Detection and quantification of *Potato mop-top virus* (PMTV) in Colombia using qRT-PCR

Detección y cuantificación del Potato mop-top virus (PMTV) en Colombia mediante qRT-PCR

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Abstract

Potato mop-top virus (PMTV) is a re-emerging virus in potato crops in Colombia. It is transmitted by *Spongospora subterranea*, the causal agent of powdery scab. Detection of PMTV is difficult due to its irregular distribution in infected plants, low titer and systemic movement of naked RNA. To increase the number of diagnostic tools available to detect the PMTV strains present in Colombia and to support seed certification schemes, a two-step qRT-PCR test using primers PMTV-1948F/ PMTV-2017R and the Taqman® probe PMTV-1970 targeted to the CP-RT gene of RNA2 from PMTV was evaluated. A standard curve was obtained from *in vitro* transcripts of a 1513 bp fragment of this gene. The qRT-PCR test was evaluated using *Nicotiana benthamiana* and *Solanum phureja* bait plants inoculated with Sss cystosori, root-tissue from plants of potato showing symptoms of powdery scab in La Unión (Antioquia, Colombia) and commercial tuber seeds. qRT-PCR was able to detect PMTV from roots in 11 out of 20 bait plants; while in the case of field samples 14 out 15 plants tested positive with concentration between $4{,}72x10^{11}$ - $7{,}60x10^{13}$ viral particles/µL. Finally, PMTV was detected in one out of 16 tuber seeds tested. These results demonstrate that qRT-PCR can be used routinely in the detection of PMTV strains affecting potato in Colombia.

Key words: Real time RT-PCR, *Solanum phureja*, *Solanum tuberosum, Spongospora subterranea*.

Resumen

El *Potato mop-top virus* (PMTV) es uno de los virus re-emergentes en cultivos de papa en Colombia. Es transmitido por *Spongospora subterranea*, el agente causal de la sarna polvosa. La detección del PMTV presenta dificultades debido a su distribución irregular en las plantas, bajo título y movimiento sistémico como ARN desnudo. Con el fin de ampliar el rango de herramientas disponibles para detectar el PMTV en los programas de certificación de tubérculo-semilla, en este estudio se evaluó la prueba de RT-PCR en tiempo real (qRT-PCR) en dos pasos: con los cebadores PMTV-1948F/PMTV-2017R y la sonda Taqman® PMTV-1970, dirigidos al gen CP-RT del ARN2 viral. Se construyó una curva estándar a partir de la transcripción *in vitro* de un fragmento de 1513 pb de este gen. Posteriormente, se evaluó la utilidad de la técnica a partir de tres tipos de muestras: plantas señuelo de *Nicotiana benthamiana* y *Solanum phureja* inoculadas con quistosoros de Sss, raíces de papa con

síntomas de sarna polvosa del municipio de La Unión (Antioquia) y tubérculos-semilla. Mediante qRT-PCR fue posible detectar el virus en 11 de las 20 muestras de raíz de plantas señuelo, mientras que 14 de las 15 muestras de raíces de papa resultaron positivas, estimándose una concentración entre 4.72 x 10^{11} y 7.60 x 10^{13} partículas virales/µl. Adicionalmente, en el ensayo de tubérculo-semilla se determinó la presencia del PMTV en una de las 16 muestras. Estos resultados indican la viabilidad de utilizar rutinariamente la técnica de qRT-PCR para la detección de PMTV en Colombia.

Palabras clave: RT-PCR en tiempo real, *Solanum phureja*, *Solanum tuberosum*, *Spongospora subterranea*.

Introduction

Potato mop-top virus (PMTV) (*Pomovirus*, *Virgaviridae*) is a virus of the potato (*Solanum tuberosum* L.) crop of the Andean region (Salazar, 2006). Its presence in Colombia was not recognized before 1970 and, was confirmed only in 2007 (Vélez, 2007). It is transmitted by *Spongospora subterranea* (Wallroth) Lagerheim f. sp. *subterranea* Tomlinson (Sss) (Jones and Harrison, 1969), causal agent of potato powdery scab, one of the most limiting diseases for this crop in Colombia (Osorio *et al.*, 2012). PMTV can be also transmitted by tuber-seed, especially when plant tubers are obtained from plants with leaf symptoms of the disease (Santala *et al.*, 2010). The disease induced by PMTV has a wide range of symptoms. In Europe and Northamerica crops is frequent the presence of rings on the tuber surface, which internally are observed as necrotic arcs 'spraing', and can be easily confused with symptoms caused by *Tobacco rattle virus* (TRV) or by the race NTN of *Potato virus Y* (PVY) (Calvert and Harrison, 1966; Santala *et al.*, 2010). At the leaf level, plants have chlorotic spots in V shape ('Acuba' type) besides of the reduction in the internode length ('mop-top') (Calvert and Harrison, 1966). In the Andean region there are not manifestation of the symptoms in the tubers; whereas at the leaf level it could be seen a yellow color, dwarfism and internode shortening (Tenorio *et al.*, 2006), although in many occasions infections could be asymptomatic (Latvala Kilby *et al.*, 2009). However, till now the symptomatology that this virus causes in the potato varieties sown in Colombia has not been clearly defined (Osorio *et al.*, 2012).

The PMTV genome comprises three single stranded RNA molecules, encapsulated individually in rigid shape of rigid rod (Savenkov *et al.*, 1999). RNA1 encodes for the RNA polymerase dependent on RNA (RdRp) (Savenkov *et al.*, 2003). RNA2 has an ORF for the capsid protein (CP) and for a CP-RT protein generated by a read-through and associated with the PMTV transmission by Sss (Sandgren *et al.*, 2001). RNA3 encodes a block of three genes (TGB) which proteins assist the viral movement from cell to cell as naked RNA; besides of a 8 kDa cysteine rich protein (CRP) (Savenkov *et al.*, 2003). Recently, it has been demonstrated that the TGBp1 protein acts in association with microtubules and plasmodesmata, which supports its role as movement protein (Shemyakina *et al.*, 2011).

Viral diseases management is supported on the availability of tools for their detection that support the seed certification programs, vector management and genetic breeding. For the PMTV case, the development of such techniques has been difficult due to its erratic distribution in the plants, low viral titration in the tissues and systemic infection as naked RNA (Xu *et al.*, 2004; Santala *et al.*, 2010). In addition, McGeachy and Barker (2000) found that virions are not present in every infected cell by PMTV, which can be explain by the wide range of symptoms induced by the virus since the capsid is required to express those symptoms.

Among the methodologies developed to detect PMTV are included bioassays with

bait plants like *N. benthamiana* (Vélez, 2007), ELISA tests with monoclonal and polyclonal antibodies (Arif y Torrance, 1996; Cerovska *et al.*, 2003), conventional RTPCR (MacKenzie, 1996; Xu *et al.*, 2004), real time RT-PCR (qRT-PCR) (Mumford *et al.*, 2000), macroarrays (Maoka *et al.*, 2010) and microarrays (Nicolaisen, 2011) that allow the detection of different virus simultaneously.

The first studies on PMTV detection in Colombia were performed using RT-PCR tests with primers for CP and TGB2 genes (Vélez, 2007) and later by partial sequencing of the RNA2 and 3 (Gil *et al.,* 2011; Osorio, 2012). Recently, Gallo (2012) and Andrade (2012) designed synthetic peptides based on the sequences of PMTV Colombian isolates to generate polyclonal antibodies for recognition of the CP N-terminal and CP-RT C-terminal. Despite the fact that the use of bait plants, together with serological or molecular techniques, has brought an alternative for PMTV detection, the time required (> 1 month) makes it unpractical to use on a routine diagnosis of the virus. To the opposite, the qRT-PCR technique offers a high speed (< 6 h), high sensibility levels and possibility to titrate the tissues (Schena *et al.*, 2004). The use of highly sensitive methods for PMTV detection is a fundamental factor to support the genetic breeding and tuber-seed certification programs for potato in Colombia, moreover when, till the moment, the effects of this virus in the potato varieties grown in the country are not known with precision. In this research was evaluated the use of the qRT-PCR technique to detect PMTV Colombian isolates by estimation of the viral contents in potato and *N. benthamiana* tissues, comparing the standard curves generated by the *in vitro* transcription of part of the viral genome.

Materials and methods

Samples used. For the initial experiments for qRT-PCR 20 total RNA samples were used, that were obtained by Osorio (2012). The validation of the technique was performed by the inoculation with Sss cystosori of 10 *Nicotiana benthamiana* Domin bait plants and 10 of *Solanum phureja* Juz. et. Buk. These plants were kept in mesh house at the Experimental Center Paysandú (6º 12' 37" N and 75º 30' 11" W), Medellín, department of Antioquia. Additionally, roots from 15 potato (*S. tuberosumand S. phureja*) plants were collected that had powderly scalb symptoms from the municipality of La Unión (5° 58' 38" N and 75° 24' 54" W), department of Antioquia, as well as 16 asymptomatic tuber-seeds of the varieties Diacol-Capiro and Criolla Colombia, commercialized in this municipality.

qRT-PCR evaluation for PMTV detection. This evaluation was done in 20 total RNA samples given by Osorio (2012), with the amplification and specificity of the primers PMTV-1948F (5'-GTGATCAGATCCGCGTC-CTT-3') and PMTV-2017R (5'-CCACTGCAA-AAGAACCGATTTC-3'), as well as the Taqman® probes PMTV-1970 (5'-FAM-ACCAGAACTACGGTGCCGCGTCGBHQ-1-3') (Mumford *et al.*, 2000). qRT-PCR reactions were performed in two steps. For retrotranscription 20 µl were used containing 40 U of M-MuLV enzyme (Thermo, EEUU), 1X RT buffer, 1 mM dNTPs, 5 mM MgCl2, 1 µM reverse primer 123end (5'-GTGAACCACGGTTTARCCCTGKAAGC-3') (Savenkov *et al.*, 1999), 20 U RNase inhibitor and 5 µl RNA. The reactions were incubated on a thermocycler T3 (Biometra, Germany) at 65 65 °C for 5 min, followed by 37 °C for 60 min and 70 °C for 10 min. qPCR was performed with the Maxima Probe/ROX kit (Thermo) in 25 µl including 12.5 µl kit, 9.5 µl water, 0.3 µM each primer, 0.2 µM Taqman® probe and 1 µl complementary DNA (cDNA). The tests were done on a Rotor-Gene Q-5plex Platform (Qiagen) equipment and consisted on 95 °C for 4 min, followed by 40 cycles at 95 °C for 25 s and 60 °C for 1 min. Ct values (threshold cycle) were defined using the default parameters of the Rotor-Gene Q ver.1.7 software, being considered as positive those samples that

surpasses the threshold value before the cycle 40 (Schena *et al.*, 2004). The efficiency of the reactions was calculated with the formula $E = 10^{(-1/m)}$, where E is the efficiency of the amplification and m is the slope. In all the experiments were used a negative control with sterile distilled water and a positive control with cDNA of the NOV114 of PMTV isolate obtained by Osorio (2012).

To corroborate the viral nature of the amplicons, the purification of five of them was done by the QIAquick Gel Extraction (Qiagen) kit, for its direct sequencing in the Macro-gen company (South Korea). Sequences were edited with the software BioEdit 6.0.6 (Hall, 1999) and confirmed by comparison with the molecular databases by BLASTn (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi).

Standard curve preparation By conventional RT-PCR a product of 1514 bp from RNA2 of the SRL5 of PMTV isolate (Santa Rosa de Osos, Antioquia), was obtained using the primer 123end in the RT and the primers PMTV_759F (5'-ACCTGAGGTCAGAGTTATCGACG-3') (Gil *et al.* 2011) and PMTV_2017R (5'-CCACTGCAAAAGAACCGATTTC-3') (Mumford *et al.*, 2000), in PCR. The last one was done on 25 µl volume including 17.8 µl water, 1X enzyme buffer (10X), 1.8 mM MgCl2, 0.2 mM dNTPs, 0.2 µM each primer, 1 U Taq DNA polymerase (Thermo) and 1 µl de cDNA. The amplification program consisted on 95 °C for 30 s, followed by 40 cycles at 95 °C for 30 s, 52 °C for 45 s, 72 °C for 1 min and final extension at 72 °C for 5 min.

This fragment was cloned using the CloneJET PCR Cloning kit (Thermo) following the manufacturer's instructions and was transformed into DH5α from *Escherichia coli* competent cells using thermal shock (Sambrook and Rusell, 2001). The selection of the recombinant bacteria was established on LB media cultures supplemented with ampicillin (100 mg/ml). Six colonies were selected and growth for 16-18 h at 37 °C in 5 ml liquid LB media with ampicillin to proceed with plasmid extraction using the Plasmid Miniprep kit (Quiagen). The plasmid concentration was measured with Nanodrop 2000C equipment (Thermo); 3 µg of this product was linearized using the NcoI enzyme (Thermo). The linearized vector was subjected to *in vitro* transcription with TranscriptAid T7 High Yield Transcription kit (Thermo). The RNA obtained was purified by the phenol:chloroform method and its further precipitation was done in absolute ethanol (Sambrook and Rusell, 2001), quantifying it again with the Nanodrop 2000C (Thermo). 600 ng/µL of transcribed RNA were used as template for retrotranscription with the primer PMTV_2017R and 20 U of M-MuLV reverse transcriptase (Fermentas). Finally, serial dilutions were prepared starting from 1 x 10^{-1} till 1 x 10^{-4}, from an initial concentration of 1000 ng/µl of cDNA, equivalent to1.20 x 10^{18} viral copies/µl, according to the formula:

$$pmol\ de\ cDNA = \mu g(of\ cDNA)\ x\ (10^6\ pg/1\mu g)\ x\ (1pmol/330\ pg)\ x\ (1/\#transcript\ bases),$$

using the Avogrado's number (6.023 x 10^{23}) to estimate the number of transcripts. In this way, the qPCR reactions were done in triplicate for each cDNA dilution and a standard curve was built by comparison between the Ct values for each dilution and the estimate concentration of viral copies. All the qRT-PCR reactions were done in the same conditions described previously.

qRT-PCR test validation. A total RNA extraction with the RNeasy Plant mini kit (Qiagen, USA) was performed from 100 mg of the youngest root and leaf tissues following manufacturer's instructions. Obtained RNA was eluted in 40 600 µl DEPC water. The validation of the qRT-PCR test was evaluated in two steps, on 20 samples of both roots and leaves of bait plants and, in the 15 root samples of potato with powdery scab symptoms. Additionally, the qRT-PCR test to detect PMTV was used in samples compound of four 2 cm buds, from eight

tuber-seeds of the Diacol-Capiro variety and eight from the Criolla Colombia variety.

Results and discussion

In the initial experiment of the qRT-PCR technique in two steps to detect PMTV in order to check the use of the primers design by Mumford *et al.* (2000) detected this virus in nine of the 20 total RNA samples (Ct between 21.72 and 38.34). The negative control presented a Ct > 40 (Figure 1). The sequencing of five of the amplicons allows the confirmation of their origin in the PMTV CP-RT domain, with identity values of 97% (e values: 0.12 and 0.20) and coverage percentages of 100% in respect to the deposited sequences of this virus in GenBank (HQ285252, FM205706 and AM503633). The standard curve obtained presented a correlation coefficient of R^2 = 0.99, -3.38 slope and 0.97 reaction efficiency, indicating a highly trustable lineal response for the detection and quantification of the PMTY Colombian isolates (Figure 2). In terms of viral copies/µl, the standard curve covers a range from 1.20 x 10^8 to 1.20 x 10^{11}. In future works it will be possible to prepare a higher number of dilutions that reach theoretical values of 10 – 100 viral copies/µl. In this sense, the original study of Mumford *et al.* (2000), indicated that this technique presented levels of sensibility 10,000 times higher when compared to elisa test; however, in that study the absolute viral copy number was not detected. Moreover, in a study by Bertolini *et al.* (2008) to detect *Citrus tristeza virus* in plants and aphids, it was determined that with the qRT-PCR technique it can be detected even 17 viral copies, giving sensitivity levels up to 100 – 500 times higher than nested IC-RT-PCR and 1,000,000 times higher than DAS-ELISA. For that reason there is no doubt that the use of this test in the potato agroindustry of Colombia will give sensitivity levels never reached by other methodologies used till now for PMTV detection.

The tool developed was based on the two-step qRT-PCR test, contrary to the one-step initially described by Mumford *et al.* (2000); moreover, a standard curve for absolute quantification of the virus was included. In comparison to the original procedure, these modifications were based on

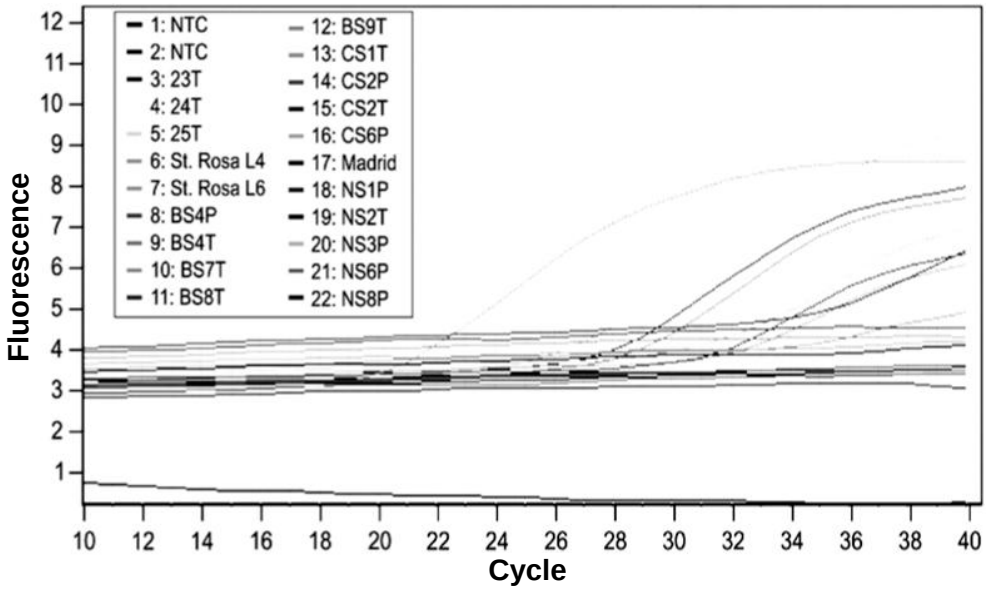

Figure 1. Amplification curve for two-step qRT-PCR using Taqman® system, in 20 total RNA samples from bait plants inoculated with *Spongospora subterranea* cystosori.

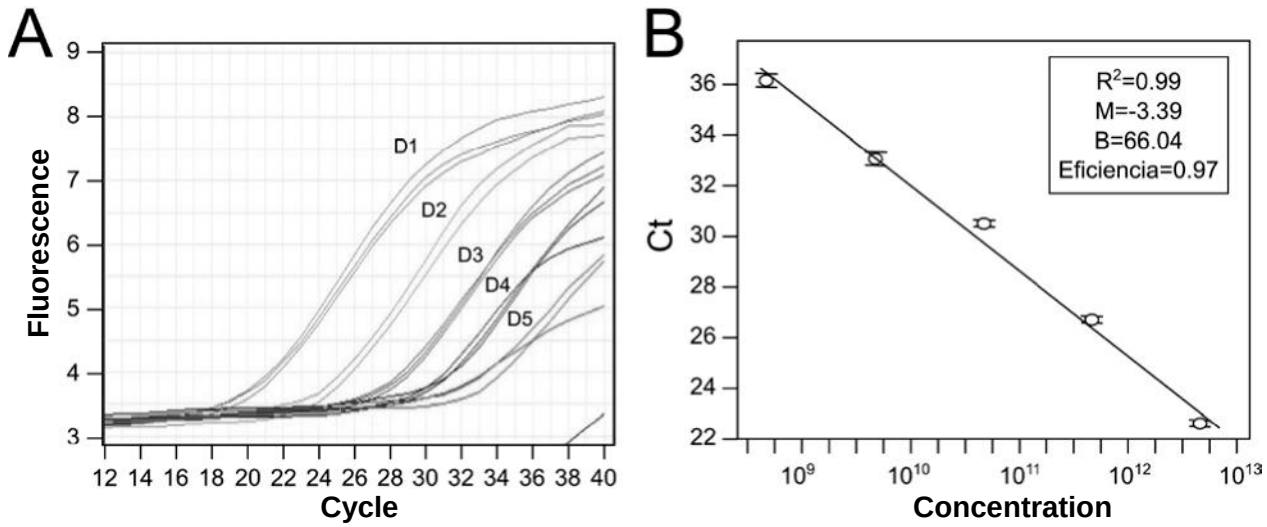

Figure 2. Standard curve build with two-step qRT-PCR for the quantification of PMTV in Colombia **(A).** Amplification curves. **(B).** Standard curve. The correlation coefficient values R^2), slope (M), intercept and (B) reaction efficiency (E) are presented.

economical and practical criteria. In the first case, there is no need to buy qRT-PCR kits that are 30% higher in cost than the ones for qPCR. From the practical point of view, the selected format uses the TaqMan® chemical system, with advantages such as high sensitivity, higher specificity levels than other systems and easiness of routine and massive use (Schena *et al.,* 2004; Bertolini *et al.,* 2008). In the same way, the two-steps procedure makes possible to use SYBR Green when the TaqMan® kit is not available and is feasible to combine it with qPCR test implement by the author of this research to detect Sss (García, 2012). This is possible, if fluorochromes used are different to the TaqMan® probes specific for each pathogen that are being used (Qu *et al.*, 2011).

The validation of the qRT-PCR in two-steps from 20 plant baits, allowed the detection of the PMTV in 11 root samples and 6 leaf samples, with the presence of Ct values between 36.74 - 39.69 and 36.26 - 39.37, respectively, -Ct for positive controls for roots and 33.62 for leaf tissue and > 40 for negative controls-. The quantification of number of viral copies/µl by comparison with the standard curve varied between 6.03 x 10^7 and 4.49 x 10^8 for roots, whereas for leaf tissue was 7.48 x 10^7 to 6.23 x 10^8 (Table 1). Therefore, it was demonstrated that the root tissue is more useful to detect PMTV that the leaf tissue. A similar result was found by Andrade (2012) who developed polyclonal antibodies for the CP-RT protein of the PMTV and observed that its use in indirect ELISA test allowed the detection of the virus in levels higher than 50% when roots were evaluated. This can be explained because of the location of the Sss vector structures in the plant roots, which supposed a higher viral titrate in that tissue; moreover when the systemic movement of the PMTV has been reported to be partial and distributed erratically (Santala *et al.*, 2010). However, that situation can change for those potato varieties, especially the ones of the temperate region, where conspicuous symptoms are presented in the foliar tissue of PMTV infected plants.

On the other hand, in the evaluation done using potato roots was possible to detect PMTV in 14 out of 15 samples, with Ct values of 25.56 a 33.04 and estimated concentrations of 4.72 x 10^{11} and 7.6 x 10^{13} viral copies/µl –Ct of 20.05 for the positive control and > 40 for the negative control– (Table 2). This result gives an experimental support for the hypothesis stated by Gil *et*

Table 1. PMTV detection and quantification by two-step qRT-PCR in root and leaf samples of *N. benthamiana* and *S. phureja* bait plants inoculated with *Spongospora subterranea* cystosori from soils of different departments of Colombia.

Sample	Host	Roots		Leaves	
		Ct*	CV/µl**	Ct	CV/ µl
BS9-P	*S. phureja*	38.4	$1.45x10^{8}$	>40	–
CS10-P	*S. phureja*	>40***	–	36.26	$6.23x10^{8}$
BS7-P	*S. phureja*	38.11	$1.76x10^{8}$	>40	–
BS7P-II	*S. phureja*	39.69	$6.03x10^{7}$	>40	–
BS10-P	*S. phureja*	36.88	$4.07x10^{8}$	>40	–
BS4-P	*S. phureja*	>40	–	>40	–
BS4-PII	*S. phureja*	>40	–	38,25	$1.61x10^{8}$
CS4-P	*S. phureja*	>40	–	>40	–
CS4-PII	*S. phureja*	>40	–	>40	–
BS2-P	*S. phureja*	39.45	$7.09x10^{7}$	>40	–
BS1-T	*N. benthamiana*	37.65	$2.42x10^{8}$	36.87	$4.10x10^{8}$
ZIPAQUIRA-T	*N. benthamiana*	36.74	$4.49x10^{8}$	37.63	$2.46x10^{8}$
TUNJA-T	*N. benthamiana*	39.4	$7.33x10^{7}$	37.88	$2.06x10^{8}$
NS12-T	*N. benthamiana*	>40	–	>40	–
NS1-T	*N. benthamiana*	>40	–	>40	–
NS10-T	*N. benthamiana*	>40	–	>40	–
Sta Rosa L4-T	*N. benthamiana*	39.69	$6.03x10^{7}$	39.37	$7.48x10^{7}$
CS6-T	*N. benthamiana*	39.31	$7.83x10^{7}$	>40	–
NS4-T	*N. benthamiana*	37.74	$2.28x10^{8}$	>40	–
Madrid-T	*N. benthamiana*	>40	–	>40	–
Pos	Control positivo	20.05	$3.79x10^{13}$	33.62	$3.74x10^{9}$
Ntc	Control negativo	>40	–	>40	–

*Ct: Threshold cycle. **CV: Viral copies. *** Ct values>40 indicate negative results.

Table 2. PMTV detection and quantification by two-stepsqRT-PCR in root samples of potato plants with powdery scab symptoms from crops in the municipality of La Unión (Antioquia).

Sample	Host	Ct*	CV/µl**
L1R1	*S. tuberosum*	28.53	$1.01x10^{13}$
L1R2	*S. tuberosum*	26.12	$5.20x10^{13}$
L1R3	*S. tuberosum*	31.53	$1.32x10^{12}$
L2R1	*S. tuberosum*	33.04	$4.72x10^{11}$
L2R2	*S. tuberosum*	30.08	$3.53x10^{12}$
L2R3	*S. tuberosum*	32.6	$6.37x10^{11}$
L3R1	*S. tuberosum*	26.08	$5.37x10^{13}$
L3R2	*S. tuberosum*	30.87	$2.07x10^{12}$
L3R3	*S. tuberosum*	31.98	$9.66x10^{11}$
L4R1	*S. tuberosum*	25.56	$7.60x10^{13}$
L4R3	*S. tuberosum*	30.04	$3.63x10^{12}$
L4R4	*S. tuberosum*	32.54	$6.61x10^{11}$
L4R2	*S. tuberosum*	>40	
L3R4	*S. tuberosum*	28.1	$1.35x10^{13}$
L3R5	*S. tuberosum*	29.31	$5.94x10^{12}$
Pos	Positive control	19.42	$4.96x10^{15}$
Ntc	Negative control	>40	

*Ct: Threshold cycle. **CV: Viral copies. *** Ct values > 40 indicate negative results.

al. (2011) in the sense that increments at the incidence level of Sss in Colombia would be accompanied by proportional increases of the associated virus PMTV. In the future it will be of great interest the use of the two-steps qRT-PCR tool presented in this study to quantitatively estimate such association.

Finally, in this research was evaluated the PMTV occurrence in potato tuber-seed using the system proposed by Latvala-Kilby *et al.* (2009), from buds and not from superficial tissues of the tuber. In this evaluation it was found that one of the tubers resulted positive for PMTV (Ct 38.63),

with a estimated concentration of 1.05 x 10^{10} viral copies/µl. This demonstrates the use of the technique for other applications, moreover when the PMTV detection in tubers leads to false negatives because of the low viral titrate presented there and the irregular distribution of the virus in the different tissues (Sokmen *et al.*, 1998; Latvala-Kilby *et al.*, 2009).

The viral detection in low titrate infected plants and the partial systemicity, is complex and requires the use of additional diagnosis methods (Xu *et al.* 2004; Santala *et al.* 2010). For the PMTV case, this is more evident since there are reports on cell to cell viral RNA movement when assembly particles are absent (Shemyakina *et al.*, 2011). For that reason, serological techniques should be accompanied by molecular evaluations for PMTV detection. The qRT-PCR methodology evaluated in this work, together with the ELISA test developed from the polyclonal antibodies generation against synthetic peptides designed by Andrade (2012) and Gallo (2012), plus the partial sequencing of viral genotypes presented in Antioquia and Boyacá (Osorio, 2012), that make it possible to design specific primers for conventional RT-PCR, offer a wide range of detection tools of this virus in Colombia. It is expected that the national plant protection organizations, the producers union and the seed producing companies make use of these technologies, especially for the quarantine processes, certification of tuber-seed and generation of resistant materials in potato breeding programs.

Conclusions

- The two-step qRT-PCR methodology was evaluated for the detection of PMTV Colombian genotypes using three different samples: bait plants inoculated with Sss cystosori, potato roots with powdery scab symptoms and tuber-seed buds. In the three cases it was possible to detect PMTV, as well as the quantification of their inoculum levels, by comparison with the standard curve based on the *in vitro* transcription of a part of RNA2 from the viral genome.
- Different that the other viruses affecting potato, to detect PMTV is important to use root tissue because in the evaluations performed on bait plants and potato, it was evident the presence of higher viral inoculum in that tissue than in the leaf tissue.

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