中国植物病理学会第十二届青年学术研讨会论文选编

植物病理学研究进展

Zhiwu Binglixue Yanjiu Jinzhan

吴学宏 主编

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·北京·

内 容 简 介

本书共收集了135篇研究论文、简报、摘要和综述。涉及植物病原真菌及真菌病害、病毒及病毒病害、原核生物及其病害、线虫等其他病原及其病害、生物防治、病害流行及预测、种子病理与杀菌剂、抗病性及抗病育种、病害综合防治、分子生物学及其应用、信息技术及其应用等方面，基本上反映了近两年来我国植物病理学青年工作者在植物病理学各分支学科基础理论、应用基础和植物病害防治实践等方面所取得的研究进展。

本书可供植物病理学、农学、生物学等相关专业师生、科研人员参考。

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前　言

中国植物病理学会第十二届青年学术研讨会论文选编《植物病理学研究进展》共收集了135篇研究论文、简报和摘要。内容涉及植物病原真菌及真菌病害、病毒及病毒病害、原核生物及其病害、线虫等其他病原及其病害、生物防治、病害流行及预测、种子病理与杀菌剂、抗病性及抗病育种、病害综合防治、分子生物学及其应用、信息技术及其应用等方面，基本上反映了近两年来我国植物病理学青年工作者在植物病理学各分支学科基础理论、应用基础和植物病害防治实践等方面所取得的研究进展。

本次大会由中国植物病理学会青年委员会主办，以“青年植病工作者与科技创新”为会议主题。大会的召开和论文集的出版得到了中国科学技术协会和中国植物病理学会的资助。挂靠单位中国农业大学给予了关心和指导。我国植物病理学界多位专家、教授给予了关心、指导和帮助。大会承办单位山东农业大学植物保护学院和山东植物病理学会，以及编辑委员会的同志们付出了辛苦的劳动，中国农业大学出版社给予了大力支持和帮助。在此，我们表示衷心的感谢！

由于大会征集论文时间仓促，同时，本着尊重作者意愿和文责自负的原则，对论文的内容一般未作改动，仅在编辑体例上进行了处理。因此，错误和不足之处在所难免，敬希论文作者和读者批评指正！

编　者

2015年10月

目 录

Type Ⅴ myosin FaMyo2B affects asexual and sexual development, reduces pathogenicity, and cooperating with myosin passenger protein FaSmy1 regulates resistance to the fungicide phenamacril in *Fusarium asiaticum*

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Fusarium head blight (FHB) or scab of wheat and other small cereal grains caused by-*Fusarium graminearum* sensu lato (teleomorph *Gibberella zeae*(Schwein.) Petch) is a disease that causes severe yield and economic losses worldwide (Bai and Shaner, 2004; Goswami and Kistler, 2004). FHB not only reduce grain yield and quality, but can also contaminate grains with a variety of potent mycotoxins that are a threat to human and animal health (Desjardins et al., 2006; Sutton et al., 1982). Although a number of *Fusarium* spp. can cause FHB, the primary etiological agents of this disease belong to the *Fusarium graminearum* species complex of B-trichothecene toxin producers, which contain at least 11 phylogenetic species, including *F. acaciae-mearnsii*, *F. asiaticum*, *F. austroamericanum*, *F. boothii*, *F. brasilicum*, *F. cortaderiae*, *F. gerlachii*, *F. graminearum*, *F. meridionale*, *F. mesoamericanum*, and *F. vorosii*, (11, 32, 34). Different *Fusarium* spp. may be associated with FHB in different regions of the world because of different cropping systems and climatic conditions (28, 36). In China, FHB was first reported in 1936 and FHB epidemics have since become more severe and frequent in the middle and lower regions of the Yangtze River and in the Heilongjiang province in the northeastern region (Chen et al., 2000).

Zhang et al. (2007) analyzed 299 isolates collected from various epidemic regions of China and found that 231 isolates (77.3%) belonged to *F. asiaticum* and the remaining 68 isolates were *F. graminearum*.

Because natural resistance against FHB pathogens is limited, which has severely hampered progress in breeding for resistance with conventional approaches (Chen et al., 2000; Parry et al., 1995; Windels, 2000), the most efficient strategy for the control of FHB is through the application of fungicides during wheat anthesis. The use of a novel cyanoacrylate fungicide phenamacril reduced both the FHB index and mycotoxin level by 80% (Li et al., 2008; Chen and Zhou, 2009; Zhang et al., 2010; Zheng et al., 2015). In vitro, phenamacril-resistant mutants were obtained easily by ultra-violet (UV) irradiation and

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fungicide domestication. Most of the resistant mutants belonged to moderately or highly resistance and exhibited similar biological fitness to the wild-type strains. In our previous studies, we found that mutations in myosin-5 confers resistance to phenamacril in *F. graminearum* (Zheng et al., 2015). In *F. graminearum*, the myosin gene family has three members, including FGSG_08719.1, which encodes myo2 (Song et al., 2013); FGSG_07469.1, which encodes myosin-2B; and FGSG_01410.1, which encodes myosin-5. All three of these myosin proteins have conserved "head" regions. The head or motor domain contains binding sites for ATP and actin. To determine whether the other myosin proteins could regulate the resistance to phenamacril in *F. asiaticum*, we evaluated the functions of myosin-2B and myo2 by gene deletion.

Myosins are molecular motors that catalyze an ATP-dependent interaction with actin filaments and generate unidirectional, chemo-mechanical force. Force generation resides in a~80 kDa motor domain that is highly conserved among all myosins. Based on genomic survey and phylogenetic analyses, 31 myosin classes have been defined (Sebe-Pedros et al., 2014). In particular, Class V myosins are processive molecular motors that transport their cargo toward the plus ends of actin filaments. They are involved in numerous membrane trafficking events (Reck-Peterson et al., 2000; Trybus, 2008). *Saccharomyces cerevisiae* has two class V myosins, the essential Myo2 and the nonessential Myo4. While Myo4 mediates the transport of mRNAs and movement of ER tubules, Myo2 plays a major role in the transport of secretory vesicles and segregation of membrane-bounded organelles including vacuoles, peroxisomes, and organelles of the secretory pathway (Matsui, 2003; Pruyne et al., 2004; Weisman, 2006; Fagarasanu et al., 2010). The Myo1 gene encodes a class Ⅱ myosin that, depending on the strain background, is either essential or nonessential for viability. However, Myo1 is important for normal cytokinesis and cell wall maintenance in yeast cells (Nitza et al., 2007). A myosin light chain that associates with Myo1 and Myo2 heavy chains is encoded by the essential Mlc1 gene (Stevens and Davis, 1998; Luo et al., 2004). In *S. cerevisiae*, the kinesin-like myosin passenger-protein Smy1 transported by myosin V is part of a negative feedback mechanism that detects cable length and prevents overgrowth (Melissa et al., 2011). And the coiled-coil interactions (CCIs) network reveals that Myo1 and Myo2 are interacting proteins and regulate Smy1p, when overexpressed, can partially compensate for defects in the Myo2 mutant, overcoming lethality and restoring polarized growth at restrictive temperature (Wang et al., 2012; Lillie and Brown, 1992, 1994; Zhang et al., 2009).

In *F. graminearum*, Song et al. (2013) identified a type Ⅱ myosin gene, designated as myo2, and demonstrated that the type Ⅱ myosin myo2 is essential for septation, conidiation and sexual reproduction, and plays a significant role in pathogenesis and mycotoxin production. In this paper, we found type Ⅴ myosin gene FaMyo2B in *F. asiaticum* affects asexual and sexual development, reduces pathogenicity, and cooperating with myosin passenger protein gene FaSmy1 regulates resistance to the fungicide phenamacril. Our data

suggest that FaMyo2B and Famyo2 could be exploited as a target for the development of novel FHB control strategies.

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番茄晚疫病菌拮抗木霉菌株的筛选与鉴定

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摘　要：采用平板对峙培养法，从土壤中筛选出对番茄晚疫病菌拮抗效果较好的 5 株木霉菌株，试验结果表明 5 株木霉对番茄晚疫病菌菌株 HMQAU150020 的拮抗率为 56%～77%。结合形态学观察和 *tef*1 的系统发育分析，5 株木霉菌株分别鉴定为绿木霉（*Trichoderma virens*）、哈茨木霉（*Trichoderma harzianum*）和棘孢木霉（*Trichoderma asperellum*）。

关键词：对峙培养；木霉；番茄晚疫病菌；生物防治

Screening and identification of *Trichoderma* strains for antagonizing tomato late blight pathogen

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Abstract: Using confrontation test, five *Trichoderma* strains were screened from soil, and had the antagonistic effect on *Phytophthora infestans*. The experimental results indicated that the inhibited rates of five *Trichoderma* strains varied from 56% to 77%. Combining morphological observation with phylogenesis analyses of *Tef*1, these five *Trichoderma* strains were identified as *Trichoderma virens*, *Trichoderma harzianum* and *Trichoderma asperellum*, respectively.

Key words: antagonistic culture; *Trichoderma*; *Phytophthora infestans*; biocontrol

木霉（*Trichoderma* spp.）属于半知菌亚门，是一种重要的生防真菌，对多种病原菌如

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疫霉、腐霉、立枯丝核菌等具有拮抗作用[1]，随着绿色农业和有机农业的发展，以木霉为来源生防制剂的应用领域越来越广泛。

番茄是我国的重要蔬菜，由 *Phytophthora infestans* 引起的晚疫病在生产上造成严重危害。目前防治晚疫病的主要措施是化学防治，但化学药剂的长期使用造成了环境污染、农药残留及抗药性等问题。番茄生产的双减需求呼唤生物防治在晚疫病害的综合防治中发挥更积极的调控作用。本研究从多种植物的种植区采集根际土样，分离纯化并鉴定保藏木霉菌株，证明了所获得本地菌株对番茄晚疫病菌有较好的拮抗作用，为番茄晚疫病的生物防治提供了新的策略。

1 材料和方法

1.1 供试木霉菌株和病原菌

2014 年夏季于山东省各地的果蔬种植区采集根际土，采用稀释平板法[2]分离得到木霉，待长出菌落后，挑取菌落边缘单菌丝纯化，获得木霉菌株 HMQAU140012tri、HMQAU140014tri、HMQAU140015tri、HMQAU140016tri 和 HMQAU140017tri，并保存于青岛农业大学真菌学研究室。

从青岛城阳的番茄种植基地采集番茄晚疫病病样，通过单孢分离技术获得纯化的番茄晚疫病菌，致病疫霉(*Phytophthora infestans*)菌株 HMQAU150020，保存于青岛农业大学真菌学研究室。

1.2 平板对峙培养

在直径 90 mm 的黑麦培养基[3]上，在同一直线相距 4 cm 的两点上分别接入直径 5 mm 的木霉菌饼与病原菌菌饼，其中处理和对照先接入番茄晚疫病菌菌饼 4 d 后再接木霉菌饼，每个处理设三个重复，以只接病原菌菌饼的平板作为对照。20℃恒温培养箱中对峙培养。每隔 24 h 观察菌落生长情况，分别测量木霉及病原菌的生长半径。待对照病原菌长满 3/4 个皿时，按照下列公式计算抑制率。

抑制率＝(对照菌落半径－处理菌落半径)/对照菌落半径×100%

1.3 木霉菌株形态学及分子鉴定

1.3.1 木霉菌株形态学观察

挑取木霉菌落边缘菌丝制作临时玻片，在 Olympus 显微镜 BX53 下观测分生孢子梗及分生孢子的形态特征并进行显微拍照。在 25℃，12 h 光暗交替条件下培养木霉菌株，观察其在 PDA 上的菌落形态特征。参照《木霉分类与鉴定》[4]对菌株进行形态鉴定。

1.3.2 基因组 DNA 的提取

将木霉菌株接种于 PS 液体培养基中，25℃、120 r/min 摇培 48 h 后收集菌丝用于 DNA 的提取。提取方法采用改良后的 CTAB 法[5]。

1.3.3 木霉菌株 *tef1* 扩增和序列分析

提取菌株基因组 DNA 后，利用 Tef 引物 EF728(5′-CATCGAGAAGTTCGAGAAGG-3′)和 Tef1(5′-GCCATCCTTGGGAGATACCAGC-3′)进行扩增。扩增产物经 1%琼脂糖凝胶电泳检测后，由生工生物工程(上海)有限公司进行纯化和双向测序，测序结果经 Sequencher5.0 软件自动装配后导出重叠群(Contig)并在 NCBI(http://www.ncbi.nlm.gov)数据库中进行 BLAST 分析后提交给 GenBank。再从 GenBank 中选取合适的序列，经 CLUSTAL