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综述

细胞骨架在植物抗锈病中的作用

宋晓贺[1] 李彦凯[1] 王 娟[2] 王 阳[2] 马 青[2]*

([1]安庆师范大学生命科学学院, 安庆 246133; [2]旱区作物逆境生物学国家重点实验室, 西北农林科技大学植物保护学院, 杨凌 712100)

摘要 锈菌是真菌中一个很大的类群, 由锈菌侵染引起的病害可造成世界范围内大多数重要作物锈病的大流行和严重的产量损失。细胞骨架包括微管和微丝, 在植物生命活动中担负着复杂的生理功能。越来越多的实验证明, 细胞骨架在植物抗锈病中起着重要的作用。该文着重对国内外有关植物与锈菌相互作用过程中细胞骨架重组、活性氧积累、过敏性坏死反应发生、细胞骨架结合蛋白功能、细胞信号转导方面的研究进展进行综述, 为深入了解植物抗锈性遗传机制并最终应用于植物锈病的防治奠定基础。

关键词 锈菌; 细胞骨架; 微管; 微丝

Roles of the Cytoskeleton in Plant-Rust Fungi Interactions

Song Xiaohe[1], Li Yankai[1], Wang Juan[2], Wang Yang[2], Ma Qing[2]*

([1]*College of Life Sciences, Anqing Normal University, Anqing 246133, China*; [2]*State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwestern A&F University, Yangling 712100, China*)

Abstract The rust fungi are a group of widely distributed fungal plant pathogens, which can cause occasional devastating epidemics and yield losses in the world's most important crops. The plant cytoskeleton, which consists of actin filaments and microtubules, plays an important role in plant resistance against rust fungi. In this review, we focus on cytoskeleton dynamic rearrangements, reactive oxidative species accumulation, hypersensitive response, actin filament and microtubule binding proteins, and signaling transduction in plant-rust fungi interactions, to facilitate our understanding of the complex plant resistant mechanisms against rust fungi.

Keywords rust fungi; cytoskeleton; microtubule; actin filament

细胞骨架(cytoskeleton)是细胞质中由微管(microtubule)、微丝(actin filament)和中间纤维(intemediate filament)构成的三维网状系统, 直接或间接参与对外界信号的应答反应过程, 与植物抵抗病原菌侵染和非寄主抗性的表达密切相关, 在植物抗病机理中起着重要作用[1-4]。真菌侵染作为外界刺激, 能够引起植物细胞骨架系统的变化, 造成侵染点周围原生质的聚集、细胞核的迁移、细胞骨架的精细重组、活性氧的迸发和防卫反应的激活等[7-11]。控制细胞骨架动态重组的关键因素是通过微管或微丝结合蛋白(microtubule/actin filament associated proteins, MAPs)来实现的[12-13]。锈菌是一类独特的

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*通讯作者。Tel: 029-87091342, E-mail: maqing@nwsuaf.edu.cn

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*Corresponding author. Tel: +86-29-87091342, E-mail: maqing@nwsuaf.edu.cn

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真菌, 全部为活体寄生菌, 具有寄主专化性, 难以人工培养, 可导致许多作物上发生严重病害, 如小麦、大麦、黑麦、燕麦、玉米、大豆等, 引起世界范围内锈病的大流行, 造成作物严重减产[14]。研究植物和锈菌互作过程中细胞骨架动态变化特征和功能, 有助于在亚细胞和分子水平上揭示其抗锈性遗传机制, 同时对于其他作物抗病性研究也具有重要指导作用。目前已报道, 有6种植物−锈菌互作系统参与了细胞骨架功能的研究, 分别是: 亚麻(*Linum usitatissimum*)-亚麻锈菌(*Melampsora lini*)、豇豆(*Vigna unguiculata*)-豇豆锈菌(*Uromyces vignae*)、小麦(*Triticum aestivum*)-小麦条锈菌(*Puccinia striiformis* f. sp. *tritici*)、小麦(*T. aestivum*)-小麦叶锈菌(*Puccinia triticina* f. sp. *tritici*)、非寄主植物拟南芥(*Arabidopsis thaliana*)-小麦条锈菌(*P. striiformis* f. sp. *tritici*)以及大麦(*Hordeum vulgare*)-小麦杆锈菌(*P. graminis* f. sp. *tritici*)互作系统。本文分别从植物与锈菌互作系统中细胞骨架动态重组、细胞骨架对活性氧(reactive oxidative species, ROS)积累和过敏性坏死反应(hypersensitive response, HR)的影响、细胞骨架结合蛋白的功能以及细胞信号转导方面阐述细胞骨架在植物抗锈病中的重要作用。

1 植物−锈菌互作过程中细胞骨架的重组

从Kobayashi等[15]首次研究细胞骨架在植物与锈菌互作中植物防卫反应的作用至今, 已有20多年的历史。在受到病原菌侵染的植物细胞中, 细胞骨架发生动态重组, 原生质在侵染点周围凝集, 并伴随着细胞核的迁移[6,8,10]。利用免疫细胞化学技术发现, 在亚麻−亚麻锈菌(*M. lini*)互作中, 健康的亚麻叶肉细胞中微管与细胞伸长轴方向垂直排列, 无微管束聚集的现象。微丝从核膜呈辐射状延伸到质膜, 形成完好的网络状结构。非亲和组合中被侵染的及其相邻叶肉细胞中细胞骨架发生明显的重排, 在锈菌侵入前期微管迅速向被侵染细胞移动, 呈辐射状聚集在侵染点周围, 随着锈菌进一步侵入, 吸器形成, 微管的数量开始减少, 直至从被侵染细胞中消失。微丝的重组在锈菌侵入前便已开始, 迅速聚集在被侵染细胞周围[15]。同样, 在豇豆−豇豆锈菌(*U. vignae*)互作系统中, 利用罗丹明−鬼笔环肽染色, 激光共聚焦显微镜观察也发现, 非亲和组合中大量的微丝和微管积累在侵染点周围, 伴随着叶肉细胞内HR的发生, 微管从侵染点周围消失的现象[7]。其他一些非锈菌的寄主或非寄主互作系统中, 包括大麦−豌豆白粉菌(*Erysiphe pisi*)[16]、大豆−大豆疫霉菌(*Phytophthora sojae*)[17]、大麦−大麦白粉菌(*Blumeria graminis* f.sp *hordei*)[18]等, 使用相同的技术, 也同样观察到了微管和微丝重新排列, 聚集在侵染点周围的现象。这说明, 不同的植物种类在抵御不同病原菌侵染时普遍会发生细胞骨架的动态重组, 这种广泛存在的细胞免疫应答反应有助于阻止病原菌的进一步扩展。与细胞骨架动态重组和分布相一致, 在植物与锈菌互作过程中, 利用电子显微镜技术观察被侵染细胞的超微结构发现, 细胞壁增厚、乳突形成、细胞核迁移至侵染位点、粗面内质网、高尔基体、囊泡等寄主细胞器在细胞内重新分布, 聚集在初始侵染点周围[7,15](图1)。说明微丝和微管骨架的重组在植物与锈菌互作过程中起到重要的作用, 参与细胞核、囊泡和内质网、高尔基体等在细胞内的重新分布以及乳突的形成发展过程, 并将这些细胞内物质运输到细胞壁增厚位点, 与植物抵御锈菌侵染的防卫反应以及细胞信号转导有关。

2 植物−锈菌互作过程中细胞骨架对ROS和HR的影响

当植物受到病原菌侵袭时, 抗病寄主会产生一系列的主动防卫反应, 包括细胞质凝集、ROS积累、乳突形成、植保素和病程相关蛋白产生等[6,8]。抗病植物细胞在侵染点处的防卫反应常伴随着细胞骨架的动态重组, 产生局部细胞程序性死亡(programmed cell death, PCD), 限制病原菌的扩展, 使得坏死点以外的组织免于病原菌的危害[19-20]。锈菌作为专性寄生菌, 是以菌丝在细胞间隙生长, 以吸器从寄主细胞内获取营养。胼底质, 以乳突的形式存在, 它的形成是寄主对锈菌侵入所做出的抗性反应, ROS积累和HR产生是植物抵抗锈菌侵染最重要的抗病机制[21-24]。细胞骨架重组与植物抗锈菌侵染过程中ROS积累和HR产生可能起着至关重要的作用。

细胞骨架特异性抑制剂药理学实验为细胞骨架在植物抗锈病中的作用研究提供了有力的证据。细胞松弛素(Cytochalasin)是微丝聚合的抑制剂, 结合在F-肌动蛋白的正(+)端, 阻抑肌动蛋白单体在该部位的聚合或解离, 特异性地抑制微丝功能, 从而加

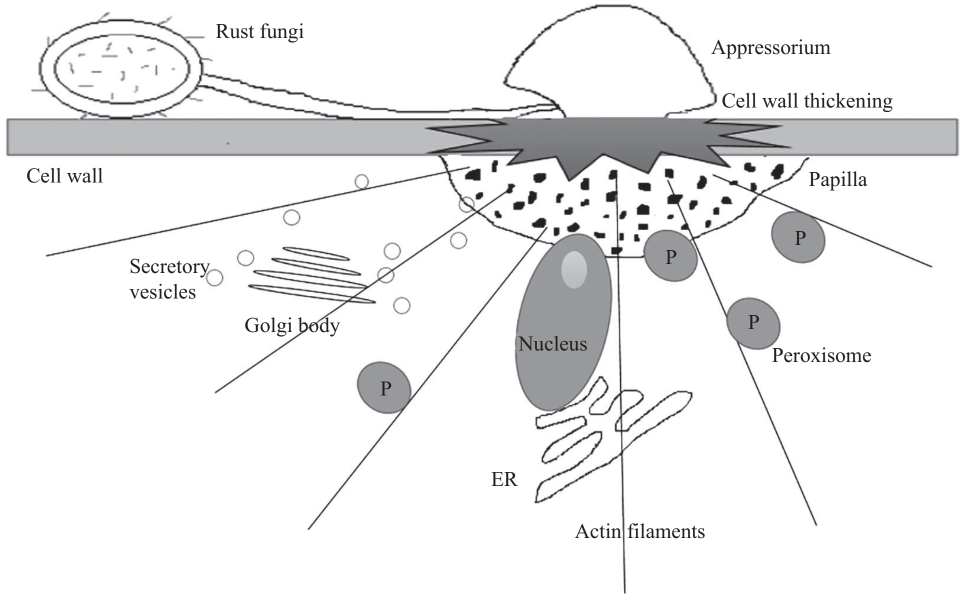

图1 植物−锈菌互作过程中微丝骨架重组和细胞内物质的极性运输

Fig.1 The response of actin cytoskeleton and polarization of secretory component in plant-fungi rust interactions

速病原菌的侵入[25]。目前, 文献报道中用于研究植物−锈菌互作中细胞骨架功能的物质有细胞松弛素A、B、D、E, 其对细胞骨架的影响见表1[26-36]。药理学实验表明, Cytochalasin E处理能够有效地抑制豇豆对豇豆锈菌(*U. vignae*)的防卫反应, 包括核位点迁移、胼底质沉积和HR产生[7,36-37], 也能够降低豌豆−豇豆锈菌(*U. vignae*)互作中H_2O_2积累, 造成锈菌侵染效率的增加[38]。Cytochalasin D处理同样能抑制抗病寄主小麦与小麦叶锈菌(*P. triticina* f. sp. *tritici*)互作中HR的产生, 并且这种抑制作用随着药物浓度的增加而增大[39]。分别用Cytochalasin A和Cytochalasin B处理抗病品种小麦叶片后接种条锈菌(*P. striiformis* f. sp. *tritici*)发现, ROS积累(包括H_2O_2和O_2^-)明显减弱且发生延迟, 叶片上坏死细胞面积增加, HR产生率下降[24,40]。以上实验结果充分说明, 细胞松弛素可明显抑制或延迟抗病寄主小麦、豌豆、豇豆叶肉细胞在抵御锈菌侵染时诱发的活性氧的积累或过敏性坏死反应, 从而抑制寄主细胞抗锈菌扩展的能力, 最终导致锈菌向周围细胞的扩展, 微丝骨架在植物抵御锈菌侵染过程中植物防卫反应的表达和HR产生起到至关重要的作用。已往药理学实验都只观察到了微丝骨架的精细重组影响ROS积累和HR产生的现象, 对微丝骨架在重组过程中是如何发挥作用的还了解甚少。关于细胞骨架抵抗植物锈菌侵染中微丝骨架如何影响HR反应, 它的具体的机制和功能值得我们深入的研究。

在自然界中, 非寄主抗病性是植物抗病性中最普遍和持久的[19]。与寄主抗病性相比, 非寄主抗病性抗病机制研究相对滞后。药理学实验表明, Cytochalasin B处理并没有使拟南芥−小麦条锈菌(*P. striiformis* f. sp. *tritici*)互作中锈菌的侵染能力增强[41], 相反却增加了小麦白粉病菌(*B. graminis* f. sp. *tritici*)对拟南芥叶肉细胞的穿透效率, 形成吸器[42], 说明拟南芥在锈菌和白粉菌互作系统中, 其非寄主抗病机制是不相同的。其他的一些研究也表明, Cytochalasin A处理可抑制大麦对豌豆白粉菌(*E. pisi*)防卫反应, 导致侵染点处胼胝质、糖类和蛋白质积累等阻止病原菌侵入的一系列抗性反应均明显减弱[16]。Cytochalasin A处理也可明显抑制或延迟小麦对黄瓜白粉菌(*Sphaerotheca fuliginea*)互作中HR产生、H_2O_2的积累产生及乳突的形成, 造成白粉菌的侵入率明显提高, 部分会形成吸器[43]。以上说明, 微丝骨架在非寄主植物抗锈病和抗白粉病中的作用是不相同的, 现有的研究结果还不能解释不同非寄主−病原菌互作系统中非寄主抗病性的作用机制, 对微丝骨架与非寄主植物抗锈性的关系还需要做深入探索。

与微丝骨架相比, 微管骨架对植物防卫反应中H_2O_2积累和HR产生的影响, 可能在不同的植物−锈菌互作系统中所起的作用是不同的(表2)。药剂黄草硝(oryzalin)、秋水仙素(colchicine)、紫杉醇(taxol)能够破坏微管骨架的结构, 影响微管蛋白的稳定性,

表1 用于研究植物−锈菌互作中细胞骨架功能的抑制剂及其属性

Table 1 A selection of inhibitors and properties used for pharmacological interference with cytoskeletal functions in plant-rust interactions

细胞骨架组分 Cytoskeletal polymer	抑制剂名称 Inhibitor name	起源 Origin	化学式 Molecular formula	对细胞骨架的影响 Effects on cytoskeleton	参考文献 References	药剂用于植物−锈菌互作研究中的参考文献 Examples of usage in the context of plant-rust interactions
Actin filaments	Cytochalasin A	*Drechslera dematioidea*	$C_{29}H_{35}NO_5$	Sulfhydryl-reactive, inhibit growth and sugar uptake	[26]	[40]
	Cytochalasin B	*Drechslera* (previously *Heiminthosporium*) *dematioideum*	$C_{29}H_{37}NO_5$	Inhibits cell movement, shortens actin filaments by blocking monomer addition at the fast growing end of the polymer, impairs maintenance of long term potentiation (LTP) of action filaments	[27-30]	[7,24,37,41]
	Cytochalasin D	*Zygosporium mansonii*	$C_{30}H_{37}NO_6$	Impairs maintenance of long term potentiation (LTP) of actin filaments, promotes conditions favorable for depolymerizing actin	[30-31]	[39]
	Cytochalasin E	*Aspergillus clavatus*	$C_{28}H_{33}NO_7$	Produces a “halo” around the nucleus	[32]	[36,38]
Microtubule	Oryzalin	Synthetic herbicide	$C_{12}H_{18}N_4O_6S$	Binds to plant tubulin and inhibits microtubule polymerization	[33]	[16,37,45]
	Colchincine	Secondary plant product of *Colchicum*	$C_{22}H_{25}NO_6$	Inhibits microtubule polymerization by binding to tubulin dimers	[34]	[36]
	Taxol	Secondary plant product of *Taxus*	$C_{47}H_{51}NO_{14}$	Stabilizes microtubules and inhibits microtubule dynamics	[35]	[7,39]

是微管作用研究中常用的药剂, 它们对细胞骨架的影响见表1。Kobayashi等[44]用Oryzalin处理亚麻叶肉细胞后接种非亲和亚麻锈菌(*M. lini*)发现, 微管的解聚会消除或延迟亚麻对非亲和小种的HR反应。同样, Oryzalin处理能够明显抑制或延迟小麦与小麦叶锈菌(*P. triticina* f. sp. *tritici*)、小麦与小麦条锈菌(*P. striiformis* f. sp. *tritici*)互作中HR的产生和H_2O_2积累[39,45]。然而在豇豆−豇豆锈菌(*U. vignae*)互作系统中, Colchicine处理并没有对胼底质的形成产生任何影响, Oryzalin和Taxol处理也没有对HR产生和核迁移有显著的影响, 但是却降低了自发荧光的乳突发生率。由于在侵染细胞内的自发荧光是细胞坏死的最后阶段, 乳突减少很可能与微管重组有关[7,37]。在一些非锈菌的寄主或非寄主互作系统中, 同样也发现, 微管解聚并未显著影响HR产生的现象。Propyzamide和Oryzalin处理并不能使白粉菌(*B. graminis*和*E. pisi*)成功地侵入大麦叶表皮细胞[46]。辣椒叶片经Oryzalin处理后接种黄瓜炭疽病菌(*Colletotrichum orbiculare*), H_2O_2积累减弱, 乳突形成率降低, 然而并未观察到完整细胞的HR产生[47]。微管的解聚在寄主植物抗病性中的作用可能是复杂的, 目前关于非寄主植物−锈菌互作中微管骨架对ROS积累和HR产生的作用还没有相关的报道, 对微管骨架与寄主和非寄主植物抗锈性的关系、微管骨架在植物与锈菌互作中的动态变化, 其调控因子以及在信号传导中的作用等, 还需要作深入研究以获得更多实验数据的支持。

3 植物−锈菌互作过程中细胞骨架结合蛋白的功能

细胞骨架在植物与病原菌互作中通过动态重组行使其功能, 这些动态变化受微管或微丝结合蛋白的调控。微管结合蛋白通过对微管动态和组织的调控作用进而影响微管骨架的功能, 微管骨架的调节因子包括MAP65家族、SPC98蛋白、γ-微管蛋白(γ-tubulin)、MOR1/MAP215蛋白、EB1蛋白、磷脂酶D(phospholipase D, PLD)、SPR1/SKU6蛋白、SPR2/TOR1蛋白[48]。微丝结合蛋白是与微丝特异结合并影响其结构与功能的一类调节蛋白, 主

表2 细胞骨架抑制剂对ROS积累和HR产生的影响

Table 2 Effectsof cytoskeletal inhibitors on ROS accumulation and HR

植物 Plant species	病原菌 Pathogens	微管解聚剂 MT inhibitors	微管解聚剂影响 Effector on MT inhibitors	微管解聚剂 MF inhibitors	微丝解聚剂影响 Effector on MF inhibitors	参考文献 Reference
Wheat	*Puccinia striiformis* f. sp. *tritici*	Oryzlain	Delay of HR; reducation of H_2O_2 accumulation	Cytochalasin A	Delay of HR; Reducation of H_2O_2 accumulation	[40,45]
			Reducation of the number of necrotic cells	Cytochalasin B	Greatly weakness the ROS accumulation and HR	[24]
Wheat	*Puccinia triticina* f. sp. *tritici*	Oryzlain	Reducation of the number of necrotic cells	Cytochalasin D	Reducation of the number of the necrotic cells	[39]
Arabidopsis	*Puccinia striiformis* f. sp. *tritici*	Not examined	–	Cytochalasin B	No significantly increase of penetration efficiency	[41]
Flax	*Melampsora lini*	Oryzlain	Formation of haustoria, delay of HR	Not examined	–	[37]
Cowpea	*Uromyces vignae*	Colchicine	No effect on callose deposit	Cytochalasin E	Inhibition of callose deposit	[36]
		Oryzlain	No effect on nuclear migration and HR	Cytochalasin B	Inhibition of nuclear migration and HR	[7,37]
Pea	*Uromyces vignae*	Not examined		Cytochalasin E	Reducation of H_2O_2 generation, increase of penetration efficiency	[38]

要包括ADFs(actin depolymerizing factors)/cofilins、profilins、formins、villins、fimbrims、thymosins、成冒蛋白和Arp2/3复合体[49]。近年来, 运用抑制性削减杂交技术(suppression subtractive hybridization, SSH), 已成功分离出不同组织、不同发育阶段以及受外界因子作用而差异表达的基因, 并利用这一技术获得了一系列微管和微丝结合蛋白的基因。然而到目前为止, 仅有少数和植物与病原菌互作相关的细胞骨架结合蛋白被报道, 且大多数是微丝结合蛋白, 其中研究的比较多的是profilins和ADF/cofilins[10,50]。Profilins是一种小分子量蛋白, 能够以1:1的比例与肌动蛋白单体(G-actin)结合组成复合物, 从而调节微丝骨架的结构和功能[51]。Bubb等[52]发现, 微丝骨架动态重组需要依靠profilins浓度调节, 低浓度下profilins加速微丝的聚合, 高浓度下profilins则会导致微丝解聚。Song等[40]克隆了一个在小麦与小麦锈菌(*P. striiformis* f. sp. *tritici*)互作中一个编码profilins的基因, 并利用RT-PCR技术分析了该基因对不同条锈菌生理小种差异性互作的影响, 发现与亲和互作相比, profilins在非亲和互作中表达量显著下调, 表明微丝骨架的解聚导致小麦感病性增强。戚拓等[53]在小麦中克隆出微丝聚合基因*TaARP3*, 发现该基因参与到了小麦抗条锈病的过程中, 并和乙烯介导的抗病反应途径相关。*TaARP3*基因在小麦抗锈病过程中是如何发挥作用的, 与Arp2/3复合体是何种关系, 对于这些问题的探索将有助于揭示小麦抗锈性机制。

ADFs/cofilins是一类分子量较小且高度保守的微丝结合蛋白, 通过提高微丝的解聚速度和加速微丝的周转率来调节微丝的动态重组[13,54]。*TaADF7*是从小麦中克隆出来的ADF基因, 利用GFP(green fluorescent proteins)技术将该基因定位于微丝骨架上。利用酵母双杂交系统发现, 该基因的过量表达诱导微丝骨架解聚。将该基因沉默后, 接种非亲和小麦条锈菌生理小种, 菌丝的长度增加, 夏孢子产生。除此之外, ROS积累和HR产生显著降低, 抗性相关蛋白*PR1*基因也呈显著降低, 表明*TaADF7*参与了小麦对条锈菌应答的防卫反应, 通过调节微丝骨架的动态重组从而影响ROS积累和HR产生。由于*PR1*基因与水杨酸(SA)信号通路相关, 且*TaADF7*受外源SA诱导表达, 因此推断*TaADF7*可能与水杨酸信号通路有关[24]。*rpg4*和*Rpg5*是从大麦中克隆出来的两个抗病基因, 呈共分离, 能够抵抗禾柄锈菌(*P. graminis*)若干生理小种转化型[55-57]。*rpg4*编码微丝结合蛋白ADF_2, *Rpg5*编码一个NBS-LRR结构类型的抗病基因[58]。在大麦与小麦秆锈菌(*P. graminis* f. sp.

tritici)互作中, 植物的防卫反应需要*rpg4*和*Rpg5*共同作用来实现, *rpg4*/*Rpg5*抗病系统是首次发现的具有小种转化的抗病基因和能够调节细胞骨架动态变化的ADF基因连锁且呈共分离现象的抗病系统[58]。分别将*rpg4*和*Rpg5*基因沉默后接种小麦秆锈菌非亲和小种, 发现该小种可成功侵染大麦[59], 这说明ADF基因和NBS-LRR基因均参与了植物抗病防卫反应。然而, *rpg4*和*Rpg5*是如何相互作用, 以及是如何调节植物对锈菌的抗病性的？对*rpg4*和*Rpg5*互作的研究将会有助于揭示微丝骨架是如何快速重组, 以及微丝骨架的重新排列在锈菌互作过程中的作用, 研究这些问题可为进一步揭示植物抗病性机制奠定基础。

4 展望

在过去的几年里, 人们主要应用免疫细胞化学技术或者罗丹明−鬼笔环肽染色来研究细胞骨架在植物防卫反应中的重要作用, 这些技术首先要求固定植物组织, 繁琐的固定方法可能会产生一些伪样本, 影响实验结果的稳定性。近年来, 随着GFP技术、酵母双杂交系统、激光共聚焦显微镜和一系列信号分子荧光探针等的应用, 为植物在抵御锈菌防卫反应中细胞骨架的形态、结构、功能和分子机制的研究开辟了新的途径。然而, 在人们普遍关注植物与锈菌互作中植物细胞骨架功能的同时, 却对锈菌细胞骨架的动态变化和重组知之甚少。这些新的实验方法和技术同样可以应用于锈菌细胞骨架功能的研究, 这对全面揭示植物与锈菌互作机制具有重要的意义。

Ca^{2+}作为细胞内最重要的第二信使, 在细胞信号转导过程中的作用日益受到关注。Ca^{2+}信号系统参与植物抗病性表达过程, 并已证实Ca^{2+}信号是病原菌侵染后产生的早期反应之一, 造成细胞骨架重新排列, 并伴随着H_2O_2的迸发、胞质pH的变化和丝裂原活化蛋白激酶(mitohen-activated protein kinases, MAPKs)的活化[60-62]。在植物与锈菌互作系统中, Ca^{2+}浓度的升高参与了细胞信号转导, 诱发豇豆、小麦叶片发生HR, 微管骨架解聚[63-65], 说明Ca^{2+}影响微管骨架成分的稳定性, Ca^{2+}和微管骨架之间存在着一定的相互作用。然而他们又是如何发挥相互作用的？目前, 关于探讨植物与锈菌互作中Ca^{2+}和微管骨架之间的关系的报道还很少。植物与锈菌互作系统中Ca^{2+}信号变化机制、微管骨架动态重组的分子机制还有待深入研究。目前还未见有关锈菌互作系统中Ca^{2+}信号转导和微丝骨架之间关系的报道, 二者之间是否存在着信号与信号之间的对话(cosstalk), 对微丝骨架在信号转导中作用的研究, 将有助于全面揭示植物抗锈性本质。

非寄主抗病性是植物抵抗大多数病原菌最常见的抗病类型, 具有显著的持久抗病性特点, 能够帮助改善作物品质, 培育广谱持久抗病品种[66-68]。已有研究发现, 大麦条锈菌(*P. striiformis* f. sp. *hordei*)某些生理小种能够成功侵入非寄主小麦[69-70]。大麦对小麦条锈菌(*P. striiformis* f. sp. *tritici*)某些生理小种则表现为感病, 从而建立了大麦和异种锈菌互作模式系统, 用于非寄主抗锈性遗传机制的研究[71]。除此之外, 拟南芥作为模式植物也已用于与小麦条锈菌(*P. striiformis* f. sp. *tritici*)互作中非寄主抗病机制的研究[41]。这些非寄主互作模式系统的建立将会加速植物抵御锈菌侵染过程中细胞骨架功能和非寄主植物防卫反应机制的研究。

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