澳洲棉 REVEILLE2 调控茉莉酸防御信号提高黄萎病抗性的分子机理

刘富杰，刘康*，周宝良*

（南京农业大学 / 作物遗传与种质改良国家重点实验室 / 省部级作物现代生产协同创新中心，南京 210095）

摘要：棉花黄萎病（Verticillium wilt, VW）是由大丽轮枝菌（*Verticillium dahlia* Kleb.，Vd）引起的一种土传性真菌病害，其菌丝能侵染入木质部维管组织中，在防治上尤为困难，植物一旦发病将很难防治。培育和利用抗病品种是最经济有效的控制黄萎病策略，而深入解析植物 - 黄萎病菌相互作用的分子机理，挖掘棉花近缘种中的优异抗病基因，能为棉花抗性设计育种提供理论基础和新的基因资源。棉花的野生近缘种澳洲棉（*Gossypium australe*，2n＝G_2G_2＝26）是 1 个对枯萎病、黄萎病兼抗的二倍体野生棉种，对黄萎病多个菌系具有广谱抗性，是棉花抗病育种的重要基因资源。为了缩小澳洲棉抗病基因的发掘范围，首先鉴定出 1 个抗黄萎病的陆地棉 - 澳洲棉易位系 TA01-7G。对 TA01-7G 根部进行 Vd 接种前后的转录组测序，分析筛选出 12 个来自于澳洲棉的候选基因。利用病毒诱导的基因沉默（VIGS）和转基因过表达技术鉴定出 MYB 类转录因子（MYB-like）RVE2 抗病基因，并对其抗性功能和机理进行深入研究。在 TA01-7G 中，沉默 *RVE2* 的表达降低了棉花对 VW 的抗性。随后，在拟南芥中异源过表达 *RVE2* 增强了拟南芥对 VW 的抗性。为了解析 RVE2 的抗性分子机理，通过酵母双杂交试验筛选 RVE2 可能的互作蛋白，结果表明 RVE2 与 TPLESS/TPL-related proteins（TPL/TPRs）和 MPK3/6 蛋白互作。随后，通过双分子萤光互补试验（BiFC）、萤火虫荧光素酶分离试验（Split-LUC）、Pull-down 和体内 Co-IP 试验验证了 RVE2 与 TPL/TPRs 存在互作。而多蛋白的 Split-LUC、Pull-down 和 Co-IP 试验均表明 RVE2 与 TPL/TPRs 的互作能干扰 NINJA 招募 TPL 和 TPR1，进而释放被 JAZ（Jasmonate ZIM-domain）抑制的 MYC2 活性。释放活性的 MYC2 正向调节茉莉酸信号通路，激活对 VW 的抗性反应。此外，ChIP-qPCR、电泳迁移率变动分析（EMSA）和双荧光素酶（Dual-LUC）试验表明，MYC2 能结合 *RVE2* 的启动子，激活其转录，形成了反馈调节回路。此外，对陆地棉自然群体的 *RVE2* 基因序列比对分析，发现了其 D 亚基因组中广泛存在 1 个独特的截短 *RVE2*，*GhRVE2D*。Dual-LUC 试验表明，GhRVE2D 能够抑制 MYC2 激活 *GhRVE2A* 启动子的活性，而不能抑制 MYC2 对 *GausRVE2* 和 *GbRVE2* 的启动子激活活性。随后，在拟南芥突变体、转 *RVE2* 基因的烟草和棉花中干扰茉莉酸信号通路导致 *RVE2* 介导的抗病能力丧失。*RVE2* 的过表达显著增强了对 VW 的抗性。最后，通过瞬时表达试验发现 *RVE2* 的抗性依赖于 BAK1 和 SOBIR1 介导的免疫反应。GhRVE2D 同样干扰 MPK3/MPK6 与 GhRVE2A 的相互作用。基于上述结果，认为 RVE2 是 1 个新的调控子，其与 MYC2 一起精细调控茉莉酸防御信号通路。本研究完善了对棉花 VW 抗性机理的理解，为棉花抗黄萎病育种提供了新基因。

关键词：棉花；黄萎病；茉莉酸信号通路；*REVEILLE*；MYC2

Molecular mechanism of *Gossypium australe* REVEILLE2 regulating jasmonic acid defense signal to enhance Verticillium wilt resistance

Liu Fujie, Liu Kang*, Zhou Baoliang*

(*State Key Laboratory of Crop Genetics & Germplasm Enhancement and Utilization/Collaborative*

第一作者简介：刘富杰（1993—），男，博士，2019201060@njau.edu.cn

* 通信作者：刘康，kangliu@njau.edu.cn；周宝良，baoliangzhou@njau.edu.cn

Innovation Center for Modern Crop Production Co-sponsored by Province and Ministry/Nanjing Agricultural University, Nanjing 210095, *China*)

Abstract: Verticillium wilt (VW) is a soil-borne fungal disease caused by *Verticillium dahliae* Kleb. (Vd). This disease is very difficult to control in infected plants owing to the long viability of resting structure, microsclerotia, and invasion of pathogen into xylem vessels. Up to now, there are no effective measure available to control VW once it occurs. Development and application of resistant varieties is the most economic and effective strategy to control VW. Therefore, based on insights into the molecular mechanisms of plant-Vd interaction, improving plant disease resistance via genetic engineering is an effective and sustainable strategy to control VW. *Gossypium australe* ($2n = G_2G_2 = 26$), a wild relative of cotton, is a diploid wild species with resistant to both Fusarium wilt and Verticillium wilt, and it also has broad-spectrum resistance to various strains of Vd, which is an important gene resource for cotton resistance breeding. To narrow the mining range of disease resistance genes in *G. australe*, we first identified a *G. hirsutum-G. australe* translocation line TA01-7G, which was resistant to VW. Before and after Vd inoculation, the transcriptome of TA01-7G roots were andyzed, and 12 candidate genes from *G. australe* were screened. The resistance gene *RVE2* was silenced in TA01-7G by using virus-induced gene silencing (VIGS) and transgenic overexpression technique in *Arabidopsis* and the resistance mechanism of *RVE2* was further studied. First, *RVE2* silencing reduced the resistance to Vd in TA01-7G. To further investigate *RVE2* resistance function, we overexpressed *RVE2* in *Arabidopsis*. The pathogenicity assays showed that overexpression of *RVE2* increased the resistance to Vd in *Arabidopsis*. To elucidate the molecular mechanism of RVE2 resistance, possible interacting proteins of RVE2 were screened by yeast two-hybrid assay (Y2H). The result revealed that RVE2 physically interacted with TPL/TPRs and disturbed JAZ (Jasmonate ZIM-domain) proteins to recruit TPL and TPR1 in NINJA-dependent manner, which regulated Jasmonicacid (JA) response by relieving inhibited-MYC2 activity. ChIP-qPCR, electrophoretic mobility shift assay (EMSA) and Dual luciferase (Dual-LUC) experiments showed that, MYC2 bound to *RVE2* promoter for activating its transcription, forming feedback loop. Interestingly, *RVE2* gene sequence analysis in natural population showed that a unique truncated *RVE2* (*GhRVE2D*) widely existing in D-subgenome of upland cotton, which represses the ability of the MYC2 to activate *GhRVE2A* promoter but not *GausRVE2* or *GbRVE2*. Subsequently, transient expression assays of different *RVE2* genes in tobacco leaves indicated that the integrity of the *RVE2* gene was of extremely importance. The result could partially explain why *G. barbadense* showed highervw resistance than *G. hirsutum*. Furthermore, disturbing the JA-signalling pathway using VIGS assays resulted in the loss of RVE2-mediated disease-resistance. *RVE2* overexpression significantly enhanced the resistance to VW. *RVE2* also physically interacted with MPK3 and MPK6, functioned in the downstream of *BAK1* and SOBIR1 mediated innate immune response. Thus, we conclude that RVE2, as a new regulatory factor, plays a pivotal role in fine-tuning JA-signaling, which improves our understanding of the mechanisms underlying the resistance to cotton VW and provides a new gene for cotton breeding for resistance to VW.

Keywords: cotton; Verticillium wilt; jasmonic acid-signalling pathway; *REVEILLE*; *MYC2*