

MYB 转录因子调控棘孢木霉 Tas653 抗杨树叶枯病的调控通路构建

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摘要:【目的】明确 MYB 转录因子在调控棘孢木霉 (Tas653) 抗杨树叶枯病菌链格孢菌 (Aa1004) 中的作用, 揭示 Tas653 抗 Aa1004 的分子机制, 为杨树叶枯病防治提供理论指导, 从调控层面上揭示生防木霉抗病原真菌的分子机理。【方法】利用真菌转基因的方法, 获得 MYB36 基因过表达型 (Ta-Oe)、敲除型 (Ta-Kn) 和抑制型 (Ta-In) 菌株; 研究转基因菌株对 Aa1004 直接和间接的生防作用; 再通过酵母单/双杂交、EMSA、RNA-seq 和 ChIP 等多种实验方法探究 MYB36 直接和间接调控的生防基因, 构建 MYB36 调控 Tas653 抗杨树叶枯病菌 Aa1004 的通路。【结果】通过真菌转基因技术共获得 20 株 Ta-Oe, 25 株 Ta-Kn 和 20 株 Ta-In, 对 MYB36 基因定量和 PCR 检测确定 Ta-Oe-8, Ta-In-1 和 Ta-Kn-1 为 MYB36 的过表达, 抑制表达和敲除型菌株。与野生型相比, Ta-Kn 菌株对 Aa1004 的抑制率下降了 11.06%, Ta-kn 菌株的 SOD, POD 和 CAT 酶活降低了 82.15 U/g, 0.19 OD470/min/g 和 1631.2 $\mu\text{mol}/\text{min}/\text{g}$ 且相关基因 S50 (SOD), C820 (CAT) 和 P120 (POD) 酶活基因分别下调 3.43, 8.1 和 2.43 倍; Ta-Oe 菌株不仅对 Aa1004 的重寄生性增强, 同时发酵液会使其菌丝膨大变形甚至破裂。MYB36 还可以通过结合 “ACAT” 和 “ATCG” 元件直接调控转录因子, 解毒和防卫基因等。其中, MYB36 调控的转录因子中主要包括 20 个锌指和 bZIP 转录因子, 其中 bZIP61.5, Zn37.5, Zn58.0, Zn67.4, Zn83.7 被 MYB36 直接调控; 调控的解毒基因包括 ABC 转运蛋白和 MFS 超家族转运蛋白, 其中 ABC174.3, MFS47.5, MFS61.5 和 MFS53.8 被 MYB36 直接调控; 调控的防卫基因包括蛋白激酶 (PK), 8 个细胞色素 P450 和 13 个水解酶基因, 其中 PK50.1, 5 个细胞色素 P450 和 3 个水解酶基因被 MYB36 直接调控。有趣的是, 在我们的试验中还发现 MYB36 还可以间接调控 dUTP、tRNA 合成和翻译后修饰等相关基因的表达。【结论】MYB 转录因子不仅可以提高生防木霉的重寄生性, 抗氧化能力等, 还可以通过直接或间接调控锌指等转录因子, MFS 等解毒基因和细胞色素 P450 和水解酶等防卫基因提高棘孢木霉 Tas653 对杨树叶枯病菌 Aa1004 的防治作用。

The Construction of Regulatory pathway of MYB36 Transcription Factor from *Trichoderma asperellum* Tas653 Resisting to Poplar leaf blight

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Abstract: 【Objective】 This study aims to clarify the role of MYB transcription factors in regulating the resistance of *T. asperellum* (Tas653) to poplar leaf blight (pathogen: *Alternaria alternata* Aa1004), reveal its molecular mechanism, provide theoretical guidance for disease prevention and control in poplar, and reveal the molecular mechanism of biocontrol *Trichoderma* against pathogenic fungi at the regulatory level. 【Method】 The MYB36 overexpression (Ta-Oe), knockout (Ta-Kn), and inhibitory (Ta-In) strains were obtained by using fungal transgenic methods. It measures the disease resistance of the MYB36 transgenic strain to study its direct and indirect biocontrol effects on Aa1004. Through various experimental methods such as yeast one/two hybridization, EMSA, RNA-seq, and CHIP, we explored the biocontrol genes directly and indirectly regulated by MYB36 and constructed a disease resistance pathway through which MYB36 regulates Tas653. 【Result】 Through fungal transformation technology, 20 Ta-Oe, 20 Ta-In, 25 Ta-Kn, and 20 Ta-Flag strains have been obtained. Quantitative and PCR detection of the *MYB36* gene confirmed that Ta-Oe-8, Ta-In-1, and Ta-Kn-1 were overexpressed, inhibited, and knockout strains of MYB36. Compared with the Wt, the inhibition rate of the Ta-Kn strain on Aa1004 decreased by 11.06%, the SOD, CAT, and POD activity decreased by 82.15 U/g, 0.19 OD470/min/g, and 1631.2 $\mu\text{mol/min/g}$, and the enzyme activity-related genes *S50* (SOD), *C820* (CAT), and *P120* (POD) were down-regulated by 3.43, 8.1, and 2.43 times, respectively. Ta-Oe strain not only enhances the hyperparasitism of Aa1004, but also its fermentation broth would cause Aa1004 hyphae to swell, deform, or even rupture. MYB36 can also directly regulate transcription factors, detoxify, and defense genes by binding to "ACAT" and "ATCG" elements. Among them, the transcription factors regulated by MYB36 mainly include 20 zinc fingers and bZIP transcription factors, among which *bZIP61.5*, *Zn37.5*, *Zn58.0*, *Zn67.4*, and *Zn83.7* are directly regulated by MYB36; the detoxification genes regulated include ABC transporters and the Major Facilitator Superfamily (MFS), with *ABC174.3*, *MFS47.5*, *MFS61.5*, and *MFS53.8* being directly regulated; the regulated defense genes include protein kinase (PK), 8 cytochrome P450, and 13 hydrolase genes, with *PK50.1*, 5 cytochrome P450, and 3 hydrolase genes being directly regulated. Interestingly, MYB36 can also regulate dUTP, tRNA synthesis, and post-translational modification genes to regulate expression of genes. 【Conclusion】 MYB transcription factors can not only enhance the parasitism and antioxidant capacity of biocontrol *Trichoderma*, but also enhance the control effect of *T. asperellum* Tas653 on poplar leaf blight pathogen Aa1004 by directly or indirectly regulating transcription factors such as zinc finger, detoxification genes such as MFS, and defense genes such as cytochrome P450 and hydrolases