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# 酿酒葡萄4种病毒多重RT-PCR检测体系的建立

苏 静,陈佰鸿*,毛 娟,左存武,胡紫璟,王 凯

(甘肃农业大学园艺学院,兰州 730070)

**摘 要**:【目的】建立快速、灵敏的葡萄病毒多重RT-PCR检测体系。【方法】通过设计6对不同引物,在退火温度分别为48.0、49.0、50.0、51.0、52.0、53.0、54.0、55.0、56.0、57.0、58.0、59.0和60.0 ℃,采用单一RT-PCR技术检测酿酒葡萄6种常见的葡萄病毒,即葡萄病毒A(*Grapevine virus A*, GVA)、葡萄斑点病毒(*Grapevine fleck virus*, GFKV)、葡萄扇叶病毒(*Grapevine fanleaf virus*, GFLV)、葡萄卷叶病毒1(*Grapevine leafroll associated virus-1*, GLRaV-1)、葡萄卷叶病毒2(*Grapevine leafroll associated virus-1*, GLRaV-2)和葡萄卷叶病毒4(*Grapevine leafroll associated virus-4*, GLRaV-4)。在此基础上,对退火温度相似、扩增片段长度不同的引物进行组合,建立能同时检测2种或2种以上病毒的多重RT-PCR检测体系。【结果】单一RT-PCR结果显示,退火温度为49 ℃、55 ℃和60 ℃时,可分别检测GVA和GLRaV、GLRaV和GFKV以及GFLV和GLRaV。多重RT-PCR结果显示,退火温度分别为49 ℃和55 ℃时,引物GVA和GLRaV-2以及引物GLRaV-1和GFKV组合所建立的2个多重RT-PCR检测体系可同时检测葡萄叶片中GVA和GLRaV以及GLRaV和GFKV。所检测的河西地区16份样品普遍携带葡萄病毒,部分葡萄样品同时携带两种病毒。其中,5份样品为GVA阳性,8份为GLRaV-1阳性,3份为GLRaV-2阳性,5份为GFKV阳性,所有样品均未检测到GFLV和GLRaV-4。退火温度分别为49 ℃和55 ℃时所建立的GVA和GLRaV以及GLRaV和GFKV两个多重PCR扩增体系检测结果与各样品单一PCR检测结果均一致。【结论】建立2套多重PCR检测体系,可同时有效地检测GVA和GLRaV-2或GLRaV-1和GFKV,可用于田间葡萄样品的快速检测。

**关键词**:酿酒葡萄;病毒;检测;多重RT-PCR

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# Establishment of multiple RT-PCR detection system for four virus in the cultivars of wine grape

SU Jing, CHEN Baihong*, MAO Juan, ZUO Cunwu, HU Zijing, WANG Kai

*( College of Horticulture, Gansu Agricultural University, Lanzhou 730070, Gansu, China)*

**Abstract**:【Objective】Grape is one of the most important fruit crops in the world. Most grape production is for wine making. Grape cultivars are uasually vegetively propagated. Hexi of Gansu province have become a large region for planting grape for wine. Virus diseases have spread widely along with the enlargement of planting area. There are 6 kinds of virus of grape in China, including *Grapevine virus A* (GVA), *Grapevine leaf roll-associated virus 1* (GLRaV-1), *Grapevine leaf roll-associated virus 2* (GLRaV-2), *Grapevine fleck virus* (GFKV), *Graperine fanleaf virus* (GFLV) and *Grapevine leaf roll-associated virus 4* (GLRaV-4).A simple, fast and efficient detection technology is necessary for controlling the virus diseases in grape.【Methods】16 leaf samples from 13 grape cultivars were collected from 4 grape production bases in Wuwei, Zhangye and Lanzhou city. All samples were frozen separately in liquid nitrogen and were stored at -80 ℃ for further use. Total RNA of all samples were extracted using modified CTAB method, and cDNA were synthesized using M-MLV First Stand DNA Synthesis Kit (TaKaRa, Bio Inc., Japan). For each virus, the sequences from different regions were downward from the National Center for Biotechnolo-

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作者简介:苏静,男,在读硕士研究生,研究方向:果树生理与栽培技术。Tel:15109318107,E-mail:1576359350@qq.com

*通信作者 Author for correspondence. E-mail: bhch@gsau.edu.cn

gy Information (NCBI, http://www.ncbi.nlm.nih.gov). After that, 6 pairs of primers from conservative sequences were designed using on online software program Primer 3 (http://primer3.ut.ee/). To detect the amplification efficiency and the existence of objective virus, each sample was amplified by simple RT-PCR with 13 annealing temperatures from 48 ℃ to 60 ℃. Furthermore, multiple RT-PCR systems were established by mixing two or more pairs of primers with same annealing temperature.【Results】16 grape samples could be effectively detected by simple RT-PCR. In the Moga Grape Base (Wuwei), GVA was positively detected from 3 out of 5 grape samples, both GLRaV-1 and GFKV were detected from 2 samples, respectively. In the Huangtai Grape Manor (Wuwei), GLRaV-1 was positively discovered from 5 samples out of 6 grape samples, GLRaV-2 was detected from 1 sample and 2 sample, respectively. In the Guofeng Organic Grape Manor (Zhangye) and Moga Grape Base (Lanzhou), GVA, GLRaV-1 and GFKV were detected from most samples while GFLV and GLRaV-4 were not found. With the annealing temperature of 49 ℃, virus GVA and GLRaV-2 in grape tissue could be positively detected by primers GVA and GLRaV-2, respectively. When the annealing temperature was up to 55 ℃, GLRaV-1 and GFKV could be detected by GLRaV-1 and GFKV, respectively. When the annealing temperature was 60 ℃, GFLV and GLRaV-4 could be detected by GFLV and GLRaV, respectively. Furthermore, three multiple PCR detection systems were established with integration of primer GVA and GLRaV-2 (system I), GLRaV-1 and GFKV (system II), and GFLV and GLRaV-4 (system III), with annealing temperature of 49 ℃, 55 ℃, 60 ℃, respectively. Within three systems, virus GVA and GLRaV-2 could be positively detected at the same time using system I, virus GLRaV-1 and GFKV using system II. In addition, system III could not positively detect the existence of GFLV and GLRaV-4 in grape tissue. Therefore, we suggested that multiple detection system I and II could be used for virus detection in wine grape.【Conclusion】Two multiple PCR systems with higher sensitivity for virus detection were established. The results of detection indicated that viral diseases in grape were ubiquitous in Hexi region. Control and prevention of the virus disease of wine grape should be taken into the consideration.

**Key words**: Vine grape; Virus; Detection; Multiple RT-PCR

葡萄(*Vitis vinifera* L.)种植面积和产量仅次于柑橘，是世界第二大水果，也是我国主栽果树之一[1]。近年来，果园的不正当管理是病虫害日益严重的原因之一。葡萄病毒种类繁多、分布广，发生普遍，不仅影响葡萄的生长，也会造成产量和品质下降。截止到2012年，世界上共报道侵染的葡萄病毒63种[2]，中国已报道11种，即5种葡萄卷叶伴随病毒(*Grapevine leaf roll-associated virus-1*，*2*，*3*，*4*，*7*，GLRaV-1，2，3，4，7)、4种皱木复合相关病毒(*Grapevine virus A*，GVA；*Grapevine virus B*，GVB；*Grapevine virus E*，GVE；*Grapevine rupestrisstem pitting associated virus*，GRSPaV)、葡萄扇叶病毒(*Graperine fanleaf virus*，GFLV)、葡萄斑点病毒(*Grapevine fleck virus*，GFKV)[3]。其中，对葡萄危害较为严重的有GFKV、GFLV、GLRaV-1、GLRaV-2、GLRaV-4和GVA，这些病毒也可复合侵染，造成更大的危害。

目前，抗病品种、生物和化学防治等措施都不能有效地防治葡萄病毒病，而建立快速、准确、灵敏的病毒检测技术对葡萄病毒病防控具有重要的现实意义。近年来，通过不断的努力，前人已建立多种葡萄病毒的检测方法，如生物学鉴定法、酶联免疫吸附法(ELISA)、基因芯片技术、逆转录环介导恒温扩增法、高通量测序法和RT-PCR法等[4]。传统的生物学鉴定方法[5]所需时间过长，不能清楚分辨所有的葡萄病毒，如葡萄卷叶病毒。传统酶联免疫吸附法[6]与部分以酶联免疫法为基础创建的检测技术，如细菌磁力珠和DAS-ELISA结合的荧光免疫分析方法可检测GFLV，并且其灵敏度要比传统ELISA高6个数量级[7]。但是，此类方法花费高，灵敏度低。基因芯片技术[8]是新型检测技术，它可以同时检测38种葡萄病毒。Abdullahi[9]建立了一种用于检测6种葡萄病毒的抗体微阵列，与常规的ELISA方法相比具有相