽红等,
Nicotiana tabacum	*Glu*	*Saccharum* spp.	Overexpression	*Sporisorium scitanminea*	It had inhibitory effect on S. scitanminea	2008
黄瓜, 烟草	*CHIT*	马铃薯	过表达	立枯丝核菌	抑制立枯丝核菌的生长	Moravčíková
Cucumis sativus, N. attenuate	*GLUC*	*Solanum tuberosum*	Overexpression	*Rhizoctonia solani*	Inhibited the growth of R. solani	et al., 2007
菜豆, 橡胶	*Chi−E, Chi−I*	棉花	过表达	枯萎病菌, 黄萎病菌	增强对枯萎病和黄萎病的抗性	程红梅等,
M. phaseolina, Hevea brasiliensis	*Glu−E, Glu−I*	*Gossypium spp*	Overexpression	*Fusarium oxysporum f. sp. vasinfectum, Verticillium dahliea*	Enhanced the resistance to Fusarium and Verticillium wilt	2005
番茄	*Chi3*	西瓜	过表达	尖孢镰刀菌	增强对枯萎病的抗性	张志忠等,
Lycopersicon esculentum	*Glu−Ac*	*Citrullus lanatus*	Overexpression	*Fusarium oxysporu*	Enhanced the resistance to blight wilt	2005
棉花	*Chi*	棉花	过表达	黄萎病菌	增强对黄萎病的抗性	吴家和等,
Gossypium spp	*Glu*	*Gossypium spp*	Overexpression	*V. dahliea*	Enhanced the resistance to Verticillium wilt	2004
芥菜, 橡胶	*Chitinase*	烟草	过表达	炭疽病菌, 黑胫病菌	增强对炭疽病的抗性	黎定军等,
Brassica juncea, H. brasiliensis	*β−1,3−glucanase*	*N. attenuate*	Overexpression	*Collectrichum, phytophthora*	Enhanced the resistance to anthracnose	2002
菜豆, 烟草	*Chi*	玉米	过表达	立枯丝核菌	增强对纹枯病的抗性	黄珏, 2013
M. phaseolina, N. tabacum	*glu*	*Zea mays*	Overexpression	*R. solani*	Enhanced the resistance to sheath blight	
菜豆, 烟草	*Chi*	甘蓝型油菜	过表达	菌核病菌	增强对菌核病的抗性	王新发, 2005
M. phaseolina, N. attenuate	*glu*	*Brassica napus*	Overexpression	*Sclerotinia sclerotiorium*	Enhanced the resistance to S. sclerotiorum	
菜豆, 烟草	*Chi*	甘蓝型油菜	过表达	菌核病菌	增强对菌核病的抗性	蓝海燕等,
M. phaseolina, N. attenuate	*glu*	*B. napus*	Overexpression	*S. sclerotiorium*	Enhanced the resistance to S. sclerotiorum	2000b
菜豆, 烟草	*Chi*	烟草	过表达	链格孢菌	增强对链格孢菌的抗性	蓝海燕等,
M. phaseolina, N. attenuate	*glu*	*N. attenuate*	Overexpression	*A. alternata*	Enhanced the resistance to A. alternata	2000a

丁质酶基因*Chi*和烟草β-1,3-葡聚糖酶基因*glu*还能提高玉米对丝核菌的抗性以及烟草对链格孢菌的抗性(王新发, 2005; 黄珏, 2013)。从小麦中分离的几丁质酶和葡聚糖酶基因在葡萄(*Vitis vinifera*)中的过表达增加了葡萄对霜霉病菌(*Plasmopara viticola*)的抗性(Nookaraju, Agrawal, 2012)。Wally等(2009)将编码酸性小麦Ⅳ类几丁质酶基因(Chi383)、小麦β-1,3-葡聚糖酶基因(*Glu638*)和水稻阳离子过氧化物酶基因(cationic peroxidase, *POC1*)单独或组合导入胡萝卜,接种灰霉病菌和菌核病菌(*Sclerotinia sclerotiorium*)后,6个单独表达*Glu638*的转基因株系对灰霉病菌的抗性没有增强,但对核盘菌有轻微的抗性;仅表达*Chi383*的六个转基因株系中有两个对这两种病原菌的耐受性增强;共表达*Glu638*和*Chi383*的两个转基因株系的病症较对照减少10%~20%。Hanin等(2020)将水稻几丁质酶基因RCH10和苜蓿β-1,3-葡聚糖酶基因*AGLU1*转化油棕榈(*Elaeis guineensis*),获得了抗白腐菌(*Ganoderma boninense*)的转基因油棕榈。Mao等(2014)将另一种水稻碱性几丁质酶基因(*RCH10*)和苜蓿β-1,3-葡聚糖酶基因(*AGLU1*)通过农杆菌导入水稻中,接菌结果表明*RCH10*和*AGLU1*的共表达赋予水稻对水稻纹枯病和稻瘟病的抗性;免疫胶体金检测结果显示,RCH10和AGLU1蛋白最初主要定位于叶绿体中,并在感染后被输送到液泡和细胞壁,表明这些亚细胞定位点是抗真菌蛋白RCH10和AGLU1的聚集和功能执行区域;此外,转基因种子表现出较低的发芽率和幼苗活力,推测转基因水稻的防御增强可能会以牺牲发育为代价来实现。

综上,几丁质酶和β-1,3-葡聚糖酶基因在植物抗真菌病基因工程中显示出良好的应用前景,并证实了双价转基因植株的抗病性普遍高于单价转基因植株,两种酶类对病原菌的抑制作用不是简单的累加效应,而是协同互补增效作用。

4 总结与展望

病虫害是农业大量减产和亏损的主要原因之一。目前,我国防治病虫害的主要方式是依靠化学药剂,对产品质量安全和生态环境造成较大影响。几丁质酶与β-1,3-葡聚糖酶作为植物防御体系中重要的防卫因子,具有广谱性、稳定性、安全性等特点,同时两者在诱导植物抗性防御反应方面具有协同增效作用。随着基因工程技术的发展,越来越多的几丁质酶和β-1,3-葡聚糖酶基因被转导目标作物,以实现既提高植物抗病虫性,又减少化学农药残留,促进环保型生态农业的建设。这凸显了几丁质酶和β-1,3-葡聚糖酶在植物抗病基因工程的重要作用。

现今,人们对植物几丁质酶和β-1,3-葡聚糖酶的结构和作用机制有了更多理解。然而,尚有很多问题需要进一步深入探究,例如,(1) 几丁质酶和β-1,3-葡聚糖酶基因是如何协同参与植物对细菌和病毒的防御? (2) 植物几丁质酶和β-1,3-葡聚糖酶的多样性及其组织器官表达特异性,及其在不同生长阶段和逆境胁迫下的差异表达,是否暗示这两种酶可能在植物生长和发育中还有未知的生物学功能? (3) 是否能够通过创建缺乏一种或几种特定类型的突变植物,了解不同类型几丁质酶或β-1,3-葡聚糖酶之间的特定相互作用? (4) 如何高效地对高酶活性的植物几丁质酶与β-1,3-葡聚糖酶成员进行挖掘鉴定、组合优化、定向改造和功能验证? (5) 几丁质酶和β-1,3-葡聚糖酶介导植物抗病性的生理生化作用和分子机制的系统解析,将能科学探究两种酶的双向交叉对话? 以上研究将有力助推植物几丁质酶和β-1,3-葡聚糖酶在农业方面的应用。

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