Sensitive detection and discrimination of WSMV, TriMV and HPV using multiplex RT-PCR

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INTRODUCTION

- ***Wheat streak mosaic virus*** **(WSMV),** ***Triticum mosaic virus*** **(TriMV) and** ***High Plains Virus*** **(HPV) are responsible for causing considerable damage to wheat. Infections of WSMV, TriMV and HPV are hard to discriminate. Multi virus species infections are very common in cereal fields.**
- **A method for early, accurate and sensitive detection of WSMV, TriMV** ***and*** **HPV in plant tissues is required by breeders, plant health officials, quarantine and extension specialists for screening resistance, and also detection, identification and management of disease outbreaks and reservoir hosts.**

MATERIALS AND METHODS

- **Three set of specific RT-PCR primers were designed using the Web interface software pathway Primer3-mFOLD-BLASTn. The CI protein gene of WSMV and TriMV, and the nucleoprotein gene of HPV were targeted.**

Table 1. Primers codes and product size designed for different viruses

Virus	Primer Name	ΔG	Product Size (bp)
HPV	HPV 3F & HPV 3R	0.2 & 1.0	178
	HPV 5F & HPV 3R	0.0 & 1.0	255
	HPV 6F & HPV 3R	0.9 & 1.0	248
	HPV 4F & HPV 4R	0.8 & 0.8	145
WSMV	WSMV 2F & WSMV 2R	1.0 & 0.6	110
TriMV	TriMV 2F & TriMV 2R	0.9 & 0.6	69

All primer sequences are available by request.

- **Total RNA was isolated from infected plant tissues using the RNeasy plant mini kit (Qiagen).**

Individual RT-PCR amplifications

- **SuperScript® III Reverse Transcriptase was used to synthesize cDNA from RNA templates of WSMV, TriMV and HPV in two step RT-PCR amplifications.**
- **GoTaq Green Master Mix® (Promega) Reactions were carried out in 20 µl containing 10 µL of 2X reaction mix, 0.2 µM of each primer, 2 µL cDNA template and nuclease free water to make volume up to 20 µL.**

Multiplex RT-PCR amplifications

- **Qiagen Multiplex PCR Kit® (Qiagen) was used for multiplex RT-PCR of WSMV, TriMV and HPV. Reactions were carried out in 50 µl containing 25 µL 2X reaction mix, 0.2 µM of each primer, 2 µL each cDNA template and nuclease free water to make volume up to 50 µL.**
- **Four primer combinations were tested seeking the best combination for multiplexing.**
- **Cycling conditions for multiplex PCR were initial denaturation 95ºC for 15 min, followed by 35 cycles (95ºC for 30 sec, 58ºC for 60 sec, and 72ºC for 45 sec. Final extension was 4 min.**
- **TOPO-TA Cloning Kit (Invitrogen) was used to clone the PCR amplicons to generate artificial positive controls and further confirmation by sequencing.**

RESULTS

- **All primers showed 100% query coverage and 100% identity** ***In silico*** **after BLASTn alignment with available GenBank accessions of corresponding viruses.**
- **Gradient PCRs were performed individually. WSMV (Fig. 1B), TriMV (Fig. 1C) and HPV (Fig. 1A). The assay perform well in a range of Tm (51.8 - 61.9 ºC),**

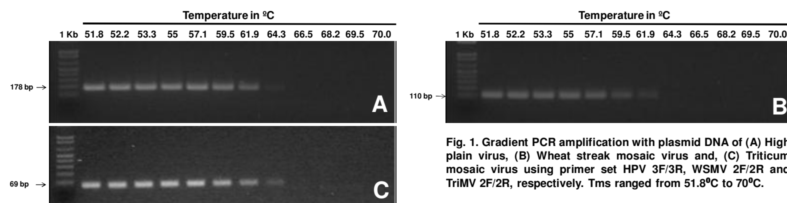

Fig. 1. Gradient PCR amplification with plasmid DNA of (A) High plain virus, (B) Wheat streak mosaic virus and, (C) Triticum mosaic virus using primer set HPV 3F/3R, WSMV 2F/2R and TriMV 2F/2R, respectively. Tms ranged from 51.8ºC to 70ºC.

- **Due to high diversity in HPV strains, five primer's combinations were made and a broad strain range detection was confirmed against isolates HPV 406, HPV 143, HPV 08-01, HPV 404-83, HPV 407-961 and HPV 404-82.**

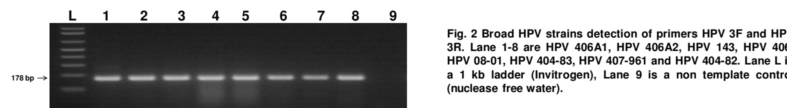

Fig. 2 Broad HPV strains detection of primers HPV 3F and HPV 3R. Lane 1-8 are HPV 406A1, HPV 406A2, HPV 143, HPV 406, HPV 08-01, HPV 404-83, HPV 407-961 and HPV 404-82. Lane L is a 1 kb ladder (Invitrogen), Lane 9 is a non template control (nuclease free water).

- **Broad range detection of WSMV primers (WSMV 2F/WSMV 2R) was confirmed against isolates WSMV 117 KS, WSMV 425 OSU, WSMV kali MT, WSMV OSU, WSMV Merredin 1 (Australia), WSMV Merredin 2 (Australia), and WSMV Type.**
- **Primers TriMV 2F and TriMV 2R were tested against three OK isolates of TriMV.**
- **Four different combinations of primers were tested against the cDNA of WSMV, TriMV and HPV (Fig. 3A).**
- **Primer combination WSMV 2F/2R, TriMV 2F/2R and HPV 3F/3R, were selected for multiplex PCR of WSMV, TriMV and HPV respectively.**

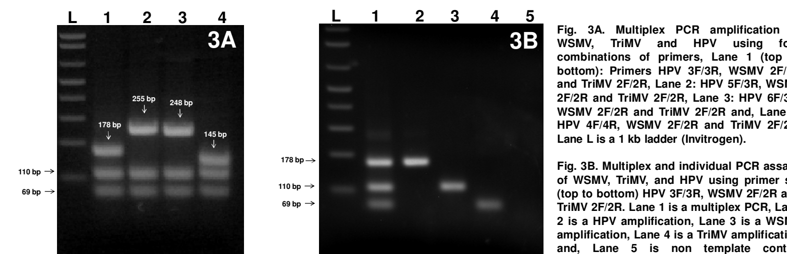

Fig. 3A. Multiplex PCR amplification of WSMV, TriMV and HPV using four combinations of primers, Lane 1 (top to bottom): Primers HPV 3F/3R, WSMV 2F/2R and TriMV 2F/2R, Lane 2: HPV 5F/3R, WSMV 2F/2R and TriMV 2F/2R, Lane 3: HPV 6F/3R, WSMV 2F/2R and TriMV 2F/2R and, Lane 4: HPV 4F/4R, WSMV 2F/2R and TriMV 2F/2R. Lane L is a 1 kb ladder (Invitrogen).

Fig. 3B. Multiplex and individual PCR assays of WSMV, TriMV, and HPV using primer set (top to bottom) HPV 3F/3R, WSMV 2F/2R and TriMV 2F/2R. Lane 1 is a multiplex PCR, Lane 2 is a HPV amplification, Lane 3 is a WSMV amplification, Lane 4 is a TriMV amplification and, Lane 5 is non template control (nuclease free water).

CONCLUSIONS

Multiplex or individual RT-PCR amplification for detection and discrimination of WSMV, TriMV and HPV are consistent, rapid and accurate. This multiplex RT-PCR has application for breeding and certification programs, biosecurity and microbial forensics and the primers can also be used for individual virus test.

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