

木霉诱导新疆野苹果抗斑点落叶病及其分子机理研究

韩静¹, 季世达², 刘志华¹

(1.沈阳农业大学林学院 沈阳 110866; 2. 沈阳农业大学园艺学院 沈阳 110866)

摘要:【目的】新疆野苹果 (*Malus sieversii*) 是我国仅存的经济果树资源的天然基因库, 利用木霉菌剂改善新疆野苹果的生存环境, 控制其生境中的病原菌数量, 解决野苹果面临的种群退化问题, 更加完整的保留新疆野苹果的遗传资源。【方法】木霉和斑点落叶病病原菌处理新疆野苹果后, 转录组数据分析得到受木霉和病原菌诱导的转录因子 MsERF105, 异源转化山新杨, 并鉴定 MsERF105 的上游调控基因和互作蛋白, 确定其在新疆野苹果抗病过程中的作用。【结果】1) 新疆野苹果根际分离获得俄罗斯木霉 Tro7 和哈茨木霉 Tha2, 对三种新疆野苹果病原菌的抑制率为 34%~58%, 并可定殖于新疆野苹果根部。木霉可以通过调节真菌和细菌群落结构进而影响病原菌种群数量和土壤代谢。2) RNA-Seq 分析新疆野苹果中大量与病原菌侵染和信号转导相关的基因差异表达, 其中 MsERF105 表达量上升 27.5 倍。MsERF105 异源表达减少了病原菌侵染导致的山新杨体内活性氧激增, 叶片相对病斑面积降低了 25%。此外, MsERF105 可以激活山新杨 PR1 和 PDF 基因表达, 验证了 MsERF105 可以诱导新疆野苹果防御响应, 进而提高对苹果斑点落叶病抗性。3) MsERF105 基因启动子区包含 5 个抗病相关的顺式作用元件, WRKY33 和 WRKY40 转录因子可以绑定 WBOXATNPR1 元件, 进而正调控 MsERF105 转录因子的表达。MsERF105 转录因子调节转录时与远端上游元件绑定蛋白 MsFUBP2 互相作用, 共同调控抗病基因的表达, 热激蛋白 MsHSP17.8 也可与 MsERF105 互作, 可能是 MsERF105 的分子伴侣。此外, MsERF105 转录因子识别 GCCBOX、CCAAT BOX 和 ERS (GCGT) 调控抗病基因和防卫响应基因的表达, 进而激活新疆野苹果对病原菌的抗性。【结论】新疆野苹果 MsERF105 转录因子是抗病系统中的关键基因, 可调控抗病基因和防卫响应基因的表达, 激活新疆野苹果对斑点落叶病病原菌的抗性, 为木霉菌剂修复真菌病害引发的新疆野苹果树种群退化提供理论基础。

关键词: 新疆野苹果, 木霉, 苹果斑点落叶病, MsERF105 转录因子, 植物防御响应

Molecular mechanism of *Trichoderma* induced MsERF105 transcription factor regulating resistance of *Malus sieversii* to *Alternaria alternata* f. sp. *mali*

Han Jing¹ Ji Shida² Liu Zhihua¹

(1. College of Forestry of Shenyang Agricultural University Shenyang 110866;

2. Horticultural College of Shenyang Agricultural University Shenyang 110866)

Abstract: 【Objective】 *M. sieversii* is the only natural gene pool of economic fruit tree resources in China. Using *Trichoderma* biofertilizer to improve the *M. sieversii* living environment, control the number of pathogenic in their habitats, solve the problem of population degradation, and more fully preserve the *M. sieversii* genetic resources. 【Method】 Following treated with *Trichoderma* biofertilizer and pathogen Aalm, the *M. sieversii* RNA-Seq data showed the transcription factor MsERF105 induced by *Trichoderma* and pathogenic, heterologously transform *Populus davidiana* × *P. alba* var. *pyramidalis*, and identify the upstream regulatory genes and interaction proteins of MsERF105 to determine its role in the disease resistance process of *M. sieversii*. 【Result】 1) *Trichoderma rossicum* Tro7 and *Trichoderma harzianum* Tha2 were isolated from the rhizosphere of *M. sieversii*. The two *Trichoderma* strains had significant inhibitory effects on *Alternaria alternata* f. sp. *mali* (Aalm), *Fusarium oxysporum* (FOSC) and *Cytospora mali* (CMA), ranging from 34% to 58%, and could colonize the roots of *M. sieversii*. *Trichoderma* can affect the number of pathogen and soil metabolism by regulating the community structure of fungi and bacteria. 2) *M. sieversii* RNA-Seq data showed that the differentially expressed genes were related to pathogen infection and signal transduction, and the differential expression of MsERF105 was the most significant. The heterologous expression of MsERF105 in *Populus davidiana* × *P. alba* var. *pyramidalis* (PdPap) showed that it could reduce the soaring reactive oxygen species caused by pathogen infection. Compared with the control, the relative lesion area of PdPap-MsERF105 was significantly smaller. Further studies showed that MsERF105 could activate the expression of defense genes, such as *PRIs* and *PDFs*, which shows that MsERF105 activates the internal defense response system of *M. sieversii*. 3) The promoter region of MsERF105 gene was analyzed and found to contain five cis-acting motifs related to disease resistance. WRKY33 and WRKY40 transcription factor can bind motif WBOXATNPR1, and signal transduction response regulator STRR and GT1 transcription factor can bind motif GT1CONSENSUS in the promoter region to regulate the expression of MsERF105 transcription factor. Yeast two hybrid showed that MsERF105 transcription factor may interact with tRNA methyltransferase MsTRMT, far upstream element binding protein MsFUBP2 or disease resistance protein MsDRP, further regulate the expression of disease resistance genes together. In addition, we also found the small heat shock protein MsHSP17.8 is the molecular chaperone of MsERF105 transcription factor. Finally, we obtained MsERF105 transcription factor can regulate the expression of disease resistance genes and defense response genes by binding to GCCBOX, CCAATBOX and ERS (GCGT), then activate the resistance of *M. sieversii* to Aalm. 【Conclusion】 The MsERF105 transcription factor of *M. sieversii* is a key gene in the disease resistance system, which can regulate the expression of disease resistance genes and defense response genes, activate the resistance of *M. sieversii* to the pathogen Aal, and provide a theoretical basis for the repair of fungal disease induced degradation of *M. sieversii* species by *Trichoderma*.

Key Words: *Malus sieversii*, *Trichoderma* sp., *Alternaria alternata* f. sp. *mali*, MsERF105 transcription factor, Plant defense respons