

芥子油苷-黑芥子酶防御系统的最新研究进展

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摘要: 芥子油苷是一类含氮、硫的植物次生代谢物质, 与其水解酶黑芥子酶共同组成了十字花目植物特有的防御系统。黑芥子酶和芥子油苷分别储藏在植物的不同细胞或同一细胞的不同区域。当植物受到机械损伤或病虫害侵袭时, 底物和酶接触, 芥子油苷被降解成多种具有生物活性的物质。这些降解产物可对昆虫及病原菌表现出较强的毒害作用, 也有可能作为信号分子启动植物其它防御机制, 如气孔的关闭、细胞程序性死亡及细胞壁胼胝质累积等。本文对近年来黑芥子酶防御系统的最新研究成果进行了综述, 介绍了芥子油苷和黑芥子酶的合成及分布、芥子油苷降解产物的多样性、芥子油苷-黑芥子酶系统对昆虫和病原菌的防御作用和防御机制及芥子油苷的代谢与初生代谢的关系, 并对目前该领域研究中存在的问题和研究趋势进行了讨论。

关键词: 芥子油苷; 黑芥子酶; 降解产物; 细胞程序性死亡; 气孔关闭; 胼胝质累积

1 芥子油苷-黑芥子酶系统的简介

芥子油苷(glucosinolates, GS)是一类特异性存在于十字花目植物中的次生代谢产物(Agerbirk和Olsen 2012)。芥子油苷类化合物的结构首次发现于1956年(Ettlinger和Lundeen 1956), 1961年“glucosinolate (芥子油苷)”一词首次出现, 迄今为止, 已确定了大约136种不同的芥子油苷结构(Agerbirk和Olsen 2015)。芥子油苷主要分布于十字花目的16个科中, 其中十字花科、白花菜科和番木瓜科中含量较多, 尤其是十字花科中的芸苔属植物中最多(Fahey等2001; Clarke 2010)。芥子油苷通常由 β -D-硫葡萄糖基、硫化脲基团以及来源于氨基酸的侧链组成。根据侧链的氨基酸来源可以将芥子油苷分为脂肪族芥子油苷(侧链来源于甲硫氨酸、丙氨酸、缬氨酸、亮氨酸和异亮氨酸)、芳香族芥子油苷(侧链来源于苯丙氨酸和酪氨酸)和吲哚族芥子油苷(侧链来源于色氨酸)(Reichelt等2002)。芥子油苷本身通常没有直接的生理功能, 需要在黑芥子酶的催化下分解成多种具有生理活性的代谢产物, 这些代谢产物在对食草性动物及多种微生物的防御性反应中起到重要作用(Stotz等2011; Calmes等2016; Dufour等2015; Tierens等2001)。

β -硫代葡萄糖苷酶, 又称黑芥子酶(myrosinase), 能催化芥子油苷降解, 于1840年首次报道(Bussy 1840), 属于糖苷水解酶家族I (thioglucoside glucohydrolases, TGG) (Rask等2000; Morant等2008)。黑芥子酶性质稳定, 通常以被糖基化的二聚体蛋白质形式存在(Zhou等2012; Natarajan等2015)。不同的植物或者相同植物不同组织部位黑芥子酶的活性

不同, 通常情况下, 在种子和幼苗时期黑芥子酶的活性最高(Lenman等1993)。一种黑芥子酶可以催化多种芥子油苷, 但效率不同(Bernardi等2003)。目前已在模式植物拟南芥中发现了7个编码黑芥子酶的基因, 其中TGG1和TGG2在地上部位表达, 编码的黑芥子酶主要分解脂肪族芥子油苷和吲哚族芥子油苷(Xue等1995); TGG3和TGG6是假基因(Zhang等2002; Andersson等2009); TGG4和TGG5仅在根部特异表达, 并且与根部的生长素合成有关(Fu等2016); 还有PEN2, 它编码的黑芥子酶能够分解吲哚族芥子油苷(Bednarek和Osborn 2009)。

芥子油苷与黑芥子酶共同组成了植物中特殊的底物-酶系统, 即芥子油苷-黑芥子酶系统(glucosinolate-myrosinase system)。该系统被认为是十字花科植物重要的防御系统, 它对植物防御病原菌侵染及动物和昆虫啃噬等方面有重要作用(徐文佳等2008; Clay等2009; Mullaney 2013; Agerbirk和Olsen 2015)。

2 芥子油苷及黑芥子酶的组织及细胞定位

通常情况下芥子油苷和黑芥子酶存在于不同的细胞或者同一细胞的不同区域, 形成“芥子油弹(mustard oil bomb)”, 当植物受到破坏后(如创伤或昆虫取食), 芥子油苷会降解成多种具有生物活性的化合物, 从而起到化学防御作用(Koroleva等2000; Andréasson等2001; Husebye等2002)。因此, 芥子

收稿 2017-10-12 修定 2017-11-16

资助 黑龙江省教育厅科学技术研究项目(12531003)和黑龙江省科学基金项目(JJ2017ZR0164)。

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油苷-黑芥子酶系统的合成部位以及储存位置与其防御作用密切相关。

2.1 芥子油苷的合成及分布

不同种类的芥子油苷合成部位不同,通常情况下,吲哚族芥子油苷的合成在韧皮部,脂肪族芥子油苷的合成在木质部和韧皮部(Mikkelsen等2000; Chen等2003; Tantikanjana等2004)。关于芥子油苷的储存部位,目前仅在拟南芥中有报道,芥子油苷主要储存在富含硫的S细胞中, S细胞中硫元素高度富集,浓度高达 $130 \text{ mmol}\cdot\text{L}^{-1}$,这些S细胞分散于拟南芥维管系统的韧皮部(Koroleva等2010)。但是其他物种是否与拟南芥一样拥有能够积累芥子油苷的S细胞存在至今没有报道。

2.2 黑芥子酶的合成及分布

早在1884年, Heinricher等就在十字花科植物中发现了一些积累蛋白质的异形细胞,这种细胞显示出异常的光折射现象,并且细胞形态和尺寸上与邻近的细胞差异明显,随后Guignard (1890)发现黑芥子酶主要存在于这种异形细胞中,并把这种储存黑芥子酶的细胞命名为芥子细胞(myrosin cells)。后来通过黑芥子酶基因启动子-报告基因*GUS* (β -glucuronidase)方法证明黑芥子酶的合成也在芥子细胞中,并且细胞内的黑芥子酶蛋白颗粒相互紧密相连以芥子酶小体的形式存在。这些芥子细胞通常分散在根、茎、叶韧皮部的韧皮薄壁细胞之间及叶肉中(Bones和Rossiter 1996; Kissen等2009)。除了芥子细胞,黑芥子酶还在保卫细胞中大量存在(Kelly等1998; Andréasson等2001; Husebye等2002; Thangstad等2004)。

2.3 Specifier proteins的分布

由于specifier proteins只有在黑芥子酶和芥子油苷存在时才具有生理活性,所以它们并不需要为了防止“芥子油弹”提前爆炸而与芥子油苷或黑芥子酶空间分离。ESP是specifier protein家族中含量最多的蛋白质,目前关于ESP的定位研究比较清楚。ESP在拟南芥中的分布与其生态型有关。Ler (*Landsberg erecta*)型拟南芥的ESP几乎完全分布在除花药外所有地上器官的表皮中,有时也会暂时存在于茎中S细胞中。Cvi (Cape Verde Island)型拟南芥的ESP存在于叶片的S细胞中。WS (*Wassilewskija*)型拟南芥的ESP似乎与NSP1和NSP5一起存在于保

卫细胞中(Burow等2007; Zhao等2008)。而其他种类specifier protein的表达模式仍不太明确。当植物遭到破坏(如昆虫咀嚼)后,在这些特定的位置上ESP会与芥子油苷以及黑芥子酶混合,从而影响芥子油苷降解产物的类型。

3 芥子油苷降解产物的多样性

芥子油苷的降解是很复杂的过程,植株在被昆虫啃噬等机械损伤情况下,组织会遭到破坏,体内的芥子油苷和黑芥子酶会相互接触,芥子油苷会先分解成非常不稳定的糖苷配基(aglycone),然后进一步分解重排成不同的物质。大体上可以把降解产物分为五类,分别是:硫代氰酸盐(thiocyanate)、异硫氰酸盐(isothiocyanate, ITC)、腈类(nitrile)、环硫腈(epithionitrile)和唑烷-2-硫酮(oxzolidine-2-thione) (Bones和Rossiter 1996; Petersen等2002) (图1),以ITC为主。影响芥子油苷最终产物类型的因素有很多,主要包括:(1)降解过程中的反应条件(pH、温度等)及是否添加金属离子。例如在有 Fe^{2+} 或 $\text{pH}<5$ 存在下,降解产物以腈为主,在中性条件下芥子油苷主要会分解成ITC (Tsao等2002)。(2) Specifier proteins的存在与否。Specifier protein于1973年首次从海甘蓝(*Crambe abyssinica*)中分离出来,命名为表皮特异硫蛋白(epithiospecifier protein, ESP), ESP并不是分解芥子油苷的酶类,但它的存在能促进芥子油苷降解形成非ITC类物质,作用机制目前尚不清楚。根据specifier proteins存在时芥子油苷降解产物的类型将其分成三类: nitrile-specifier proteins (NSPs)、epithiospecifier proteins (ESPs)和thiocyanate-forming proteins (TFPs)。NSPs可以促进不同类型的芥子油苷形成单腈; ESPs不仅促进芥子油苷形成单腈,还可以促进侧链末端含有双键的芥子油苷形成环硫腈(Burow等2009; Kissen和Bones 2009),而TFPs除了促进芥子油苷形成单腈外还可以形成硫代氰酸盐(Lüthy等1977)。绝大多数specifier proteins含有保守的 Fe^{2+} 结合位点(Brandt等2013), Fe^{2+} 对specifier proteins活性至关重要。当ESP存在时,向黑芥子酶、芥子油苷的混合物中加入 Fe^{2+} 或其他金属离子会减少ITC的形成而增加其他产物的形成,但芥子油苷降解产物总量不变。而向由AtESP、烯丙基芥子油苷、黑芥子酶、 Fe^{2+} 组成的混合物加入黑芥子酶激活剂抗坏血酸会增加ITC

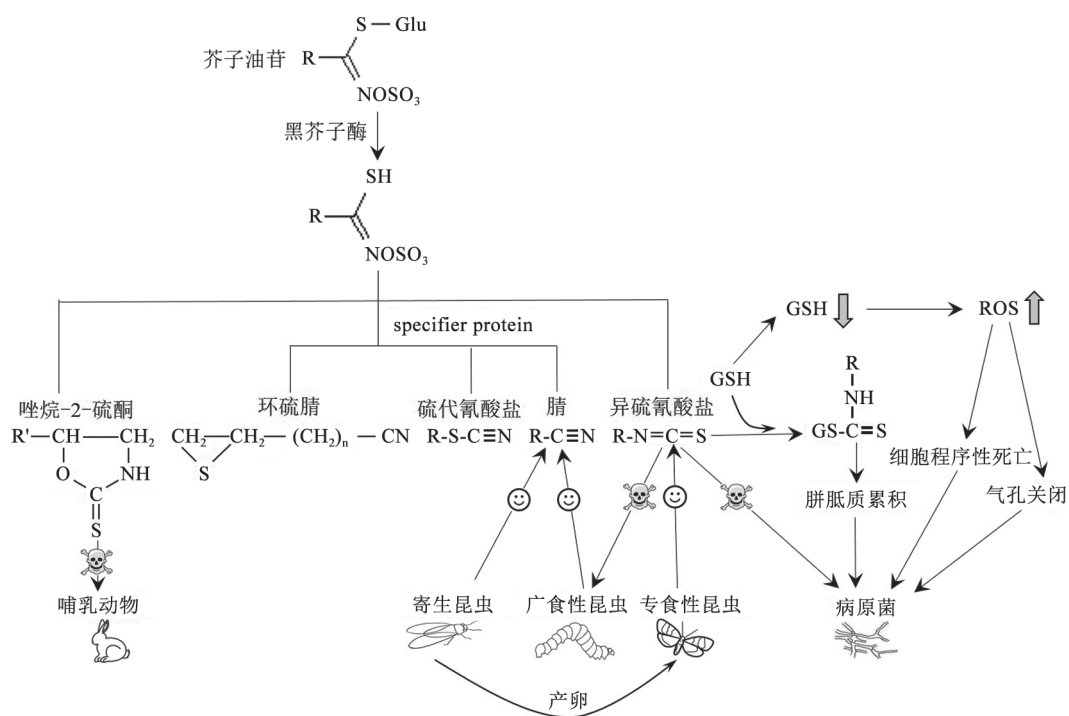


图1 芥子油苷的降解及其产物在植物防御中的作用

Fig.1 The breakdown of the glucosinolate and the role of its products in plant defense

的形成, 而其他产物的含量不变, 导致最终分解产物总量增加(Matusheski等2006; Burow等2007; Kuchernig等2011)。根据这些证据推测也不是黑芥子酶的辅助因子, 很可能是一种可以催化芥子油苷中间产物形成非ITC类物质的酶(Wittstock和Burow 2007), 但是由于中间产物的极度不稳定性, 至今分离不出来, 产物形成的机制也至今未知。

在这五类芥子油苷降解产物中, ITC对病原菌和昆虫等具有很高的毒性, 被认为是含有芥子油苷植物的主要防御化合物。然而, 由于specifier protein的存在而形成的单腈等化合物, 不仅毒性很小, 甚至还会吸引一些广食性昆虫的取食(Burow等2006)。那么specifier protein的产生在进化上到底有什么意义呢? 植物与昆虫的相互作用会引起两者的协同进化, 现在一些专食性昆虫已经进化出对ITC的解毒能力, 甚至ITC会吸引专食性昆虫对植株进行取食及产卵。这时植物通过产生specifier protein减少ITC的形成会减少对专食性昆虫的吸引, 例如研究发现专食性菜粉蝶(*Pieris rapae*)会在产生ITC较少而单腈较多的植物上产更少的卵。此外, 这些单

腈类产物可吸引某些专食性昆虫天敌如寄生蜂等, 这些寄生性昆虫会在专食性昆虫体内产卵并最终消灭寄主昆虫(Mumm等2008; Heil 2014)。可见, 虽然specifier protein诱导产生的单腈化合物不能对取食昆虫产生毒性, 但却可以通过减少对专食性昆虫的吸引以及吸引其天敌来进行防御(图1)。由此可见, 在植物与昆虫的共同进化过程中, 植物已经形成了一套对付昆虫的防御系统。芥子油苷分解产物的多样性为植物提供了更多的选择, 使植物在不同的环境下具有更多的生存优势。

4 芥子油苷-黑芥子酶系统的防御机制

4.1 直接的化学防御

黑芥子酶催化芥子油苷降解后产生的ITC具有最高的化学活性, 它对昆虫、食草动物、线虫、真菌和卵菌等均有毒性(Tierens等2001; Agrawal和Kurashige 2003; Stotz等2011), 甚至浓度很低的ITC也会对病原菌的生长产生负面影响(Calmes等2015; Dufour等2015)。在体外和植物体内用生物检定法检测了几种由芥子油苷降解产生的ITCs, 包括烯丙基- (allyl-)、苄基- (benzyl-)和4-甲基亚磺酰基丁

基-(4-methylsulfinylbutyl)-ITC, 发现这些化合物对细菌和真菌植物病原体具有广泛抗菌活性(Tierens等2001; Sellam等2007)。相比需要specifier protein才能生成的腈类等降解产物而言, 芥子油苷在多数情况下会降解生成ITC, 甚至程度很少的组织损害(如寄生菌的侵染等)也会激活植物体内的脂肪族芥子油苷产生ITC, 从而影响植物与寄生菌的相互作用(Sonderby等2007; Buxdorf等2013)。同时在*myb28* (长链和短链芥子油苷含量均减少)和*myb29* (仅短链芥子油苷含量减少)突变体拟南芥的叶片上接种核盘菌(*Sclerotinia sclerotiorum*), 发现*myb28*突变体对核盘菌更敏感, 但*myb29*突变体则没有明显表型。这说明不同侧链长度的ITC可能对病原菌的抗菌活性是不同的, 长链脂肪族芥子油苷对核盘菌有更强的抗性。体外实验也证明ITC对核盘菌的抗性与侧链长度正相关, 其中8-甲基亚磺酰基辛基(8-methylsulfinyloctyl) ITC对核盘菌生长的抑制能力最强。但是长链ITC是否仅对核盘菌的抗菌能力强或者它对其他菌也具有普遍较高抗菌性尚不清楚(Sønderby等2007; Stotz等2011)。

ITC除了对病原菌产生化学毒性外, 也可对某些啃噬植物的广食性昆虫产生毒性; 其他的降解产物如单腈可以吸引十字花科植物专食性昆虫的天敌, 而一些脂肪族芥子油苷在侧链经过修饰后降解产生的噻烷-2-硫酮可对哺乳动物产生化学毒害作用(Mumm等2008; Heil 2014) (图1)。总之, 芥子油苷的降解产物可通过其化学活性对多种昆虫、病原菌及食草动物产生直接的化学防御反应。

4.2 诱导气孔关闭

芥子油苷的降解产物除了可以通过自身的化学性质来参与植物的直接防御外, 也参与调控植物界高度保守的免疫过程(Bednarek 2012; Maag等2014)。许多致病微生物可通过叶片上存在的天然开口——气孔来实现对植物最初的感染, 因此抵御微生物入侵的气孔闭合机制被认为是植物界保守的免疫机制之一(Melotto等2006; Zeng等2010)。黑芥子酶TGG1是拟南芥保卫细胞中含量最丰富的蛋白质之一, 在*tgg1 tgg2*双缺失突变体中, ABA诱导气孔关闭的过程受到了抑制(Zhao等2008; Ahuja等2016)。进一步的研究证明外源的ITCs能够诱导气孔闭合, 其机理与谷胱甘肽(glutathione, GSH)的

代谢密切相关(Khokon等2011; Hossain等2013)。谷胱甘肽是植物体内维持细胞氧化还原动态平衡的重要调节因子, 它可以与活性氧(reactive oxygen species, ROS)结合参与植物体内的氧化还原过程, 影响细胞程序性死亡(Andersson等2015; Johansson等2014)。谷胱甘肽还可以作为气孔关闭的ABA信号途径中的负调节因子, 它可以通过抑制ROS的产生而抑制ABA诱导气孔关闭过程(Munemasa等2013)。研究发现ITCs可以与谷胱甘肽结合, 例如一些广食性鳞翅目幼虫可以通过ITC与谷胱甘肽结合而解除ITC的毒性(Schramm等2012; Jeschke等2016)。ITCs通过与谷胱甘肽结合, 促进了ROS的产生, 而引发气孔关闭(图1)。

4.3 诱导细胞程序性死亡

通过识别病原体分泌的效应蛋白引发超敏反应(hypersensitive response, HR)也是植物中一种复杂而且保守的免疫机制, 可引发感染部位细胞的程序性死亡(programmed cell death, PCD)并且激活其他部位和全身组织的防御反应(Mur等2008; Shah 2009; Coll等2011)。研究表明拟南芥叶组织在经历HR时脂肪族芥子油苷(4-甲基硫氧丁基芥子油苷)的降解产物萝卜硫素(sulforaphane)会被释放, 并且该化合物能够诱导细胞程序性死亡以及组织中的防御反应(Andersson等2015)。在萝卜硫素缺失的*tgg1 tgg2*突变体和*myb28 myb29*突变体上接种细菌菌株丁香假单胞菌(*Pseudomonas syringae*)和霜霉病海乳白僵菌(*Hyaloperonospora arabidopsidis*), 会发现这两种突变体均表现出减少或延迟细胞程序性死亡现象(Andersson等2015)。这表明脂肪族芥子油苷的降解产物ITCs参与了HR过程。研究还发现*pen2*和*pen3*突变体拟南芥在被丁香假单胞菌和霜霉病海乳白僵菌感染后也会影响HR过程。PEN2是分解吲哚族芥子油苷的黑芥子酶, 而PEN3是将由PEN2分解的产物运到细胞外的载体(Johansson等2014)。因此推测吲哚族芥子油苷的降解产物也参与了HR过程(Zhao等2015)。而ITCs在PCD过程中的作用可能也是通过减少谷胱甘肽含量实现的(图1) (Andersson等2015)。

4.4 参与胼胝质累积

植物界的另一个保守的防御反应胼胝质的累积, 被认为是细胞壁防御病原菌的物理屏障(EI-

linger和Voigt 2014)。研究表明吲哚族芥子油苷的代谢过程参与了flg22诱导叶片细胞胼胝质沉积过程(Bednarek和Osborn 2009; Clay等2009)。在吲哚族芥子油苷或其代谢产物缺失的各种突变体中flg22诱导的胼胝质累积反应受损,而脂肪族芥子油苷及其降解产物的缺失则没有相似的表型。这表明在响应flg22过程中胼胝质的积累主要受吲哚族芥子油苷代谢的影响,而脂肪族芥子油苷对胼胝质的积累不起主要作用(Clay等2009)。其中4-甲氧基-吲哚-3-甲基芥子油苷(4MI3G)在恢复flg22诱导胼胝质累积过程起到了关键作用。进一步的研究发现真正在flg22诱导胼胝质积累过程中起作用的是4MI3G的降解产物,其降解产物由PEN3当作载体运输到体外作为诱导胼胝质积累的信号分子,但其降解产物至今未知。由此可见除了芥子油苷的降解产物外, PEN2和PEN3也是胼胝质累积和对抗病原体所必需的(Clay等2009; Luna等2011; Fuchs等2016)。又有研究发现吲哚族芥子油苷降解产物也参与了拟南芥根中胼胝质累积过程,在吲哚族芥子油苷不完全缺失的突变体中由几丁质诱导的根部胼胝质累积反应会受损(Millet等2010)。这些研究结果表明,吲哚族芥子油苷降解产物可以作为触发胼胝质累积机制的信号分子参与植物广谱抗病性反应(图1)。

5 芥子油苷在完整组织里的代谢

芥子油苷在植物体的含量不是一成不变的,在植物的某些发育阶段芥子油苷的含量可能会下降(McGregor 1988; Petersen等2002; Brown等2003)。这暗示芥子油苷可以在完整组织里发生降解,说明芥子油苷可通过运输作用与黑芥子酶接触来完成分解过程(Maruyama等2006; Ahn等2010)。拟南芥和油菜的种子萌发和幼苗发育过程中芥子油苷的总含量均会减少约30%,但不同种类的芥子油苷含量减少程度是不同的,这表明芥子油苷的降解存在特异性,并且这种降解特异性的现象在植物的不同发育阶段均可观察到(McGregor 1988; Petersen等2002; Brown等2003)。此外,芥子油苷也会随着昼夜交替产生节律性的变化,芥子油苷的含量白天比夜晚高,且长时间的黑暗会导致芥子油苷的含量大量减少,而再重新照射会使得芥子油苷生物合成增加(Rosa和Rodrigues 1998; Huseby等

2013)。而当植物在缺乏氮和硫情况下,黑芥子酶的转录水平被上调,芥子油苷含量会降低,从而释放储存在芥子油苷中的硫。因此人们认为芥子油苷除了可以作为化学防御物质外还可以作为硫的储存库(Hirai等2004; Falk等2007);而硫作为植物生长发育不可缺少的营养元素之一,它在植物的生长发育及代谢过程中有重要的生理功能,参与植物体内许多的重要反应。半胱氨酸(cysteine, Cys)、甲硫氨酸(methionine, Met)是合成蛋白质的必需氨基酸,充足的硫有利于提高富含Cys、Met蛋白质的含量(Tab等2002)。因此可以说芥子油苷代谢是初生代谢的延伸,其整个代谢途径与其他物质代谢途径有着紧密的联系(朱凤羽等2007)。

6 小结和展望

芥子油苷虽然代表相对含量较低的一类次生代谢产物,但是芥子油苷降解产物的多样性为植物提供了很多具有不同物理化学性质的化合物,使芥子油苷-黑芥子酶系统参与了许多植物与环境相互作用过程。本文对芥子油苷-黑芥子酶系统的研究进展、在植物中防御作用及机制进行综述,以期对芥子油苷-黑芥子酶系统有更系统深入的认识,并为未来芥子油苷-黑芥子酶系统的研究提供借鉴。

迄今为止,关于芥子油苷-黑芥子酶系统的研究已经取得了一定的进展,但仍有很多方面的问题不甚了解。例如在完整组织里降解芥子油苷的具体是哪个黑芥子酶至今仍不清楚。此外,吲哚族芥子油苷在植物抗菌过程中的研究相对比较完善,而脂肪族芥子油苷在抗菌方面的研究较少。现有关于芥子油苷的防御功能的研究主要集中在叶片中,对于芥子油苷及其降解产物在根中的功能研究较少。芥子油苷降解产物作为信号分子引发植物各种防御反应的途径还很不完善,其分子机制还有待深入探讨。

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Recent research advances on glucosinolate-myrosinase defense system

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Abstract: Glucosinolates are secondary metabolites containing nitrogen and sulfur. Together with their hydrolyase myrosinase, glucosinolates provide a specific defensive mechanism in Brassicaceae order. Glucosinolates and myrosinase are harboured either in different cells or in different compartments of the same cell. When plant tissue is damaged, glucosinolates come into contact with myrosinase and are broken down into a variety of biologically active metabolites. These breakdown products are demonstrated to be very toxic to insects and pathogens. In addition to direct defense, the glucosinolates are found to be functional as signaling molecules, and initiate other plant defense pathways, such as stomatal closure, programmed cell death and callose accumulation. In this paper, we summarized the recent research achievements of the glucosinolate-myrosinase defense system and the several important aspects were reviewed. The biosynthesis and distribution of glucosinolates and myrosinase, the diversity of glucosinolate breakdown products, the defense mechanisms of the glucosinolate-myrosinase system against insects and pathogens and the relationship between glucosinolate degradation and primary metabolism were reviewed. Open questions and future perspectives in this research field were also discussed.

Key words: glucosinolates; myrosinase; degraded products diversity; programmed cell death; stomatal closure; callose deposition

Received 2017-10-12 Accepted 2017-11-16

This work was supported by the Science and Technology Research Project of Heilongjiang Provincial Education Department (Grant No. 12531003) and the Heilongjiang Province Science Foundation Project (Grant No. JJ2017ZR0164).

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