

基于 PCR 技术的植物病原菌分子定量检测技术研究进展

曹学仁¹, 周益林^{2*}

(1. 中国热带农业科学院环境与植物保护研究所, 农业农村部热带作物有害生物综合治理重点实验室, 海口 571101;
2. 中国农业科学院植物保护研究所, 植物病虫害生物学国家重点实验室, 北京 100193)

摘要 植物病原菌的菌源量是病害发生和流行的重要因子之一, 对其精准的定量测定或检测可大大提高植物病害预测的准确性, 本文对实时荧光定量 PCR (qPCR) 与数字 PCR 在植物病原菌定量检测、以及基于 RNA 水平的 real-time PCR 和基于核酸染料(EMA/PMA)与 qPCR 相结合的技术在植物病原菌活体定量检测中的应用进行了综述, 并展望其在植物病害流行和预测中的应用前景。

关键词 植物病原菌; 定量检测; 实时定量 PCR; 数字 PCR; 活体检测

中图分类号: S 432 **文献标识码:** A **DOI:** 10.16688/j.zwbh.2019463

Research progress in quantitative detection of plant pathogens using PCR technique

CAO Xueren¹, ZHOU Yilin^{2*}

(1. Key Laboratory of Integrated Pest Management on Tropical Crops, Ministry of Agriculture and Rural Affairs, Institute of Environment and Plant Protection, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China; 2. State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China)

Abstract The inoculum of plant pathogens is an important factor related with disease epidemics. Precise quantification of inoculum greatly help the prediction of diseases. This review summarized the applications of real-time quantitative PCR (qPCR) and digital PCR in the quantification of plant pathogens. The use of real-time reverse-transcriptase PCR and the combination of viability dyes and qPCR in viability detection of plant pathogens were also reviewed. The future perspectives of molecular quantitative detection of the pathogens in plant disease epidemics were discussed.

Key words plant pathogen; quantitative detection; real-time quantitative PCR; digital PCR; viability detection

在植物病害流行过程中, 病原菌的菌源量是一种重要的驱动因子, 是病害预测的一个重要参数, 如土壤中芸薹根肿菌 *Plasmodiophora brassicae* 的含量与病害发生程度显著相关, 小麦条锈菌 *Puccinia striiformis* f. sp. *tritici* 和白粉菌 *Blumeria graminis* f. sp. *tritici* 的越冬菌量和第二年早春的病情也存在显著的相关性等^[1-3], 因此准确获得病原菌的菌源量对于一些病害的预测和治理有十分重要的作用。

传统病原菌菌源量数据的获取主要依靠田间调查, 但是一方面仅根据症状来判断病情容易出现误判, 如苗期小麦条锈病与叶锈病容易混淆; 另一方面对在寄主组织内潜伏侵染或未显症或隐症病原菌来说, 由于此时尚未显症, 无法准确估计病原菌的菌源量, 从而影响对病害的预测。近年来, 快速发展的分子生物学技术如实时荧光定量 PCR、数字 PCR 等为植物病原菌的定量检测提供了新工具。

收稿日期: 2019-09-05

修订日期: 2019-10-11

基金项目: 国家自然科学基金(31701731, 31972212); 中国热带农业科学院基本科研业务费专项(1630042017003)

* 通信作者 E-mail: yilinzhou6@163.com

1 基于实时荧光定量 PCR (qPCR) 的植物病原菌定量检测

实时荧光定量 PCR (real-time quantitative PCR, qPCR) 技术通过在 PCR 反应体系中加入特定的荧光结合物质或者荧光探针, 实时监测荧光量的变化, 获得待测样品达到荧光检测阈值的循环数 (cycle threshold, C_t 值), 再根据已知浓度标准品的 C_t 值与其浓度对数建立的标准曲线计算样品中模板 DNA 的浓度。该技术实现了从定性研究到定量研究, 在寄主组织内、空气中、土壤中以及种子中的植物病原菌的定量检测中得到了广泛应用, 如 Yan 等^[2] 和 Zheng 等^[3] 分别利用 qPCR 技术定量检测了小麦叶片中条锈病菌 *P. striiformis* f. sp. *tritici* 和白粉病菌 *B. graminis* f. sp. *tritici* 的越冬菌量; 结合孢子捕捉器和 qPCR, Rogers 等^[4] 和 Cao 等^[5] 分别建立了空气中油菜菌核病菌 *Sclerotinia sclerotiorum* 和小麦白粉病菌 *B. graminis* f. sp. *tritici* 孢子浓度的检测技术; 关于土壤中芸薹根肿菌 *P. brassicae*、*Pythium tracheiphilum* 和穿刺短体线虫 *Pratylenchus neglectus* 的 qPCR 定量检测研究均有报道^[1,6-7]; 菠菜种子中黄萎病菌 *Verticillium dahliae* 和水稻种子中恶苗病菌 *Fusarium fujikuroi* 的 qPCR 定量检测技术也已建立^[8-9], 并且国内外有多篇文章对该方面的研究进展进行了综述^[10-13]。

2 基于数字 PCR 的植物病原菌定量检测

数字 PCR (digital PCR) 是近年来发展起来的一种定量分析技术, 与 qPCR 技术不同的是它采用直接计数或泊松分布公式来计算每个反应单元的平均浓度 (含量), 从而进行定量分析, 不需要依赖于扩增曲线的循环阈值 (C_t), 不受扩增效率的影响。其原理是通过将一个样本分成几十到几万个不同的反应单元, 每个单元包含一个或多个拷贝的目标分子 (DNA 模板), 并进行 PCR 扩增, 扩增结束后对采集每个反应单元的荧光信号进行统计学分析^[14]。近年来该技术也开始应用于植物病原菌的定量检测研究。Blaya 等对烟草疫霉 *Phytophthora nicotianae* qPCR 和数字 PCR 定量检测结果的比较发现, 数字 PCR 可检测的浓度更低, 受样品的影响更小^[15]。对柑橘溃疡病菌 *Xanthomonas citri* subsp. *citri* 和木质部难养菌 *Xylella fastidiosa* 的研究也得到类似的研

究结果^[16-17]。另外梨火疫病病菌 *Erwinia amylovora*、马铃薯青枯病菌 *Ralstonia solanacearum*^[18]、葡萄土壤杆菌 *Agrobacterium vitis*^[19]、柑橘黄龙病菌 *Candidatus Liberibacter asiaticus*^[20-21]、芸薹根肿菌 *P. brassicae*^[22] 等植物病原菌的数字 PCR 定量检测技术也有报道。但是目前由于数字 PCR 设备价格昂贵、检测成本高、分析的样品通量低等, 制约了其推广应用。

3 基于 RNA 水平的 real-time PCR (qRT-PCR) 技术的植物病原菌活体定量检测

由于细胞死亡后 DNA 仍能保留较长时间, 如细菌死亡数周后还能用 PCR 技术检测到^[23]。因此若以 DNA 为材料检测寄主组织内病原菌的菌源量, 很容易将组织内已死亡的病原菌统计在内, 从而导致获得的菌源量数据偏高^[24-25]。为了准确估计病原菌的菌源量, 需要将样品中的“死菌”与“活菌”区分, 近年来在这方面的研究也取得了一定的进展。和 DNA 不同, RNA 特别是 mRNA 的半衰期较短, 一般只存在于活细胞中。细胞死亡后, RNA 会迅速地被降解成寡核苷酸片段^[26]。Chimento 等^[27] 研究结果表明, 柞树猝死病菌 *Phytophthora ramorum* 在死亡 7 d 后, 就检测不到病菌中的 mRNA, 而死亡 3 个月后, 病原菌中的 DNA 还能被检测到。因此通过提取样品中的 RNA, 利用 RNA 反转录试剂盒将 RNA 反转录成 cDNA, 设计特异性引物, 以 cDNA 为模板进行 real-time PCR 反应, 可以用来定量检测样品中活的病原菌的含量。近年来有利用 qRT-PCR 技术定量检测寄主中活的植物病原真菌的研究报道, Pavón 等^[28] 建立了链格孢属 *Alternaria* spp. 真菌的 qRT-PCR 检测技术, 并利用该技术对新鲜果蔬样品和加工后样品中活的病原菌进行了定量检测, 结果与传统培养方法得到的结果存在极显著的相关性。Ma 等^[29] 建立了可用来定量检测叶片中活的条锈菌 *P. striiformis* f. sp. *tritici* 的 qRT-PCR 技术, 并利用该技术对我国甘肃和青海不同海拔地区的小麦条锈菌越冬菌量进行了定量检测。Fan 等^[30] 建立了苹果树腐烂病菌 *Valsa mali* 的 qRT-PCR 检测技术并定量检测接种生防菌后苹果枝条中的 *V. mali* 活菌含量, 从而用来研究生防菌的防效和防治机制。另外, 基于 qRT-PCR 的栗黑水疫霉 *Phytophthora cambivora*

和樟疫霉 *P. cinnamomi* 的活体定量检测研究也已报道^[31-32]。此外该技术在植物病原细菌如柑橘溃疡病菌 *X. citri* subsp. *citri*^[33]、植物病原线虫如松材线虫 *Bursaphelenchus xylophilus*^[34] 和马铃薯孢囊线虫 *Globodera* spp.^[35] 的活体定量检测中均有报道。qRT-PCR 技术最明显的不足之处在于 RNA 提取过程中的损失、污染及降解问题,不同的 RNA 提取方法获得的 RNA 的质量和浓度均有差异。其次靶基因的选择对结果也有很大的影响,有些基因在病原菌不同发育阶段的表达量存在较大差异,从而影响对活体和死体病原菌的区分,如 Ma 等的研究表明利用小麦条锈病菌延伸因子 EF1 引物,采用 qRT-PCR 可以定量检测小麦叶片组织内活的条锈菌的生物量,但不能区分活的和死的小麦条锈病菌的夏孢子^[36];对 4 个柑橘溃疡病菌 *X. citri* subsp. *citri* 靶标基因(*gumD*、*rpfB*、*avrBs2* 和 *gyrB*)的研究也发现,只有 *gumD* 基因适合用来区分死的和活的 *X. citri* subsp. *citri* 细胞^[33]。

4 基于核酸染料(EMA/PMA)和 qPCR 的植物病原菌活体定量检测

叠氮溴化乙锭(ethidium monoazide, EMA)和叠氮溴化丙锭(propidium monoazide, PMA)是两种对 DNA 分子具有高度亲和力的光敏染料,它们不能透过完整的细胞膜,但可以穿过受破坏的细胞膜进入细胞内,选择性地结合细胞膜受损伤的死细胞的 DNA 并抑制其进行 PCR 扩增。其中 EMA 在进入膜损伤细胞并插入双链 DNA 后,在可见光的作用下,通过与 DNA 双螺旋发生不可逆的共价交叉偶合,从而抑制 PCR 反应中引物与死菌 DNA 的结合,达到区分死菌和活菌的目的^[37];而 PMA 在进入膜损伤细胞并插入双链 DNA 后,在可见光激活下, PMA 分子中具有光敏性的叠氮基团会生成高反应性的 nitrene 基,很容易地在结合部位与碳氢化合物部分结合生成稳定牢固的共价氮碳键,产生稳定的共价交联沉淀物,有效地抑制死菌细胞 DNA 的扩增^[38]。2003 年 Nogva 等^[39]提出了 EMA-PCR 方法用于区分死菌和活菌,随后 2006 年 Nocker 等^[40]研究发现 EMA 在一定程度上对某些种属细菌的活细胞也产生影响,提出了与 EMA 结构类似的 PMA 结合 qPCR 的活菌检测技术。此后将 EMA/PMA 与 PCR、qPCR 和 LAMP 技术相结合在食源性致病菌

的活菌检测研究中得到了广泛的应用^[25,41-44]。在活的植物病原菌的定量检测方面,目前关于 EMA/PMA 和 qPCR 相结合定量检测植物病原细菌的研究报道较多。其中基于 EMA-qPCR 和基于 PMA-qPCR 的活柑橘黄龙病菌 *Candidatus Liberibacter asiaticus* 定量检测技术都已建立^[45-46]。此外基于 EMA-qPCR 的番茄细菌性溃疡病菌 *Clavibacter michiganensis* subsp. *michiganensis* 活菌^[47]和基于 PMA-qPCR 的黄瓜细菌性角斑病菌 *Pseudomonas syringae* pv. *lachrymans*^[48]、胡萝卜细菌性枯萎病菌 *X. hortorum* pv. *carotae*^[49]、玉米细菌性枯萎病菌 *Pantoea stewartii* subsp. *stewartii*^[50]、植物细菌性青枯病菌 *Ralstonia solanacearum*^[51]、猕猴桃溃疡病菌 *P. syringae* pv. *actinidia*^[52] 活菌的定量检测技术均有报道。在其他植物病原菌方面, Vilanova 等^[53]报道了用来定量检测果实和花上存活的褐腐病菌 *Monilinia fructicola* 的 PMA-qPCR 技术; Christoforou 等^[54]建立了可以用来定量检测田间存活的马铃薯孢囊线虫(*Globodera pallida* 和 *G. rostochiensis*) 的 PMA-qPCR 检测技术; Al-Daoud 等^[55]利用 PMA 与 qPCR 相结合的技术定量检测土壤中共存的芸薹根肿菌 *P. brassicae* 的休眠孢子。虽然基于 EMA/PMA 的活体定量检测技术解决了 qRT-PCR 技术中 RNA 提取过程中的问题,但是影响其效率的因素也有不少^[41-42],包括 1) 染料的浓度、孵育时间和处理温度;2) 光源、光照时间等;3) 靶基因的长度和序列;另外样品中微生物的浓度、死细胞和活细胞的比例等都会影响 EMA/PMA 的效率。

5 展望

建立精准、可靠的植物病原菌定量检测方法,准确估计病原菌的种群数量,对于深入研究植物病害的流行规律,提高病害预测的准确性具有重要意义。qPCR 技术目前在植物病原菌定量检测中得到了比较广泛的应用,而数字 PCR 技术由于检测样品的通量很低、成本高,在植物病原菌的定量检测研究中应用还较少。但是数字 PCR 技术和 qPCR 相比具有独特的优势,且灵敏度和稳定性更高,随着技术不断发展,低成本的数字 PCR 产品将被开发出来,其应用范围会越来越广泛。

利用常规分离培养和一般的分子检测方法,均无法实现对寄主中活的病原菌越冬和越冬菌源、土

壤中存活的病原菌以及活的非可培养(viable but nonculturable, VBNG)状态细菌的定量检测。qRT-PCR技术、核酸染料(EMA/PMA)和qPCR相结合的技术是目前用于病原菌活体定量检测的两种主要技术,不仅实现了对样品中病原菌的定量检测,还可以区分样品中的死活病原菌细胞,较好地解决上述难题。虽然这两种技术在植物病原菌的检测中还处于起步阶段,但是随着这些技术的进一步发展和完善,将具有广阔应用前景。另外,随着一些高通量、快速精准、检测灵敏度高的新型检测方法如多重荧光定量PCR的开发,可为植物病原菌活体定量检测提供新技术。

参考文献

- [1] WALLENHAMMAR A C, ALMQUIST C, SÖDERSTRÖM M, et al. In-field distribution of *Plasmodiophora brassicae* measured using quantitative real-time PCR [J]. *Plant Pathology*, 2012, 61(1): 16–28.
- [2] YAN Jiahui, LUO Yong, CHEN Tingting, et al. Field distribution of wheat stripe rust latent infection using real-time PCR [J]. *Plant Disease*, 2012, 96(4): 544–551.
- [3] ZHENG Yaming, LUO Yong, ZHOU Yilin, et al. Real-time PCR quantification of latent infection of wheat powdery mildew in the field [J]. *European Journal of Plant Pathology*, 2013, 136(3): 565–575.
- [4] ROGERS S, ATKINS S, WEST J. Detection and quantification of airborne inoculum of *Sclerotinia sclerotiorum* using quantitative PCR [J]. *Plant Pathology*, 2009, 58(2): 324–331.
- [5] CAO Xueren, YAO Dongming, ZHOU Yilin, et al. Detection and quantification of airborne inoculum of *Blumeria graminis* f. sp. *tritici* using quantitative PCR [J]. *European Journal of Plant Pathology*, 2016, 146(1): 225–229.
- [6] VAN DER HEYDEN H, WALLON T, LEVESQUE C A, et al. Detection and quantification of *Pythium tracheiphilum* in soil by multiplex real-time qPCR [J]. *Plant Disease*, 2019, 103(3): 475–483.
- [7] BAIDOO R, YAN Guiping, NAGACHANDRABOSE S, et al. Developing a real-time PCR assay for direct identification and quantification of *Pratylenchus penetrans* in soil [J]. *Plant Disease*, 2017, 101(8): 1432–1441.
- [8] DURESSA D, RAUSCHER G, KOIKE S T, et al. A real-time PCR assay for detection and quantification of *Verticillium dahliae* in spinach seed [J]. *Phytopathology*, 2012, 102(4): 443–451.
- [9] AMARAL CARNEIRO G, MATIC S, ORTU G, et al. Development and validation of a TaqMan real time PCR assay for the specific detection and quantification of *Fusarium fujikuroi* in rice plants and seeds [J]. *Phytopathology*, 2017, 107(7): 885–892.
- [10] SCHENA L, NIGRO F, IPPOLITO A, et al. Real-time quantitative PCR: a new technology to detect and study phytopathogenic and antagonistic fungi [J]. *European Journal of Plant Pathology*, 2004, 110(9): 893–908.
- [11] LUCHI N, CAPRETTI P, PAZZAGLI M, et al. Powerful qPCR assays for the early detection of latent invaders: interdisciplinary approaches in clinical cancer research and plant pathology [J]. *Applied Microbiology and Biotechnology*, 2016, 100(12): 5189–5204.
- [12] 骆勇. 植物病害分子流行病学概述[J]. *植物病理学报*, 2009, 39(1): 1–10.
- [13] 郑亚明, 骆勇, 周益林, 等. 实时荧光定量PCR在植物病害流行病学中的应用[J]. *植物保护*, 2011, 37(4): 18–22.
- [14] VOGELSTEIN B, KINZLER K W. Digital PCR [J]. *Proceedings of the National Academy of Sciences of the United States of America*, 1999, 96(16): 9236–9241.
- [15] BLAYA J, LLORET E, SANTISIMA-TRINIDAD A B, et al. Molecular methods (digital PCR and real-time PCR) for the quantification of low copy DNA of *Phytophthora nicotianae* in environmental samples [J]. *Pest Management Science*, 2016, 72(4): 747–753.
- [16] ZHAO Yun, XIA Qingyan, YIN Youping, et al. Comparison of droplet digital PCR and quantitative PCR assays for quantitative detection of *Xanthomonas citri* subsp. *citri* [J/OL]. *PLoS ONE*, 2016, 11(7): e0159004. DOI: 10.1371/journal.pone.0159004.
- [17] DUPAS E, LEGENDRE B, OLIVIER V, et al. Comparison of real-time PCR and droplet digital PCR for the detection of *Xylella fastidiosa* in plants [J]. *Journal of Microbiological Methods*, 2019, 162: 86–95.
- [18] DREO T, PIRC M, RAMŠAK Ž, et al. Optimising droplet digital PCR analysis approaches for detection and quantification of bacteria: a case study of fire blight and potato brown rot [J]. *Analytical and Bioanalytical Chemistry*, 2014, 406(26): 6513–6528.
- [19] TANJA V, LOUISE N. Quantification of *Agrobacterium vitis* from grapevine nursery stock and vineyard soil using droplet digital PCR [J]. *Plant Disease*, 2018, 102(11): 2136–2141.
- [20] ZHONG Xi, LIU Xuelu, LOU Binghai, et al. Development of a sensitive and reliable droplet digital PCR assay for the detection of ‘*Candidatus Liberibacter asiaticus*’ [J]. *Journal of Integrative Agriculture*, 2018, 17(2): 483–487.
- [21] SELVARAJ V, MAHESHWARI Y, HAJERI S, et al. Development of a duplex droplet digital PCR assay for absolute quantitative detection of ‘*Candidatus Liberibacter asiaticus*’ [J/OL]. *PLoS ONE*, 2018, 13(5): e0197184. DOI: 10.1371/journal.pone.0197184.
- [22] GOSSEN B D, AL-DAOUD F, DUMONCEAUX T, et al. Comparison of techniques for estimation of resting spores of *Plasmodiophora brassicae* in soil [J]. *Plant Pathology*, 2019, 68(5): 954–961.

- [23] JOSEPHSON K L, GERBA C P, PEPPER I L. Polymerase chain reaction detection of nonviable bacterial pathogens [J]. *Applied and Environmental Microbiology*, 1993, 59(10): 3513–3515.
- [24] KEER J, BIRCH L. Molecular methods for the assessment of bacterial viability [J]. *Journal of Microbiological Methods*, 2003, 53: 175–183.
- [25] FITTIPALDI M, NOCKER A, CODONY F. Progress in understanding preferential detection of live cells using viability dyes in combination with DNA amplification [J]. *Journal of Microbiological Methods*, 2012, 91: 276–289.
- [26] TOURRIÈRE H, CHEBLI K, TAZI J. mRNA degradation machines in eukaryotic cells [J]. *Biochimie*, 2002, 84(8): 821–837.
- [27] CHIMENTO A, CACCIOLA S O, GARBELOTTO M. Detection of mRNA by reverse-transcription PCR as an indicator of viability in *Phytophthora ramorum* [J]. *Forest Pathology*, 2012, 42(1): 14–21.
- [28] PAVÓN M Á, GONZÁLEZ I, MARTÍN R, et al. A real-time reverse-transcriptase PCR technique for detection and quantification of viable *Alternaria* spp. in foodstuffs [J]. *Food Control*, 2012, 28(2): 286–294.
- [29] MA Lijie, KONG Xinyu, QIAO Jiaxing, et al. Overwintering of *Puccinia striiformis* f. *tritici* on winter wheat at varying altitudes in Gansu and Qinghai provinces [J]. *Plant Disease*, 2016, 100(6): 1138–1145.
- [30] FAN Dongying, LI Yanfang, ZHAO Lingyun, et al. Study on interactions between the major apple Valsa canker pathogen *Valsa mali* and its biocontrol agent *Saccharothrix yanglingensis* Hhs. 015 using RT-qPCR [J/OL]. *PLoS ONE*, 2016, 11(9): e0162174. DOI: 10.1371/journal.pone.0162174.
- [31] VETTRAINO A M, TOMASSINI A, VANNINI A, et al. Use of mRNA as an indicator of the viability of *Phytophthora cambivora* [J]. *Acta Horticulturae*, 2010, 866: 431–434.
- [32] KUNADIYA M, DUNSTAN W, WHITE D, et al. A qPCR assay for the detection of *Phytophthora cinnamomi* including an mRNA protocol designed to establish propagule viability in environmental samples [J]. *Plant Disease*, 2019, 103(9): 2443–2450.
- [33] GOLMOHAMMADI M, LLOP P, SCUDERI G, et al. mRNA from selected genes is useful for specific detection and quantification of viable *Xanthomonas citri* subsp. *citri* [J]. *Plant Pathology*, 2012, 61(3): 479–488.
- [34] LEAL I, FOORD B, ALLEN E, et al. Development of two reverse transcription-PCR methods to detect living pinewood nematode, *Bursaphelenchus xylophilus*, in wood [J]. *Forest Pathology*, 2013, 43(2): 104–114.
- [35] MENDES O, VAN GENT-PELZER M P E, VAND L T A J, et al. Quantification of viable eggs of the potato cyst nematodes (*Globodera* spp.) using either trehalose or RNA-specific real-time PCR [J]. *Nematology*, 2014, 16(10): 1219–1232.
- [36] MA Lijie, QIAO Jiaxing, KONG Xinyu, et al. Effect of low temperature and wheat winter-hardiness on survival of *Puccinia striiformis* f. sp. *tritici* under controlled conditions [J/OL]. *PLoS ONE*, 2015, 10(6): e0130691. DOI: 10.1371/journal.pone.0130691.
- [37] KNUT R, BIRGITTE M, SIGNE M D, et al. Use of ethidium monoazide and PCR in combination for quantification of viable and dead cells in complex samples [J]. *Applied and Environmental Microbiology*, 2005, 71(2): 1018–1024.
- [38] NOCKER A, CAMPER A K. Novel approaches toward preferential detection of viable cells using nucleic acid amplification techniques [J]. *FEMS Microbiology Letters*, 2008, 291(2): 137–142.
- [39] NOGVA H K, DRØMTORP, S M, NISSEN H, et al. Ethidium monoazide for DNA-based differentiation of viable and dead bacteria by 5'-nuclease PCR [J]. *BioTechniques*, 2003, 34(4): 804–813.
- [40] NOCKER A, CHEUNG C Y, CAMPER A K. Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells [J]. *Journal of Microbiological Methods*, 2006, 67(2): 310–320.
- [41] ELIZAQUIVEL P, AZNAR R, SÁNCHEZ G. Recent developments in the use of viability dyes and quantitative PCR in the food microbiology field [J]. *Journal of Applied Microbiology*, 2014, 116(1): 1–13.
- [42] ZENG Dexin, CHEN Zi, JIANG Yuan, et al. Advances and challenges in viability detection of foodborne pathogens [J/OL]. *Frontiers in Microbiology*, 2016, 7: 1833. DOI: 10.3389/fmicb.2016.01833.
- [43] 吕婧, 姜兆兴, 王海艳, 等. 叠氮溴化乙锭与 PCR 技术结合检测活菌研究进展 [J]. *食品研究与开发*, 2017, 38(24): 220–224.
- [44] 肖莉莉, 张昭寰, 娄阳, 等. 叠氮溴化丙锭(PMA)在食源性致病菌检测中的应用[J]. *中国食品学报*, 2016, 16(6): 187–194.
- [45] TRIVEDI P, SAGARAM U S, KIM J S, et al. Quantification of viable *Candidatus Liberibacter asiaticus* in hosts using quantitative PCR with the aid of ethidium monoazide (EMA) [J]. *European Journal of Plant Pathology*, 2009, 124(4): 553–563.
- [46] HU H, DAVIS M J, BRLANSKY R H. Quantification of live '*Candidatus Liberibacter asiaticus*' populations using real-time PCR and propidium monoazide [J]. *Plant Disease*, 2013, 97(9): 1158–1167.
- [47] LUO Laixing, WALTERS C, BOLKAN H, et al. Quantification of viable cells of *Clavibacter michiganensis* subsp. *michiganensis* using a DNA binding dye and a real-time PCR assay [J]. *Plant Pathology*, 2008, 57(2): 332–337.
- [48] MENG Xianglong, CHAI Ali, CHEN Lu, et al. Rapid detection and quantification of viable *Pseudomonas syringae* pv. *lachrymans* cells in contaminated cucumber seeds using propidium monoazide and a real-time PCR assay [J]. *Canadian Journal of Plant Pathology*, 2016, 38(3): 296–306.

布研究[J]. 浙江农业科学, 2016, 57(12): 1976-1979.

- [19] 杨帅. 无人机低空喷雾雾滴在作物冠层的沉积分布规律及防治效果研究[D]. 北京: 中国农业科学院, 2014.
- [20] 高圆圆, 张玉涛, 张宁, 等. 小型无人机低空喷洒在小麦田的雾滴沉积分布及对小麦吸浆虫的防治效果初探[J]. 作物杂志, 2013(2): 139-142.
- [21] ALM S R, REICHARD D L, HALL F R. Effects of spray drop size distribution of drops containing bifenthrin on *Tetranychus urticae* (Acari: Tetranychidae) [J]. Journal of Economic Entomology, 1987, 80(2): 517-520.

(责任编辑: 田 喆)

(上接 11 页)

- [49] TEMPLE T N, DU TOIT L J, DERIE M L, et al. Quantitative molecular detection of *Xanthomonas hortorum* pv. *carotae* in carrot seed before and after hot-water treatment [J]. Plant Disease, 2013, 97(12): 1585-1592.
- [50] 冯建军, 刘杰, 王飞, 等. PMA 结合实时荧光 PCR 进行玉米细菌性枯萎病菌细胞活性检测初步研究[J]. 植物检疫, 2014, 28(2): 27-32.
- [51] 王帅, 徐进, 许景升, 等. PMA-qPCR 定量检测青枯菌活菌方法的建立[J]. 植物保护, 2018, 44(6): 122-128.
- [52] 肖妍, 刘芸宏, 高贵田, 等. PMA-qPCR 方法检测陕西猕猴桃溃疡菌优势病原菌活菌的研究[J]. 食品与机械, 2019, 35(4): 48-53.

(责任编辑: 田 喆)

(上接 24 页)

- [10] 巩焕然. 云南和广西省胜红蓟黄脉病的病原研究[D]. 杭州: 浙江大学, 2010.
- [11] XIONG Qing, FANG Sanwei, WU Jianxiang, et al. Ageratum yellow vein China virus is a distinct begomovirus species associated with a DNA β molecule [J]. Phytopathology, 2007, 97(4): 405-411.
- [12] JIAO Xiaoyang, GONG Huanrang, LIU Xuejian, et al. Etiology of Ageratum yellow vein diseases in South China [J]. Plant Disease, 2013, 97(11): 1497-1503.
- [13] YANG Xiuling, GUO Wei, MA Xinying, et al. Molecular characterization of tomato leaf curl China virus, infecting tomato plants in China, and functional analyses of its associated betasatellite [J]. Applied and Environmental Microbiology, 2011, 77(9): 3092-3101.
- [14] MARTIN D P, MURRELL B, GOLDEN M, et al. RDP4: Detection and analysis of recombination patterns in virus genomes [J/OL]. Virus Evolution, 2015, 1(1): vev003. DOI: 10.1093/ve/vev003.
- [15] LI Zhenghe, XIE Yan, ZHOU Xueping, et al. Tobacco curly shoot virus DNA β is not necessary for infection but intensifies symptoms in a host-dependent manner [J]. Phytopathology, 2005, 95(8): 902-908.
- [16] ROJAS M R, MACEDO M A, MALIANO M R, et al. World

- [22] 袁会珠, 王国宾. 雾滴大小和覆盖密度与农药防治效果的关系[J]. 植物保护, 2015, 41(6): 9-16.
- [23] 杨帅, 王国宾, 杨代斌, 等. 无人机低空喷施苯氧威防治亚洲玉米螟初探[J]. 中国植保导刊, 2015, 35(2): 59-62.
- [24] 高赛超, 周晓欣, 秦维彩, 等. 利用风洞评价助剂对杀虫剂航空喷雾雾滴飘移的影响[J]. 应用昆虫学报, 2018, 55(4): 654-658.
- [25] 张龙, 马金龙, 陈树茂, 等. 小型多旋翼无人机在玉米植株上雾滴沉积分布及对玉米锈病防治效果的影响初探[J]. 农业工程技术, 2018(9): 84-87.

- [53] VILANOVA L, USALL J, TEIXIDÓ N, et al. Assessment of viable conidia of *Monilinia fructicola* in flower and stone fruit combining propidium monoazide (PMA) and qPCR [J]. Plant Pathology, 2017, 66(8): 1276-1287.
- [54] CHRISTOFOROU M, PANTELIDES I S, KANETIS L, et al. Rapid detection and quantification of viable potato cyst nematodes using qPCR in combination with propidium monoazide [J]. Plant Pathology, 2014, 63(5): 1185-1192.
- [55] AL-DAOUD F, GOSSEN B D, ROBSON J, et al. Propidium monoazide improves quantification of resting spores of *Plasmidiophora brassicae* with qPCR [J]. Plant Disease, 2017, 101(3): 442-447.

(责任编辑: 田 喆)

- management of geminiviruses [J]. Annual Review Phytopathology, 2018, 56: 637-677.
- [17] QING Ling, ZHOU Xueping. Trans-replication of, and competition between, DNA β satellites in plants inoculated with *Tomato yellow leaf curl China virus* and *Tobacco curly shoot virus* [J]. Phytopathology, 2009, 99(6): 716-720.
- [18] ZHANG Tong, XU Xiongbiao, HUANG Changjun, et al. A novel DNA motif contributes to selective replication of a geminivirus-associated betasatellite by a helper virus-encoded replication-related protein [J]. Journal of Virology, 2016, 90(4): 2077-2089.
- [19] XU Xiongbiao, QIAN Yajuan, WANG Yaqin, et al. Iterons homologous to helper geminiviruses are essential for efficient replication of betasatellites [J/OL]. Journal of Virology, 2019, 93(5): e01532-18. DOI: 10.1128/JVI.01532-18.
- [20] ZHOU Xueping, LIU Yule, CALVERT L, et al. Evidence that DNA-A of a geminivirus associated with severe cassava mosaic disease in Uganda has arisen by interspecific recombination [J]. Journal of General Virology, 1997, 78(Pt8): 2101-2111.
- [21] MUBIN M, BRIDDON R W, MANSOOR S, et al. Diverse and recombinant DNA betasatellites are associated with a begomovirus disease complex of *Digera arvensis*, a weed host [J]. Virus Research, 2009, 142(1/2): 208-212.

(责任编辑: 田 喆)