



Incidence of begomovirus infecting tomato (*Solanum lycopersicum*) and pepper (*Capsicum* spp.) crops in selected areas in Cebu, Philippines

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ABSTRACT

Begomoviruses (genus *Begomovirus*, family *Geminiviridae*) are groups of plant viruses causing destructive diseases of vegetable crops worldwide. Several tomato- and pepper-infecting begomoviruses pose a major concern in crop production. In this study, the incidence of begomoviruses associated with tomato and pepper samples from Cebu, Philippines was determined through visual assessment of symptoms and detection of the virus by polymerase chain reaction (PCR) using two degenerate primers (TY1F/TY2R and PAL1v1978/PAR1c715H). Symptoms of begomovirus infection were observed in all the tomato and pepper farms visited. Infected tomato varieties exhibited severe to mild leaf curling and crinkling along with interveinal yellowing and plant stunting at early to late vegetative stage. In pepper crops, symptoms included severe leaf deformations (upward curling/cupping, crinkling and puckering) in addition to leaf discolorations (interveinal yellowing/mosaic). Results of PCR assays confirmed the presence of begomoviruses on both symptomatic tomato and pepper samples. In tomato, the expected DNA fragments of ~585 bp were successfully amplified in all samples while DNA-A component (~1.5 Kb fragment size) of the virus was generated in 88% of the total samples. In pepper, begomoviral coat protein (~565 bp) was detected in 42.5% of the samples with the gDNA as template. Using RCA-amplified DNA as a template, 75% (3/4) and 50% (2/4) of the RCA-amplified samples were positive of begomovirus. This study confirmed the prevalence of begomovirus on tomato and pepper crops in Cebu. Thus, early and rapid detection of these plant viruses provides a proactive strategy in dealing the disease and to mitigate yield losses.

KEYWORDS: *begomovirus, detection, incidence, pepper, polymerase chain reaction, tomato, Cebu, Philippines*

1 INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to the nightshade family Solanaceae and is the most widely grown and consumed vegetables worldwide. They are consumed fresh, alone or as part of a vegetable salad, as well as cooked or variously processed as paste, peeled or diced, juice, sauces, ketchup, etc. Tomatoes are usually grown in the field, under plastic covers or in greenhouses. In the Philippines, tomato is the most economically important vegetable crop grown covering an area of about 16,197 ha with a total production of 210,720 metric tons in 2016 (PSA, 2017). Meanwhile pepper is an important spice and vegetable crop in the tropics and subtropics for its spicy taste and one of the richest sources of vitamin A, vitamin C and acidic phenolic compounds (Howard, 2000). There are diverse types of peppers grown and fruits come in a wide range of shapes, sizes, colors and pungency. In the Philippines, pepper is considered a minor crop but grown throughout the year and with an increasing demand.

One of the important limiting factors in tomato and pepper production is the occurrence of viral diseases especially begomoviruses. The genus *Begomovirus* (family *Geminiviridae*) is one of the most important groups of plant viruses that cause diseases to a variety of vegetable crops including tomato and pepper. *Begomovirus* is transmitted by whitefly, *Bemisia tabaci* and constitutes the largest genus of plant viruses comprising more than 300 species. They have circular, single-strand DNA genomes consisting of one (monopartite) or two (bipartite) components of 2.6 to 3 Kb encapsulated in twinned icosahedral particles of about 22 nm x 38 nm (Brown et al., 2015). This group of plant viruses can inflict severe losses in vegetable production that ranged from 20 to 100% or near crop loss in severe epidemics (Saikia and Muniyappa, 1989; Polston and Anderson, 1997; Sulandari et al., 2006; De Barro et al., 2008). In the Philippines, tomato leaf curl begomoviruses have been reported in the country and accounted for 50 to 80% reduction in tomato yield (Retuerta et al., 1971; Mendoza, 2005).

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Several begomoviruses have emerged as important plant viruses to affect tomato and pepper throughout the world. Tomato-infecting begomoviruses have been reported in West and Central Africa (Leke et al., 2015), USA particularly in Brazil (Inuoe-Nagata et al., 2016), Florida (Polston et al., 1996), Texas and Arizona (Polston and Aderson, 1997), Mexico (Ascencio-Ibanez et al., 1999), Nicaragua (Rojas et al., 2000) and throughout Central America (Morales and Anderson, 2001; Jones 2003). Occurrence of begomoviruses has also been reported in many Asian countries like China, Indonesia, India, Bangladesh, Iran, Laos, Thailand, Vietnam, Korea and the Philippines (Tsai et al., 2009; Tsai et al., 2011a; Varma et al., 2011). While, begomovirus infecting pepper was first reported in the United States (Brown et al., 1989; Brown and Poulos, 1990) and Mexico (Leal and Quintero, 1989). It has also been detected in the northern coastal region of Oman (Khan et al., 2007) and in India (Green and Kalloo, 1994).

In the Philippines, begomovirus infecting tomatoes was first reported in 1971 (Retuerma et al., 1971). Since then, several isolates, variants and/or distinct species were detected in the country particularly in Laguna (Kon et al., 2002, Stanley et al., 2005; Fauquet et al., 2008; Tsai et al., 2011a), Ilocos Norte (Sta. Cruz et al., 2016), Cebu as well as in Mindanao (Tsai et al., 2011a). In pepper, the presence of begomovirus was noted in 1994 from samples collected in Laguna (Dolores and Pissawin, 1994). Recently, Tomato leaf curl Philippines virus and Tomato leaf curl Cebu virus were also reported in the country infecting pepper (Tsai et al., 2011). So far, only limited studies have been done on the occurrences and distribution of begomovirus infecting tomato and pepper in the country and in Cebu province in particular.

Knowledge of virus occurrences and its early detection constitute a proactive strategy in dealing with the disease. This would greatly help in coping up disease threats before it reaches severe infections or epidemic proportions thereby mitigate yield losses. Current plant virus detection technologies mainly rely on molecular techniques such as polymerase chain reaction (PCR). It provides a powerful tool for the detection of begomoviruses as it allows amplification of the specific nucleic acid sequences using two short oligonucleotide primers that flank the target sequence (Henson and French, 1993). PCR has been widely used in detection and diagnosis of plant viruses due to its rapidity, sensitivity, specificity and reliability (Hull, 2002). In the Philippines, most of the begomoviruses were detected through PCR using degenerate primers. In this study, the prevalence of begomovirus associated with tomato and pepper plants in Cebu, Philippines was determined through visual assessment of symptoms and virus detection by PCR.

2 MATERIALS AND METHOD

Surveys and Sample Collection

Field visits and sample collections were conducted in tomato and pepper growing municipalities in Cebu (Cebu City, Barili, and Dalaguete) last May 2017. Five (5) leaf samples per farm were randomly taken from plants showing virus-like symptoms of leaf curling/crinkling, interveinal and leaf margin yellowing, mosaic/mottle and stunting. Additional information such as crop growth stage, presence of vectors (whiteflies), crop variety, etc. were recorded for each crop. The collected samples were dried from any moisture and immediately placed into plastic bags lined with tissue paper. Samples were brought to the laboratory (Plant Virology Laboratory, IWEP, CAFS, UPLB) and kept at 4°C until molecular analysis was done.

Visual detection of begomoviral infection in the field

Occurrence of begomovirus infection in tomato and pepper crops was assessed in the field through visual detection of typical leaf curl symptoms (leaf curling/cupping/ crinkling along with interveinal and marginal yellowing) and other virus-like symptoms (mosaic/mottle, leaf chlorosis, leaf crumpling, and stunting). The observed symptoms were photo-documented and were classified according to type, crop stage, and location.

Virus Detection by Polymerase Chain Reaction

DNA Extraction

Total genomic DNA was extracted from symptomatic leaf samples following Dellaporta et al. (1983) protocol. Two leaf punches (~0.2 g) were placed in a 1.5 ml microcentrifuge tube and were immersed into liquid N for a few minutes and were crushed using sterile Kontis pestle. The crushed samples were added with 500 µL Dellaporta extraction buffer (100 mM Tris pH 8.0, 500 mM EDTA pH 8.0, 500 mM NaCl, 10 mM β-mercaptoethanol). The homogenate was added with 60 µL SDS (20%), vortexed for 2 min and incubated for 10 min at 65°C in the thermoshaker. Then, 0.3 V (160 µL of 5M KoAC) of Potassium acetate were added into the tube and vortexed for 2 min before spinning at 14,000 rpm for 10 min at microcentrifuge. About 450 µL of the supernatant were carefully collected and transferred into a new 1.5 ml microfuge tube which were added with 0.5 V ice-cold isopropanol (225 µL), vortexed for few seconds and spinned at 14,000 rpm for 10 min to decant easily the supernatant leaving the DNA pellet at the bottom of the tube. The supernatant was removed and discarded and remaining nucleic acid pellet were washed with 500 µL 70% ETOH and were spinned at 14,000 rpm for 5 min to discard the ETOH and collect the DNA pellet at the bottom of the tube. The supernatant was discarded and extracted DNA pellet were air-dried overnight and

were re-suspended with 50 µL TE buffer and were kept in the freezer. The extracted genomic DNA was run in 1.0% agarose gel at 95 volts to check its quality and viewed under the AlphaInotech Imager. The genomic DNA was quantified using Nanodrop spectrophotometer (SPECTROstar Nano) at Molecular Biology Lab, NCPC, UPLB.

PCR Amplification

Presence of begomovirus on symptomatic tomato and pepper leaf samples was molecularly detected through polymerase chain reaction (PCR) using two degenerate primer pairs TY1F/TY2R (Acotto et al., 1993) and PAL1v1978B/PAR1c715H (Gilbertson et al., 1991; Tsai et al., 2011a) (Table 1) that amplified ~585 bp coat protein coding region of the viral genome and ~1.5 kb DNA-A product, respectively.

PCR was carried out in 25 µL reaction mixture containing 2.5 µL 1X PCR buffer, 1.0 µL 2 mM MgCl₂, 0.5 µL dNTPs, 1.0 µL each of the forward and reverse primers (10 µM), 0.2 µL 1U Taq DNA Pol (Invitrogen), 16.8 µL sterile nanowater, and 2 µL DNA template (50 ng/µL). The same concentration and volume of PCR components were used with primers PAL1v1978B/PAR1c715H except in MgCl₂ (0.75 µL 1.5 mM), dNTPs (0.625 µL 25 µM), and primers (0.5 µL 0.2 µM). All sets of reactions included: DNA samples from healthy plants as negative control, sterile distilled water as blank control, and DNA from positively ToLCPV-infected plants as positive control. PCR amplification was performed in a Veriti 96 Well Thermal Cycler (Applied Biosystems) with the following conditions: initial denaturation of 94 °C for 1 min; 35 cycles of denaturation at 94 °C for 15 sec, annealing at 55 °C for 15 sec and extension at 72 °C for 1 min; and a final extension at 72 °C for 10 min using primer TY1F/TY2R. For detection using primer PAL1v1978/PAR1c715H, the PCR reaction was carried out with the following conditions: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 2 min, and extension at 72 °C for 2 min; and a final extension at 72 °C for 10 min.

loading dye were separated by electrophoresis on 1% agarose gel in 1x Tris borate (TBE) buffer (0.089 M Tris base, 0.25 M boric acid and 0.5 M EDTA, pH 8.0) and DNA fragments were visualized using 0.6 µL gel red stain (Biotum) and analyzed by alpha imager gel documentation and analysis system (Alpha Innotech). DNA ladder set (1 Kb plus, Invitrogen, USA) was included as a sized molecular marker.

Rolling circle amplification (RCA)

Rolling circle amplification was run on some pepper samples to increase the amount of circular viral DNA. This method can amplify all infecting circular viral DNA in the samples by a single reaction (Haible et al., 2006; Inoue-Nagata et al., 2004). Circular single stranded DNA was amplified from the total nucleic acid extracted from symptomatic pepper leaf using TempliPhi Amplification Kit (GE Healthcare Life Sciences) following the manufacturer’s protocol. In each reaction, 1.0 µL of undiluted total DNA were mixed first with 5.0 µL sample buffer and incubated at 95 °C for 3 min in a thermoshaker and cooled down on ice. Each sample mixture was then added with 5 µL reaction buffer plus 0.2 µL enzyme mix and incubated at 30 °C for 18 h in a Biometra Thermoshaker. After incubation, enzyme inactivation followed by which samples were subjected to 65 °C for 10 min. The RCA products (1 µL RCA product + 5 µL 1x loading dye) were run in 0.8% agarose gel pre-stained with 1.0% GelRed at 95V for 40 min. The gel was then viewed under the AlphaInotech Imager. Then RCA products were used as template for PCR detection of begomovirus on some of the pepper samples.

3 RESULTS AND DISCUSSION

Field sampling was done to determine the occurrence of begomoviral diseases of tomato and pepper crops in Cebu. Presence of the disease was initially assessed by visual observation of symptoms and later confirmed by PCR detection of the virus. A total of 40 leaf samples with suspected symptoms of tomato leaf curl

Table 1. Primers used for detection of begomoviruses by polymerase chain reaction.

Primers	Sequence (5’ to 3’)	arget Region	Expected Amplicon (bp)
TY-1F/ TY-2R	GCCCATGTA(T/C)CG (A/G)AAGCC GG(A/G)TTAGA(A/G)GCATG(A/C)GTA	Coat protein	~ 585
PAL1v1978B/ PAR1c715H	GCATCTGCAGGCCACAT BGYTT HCC NGT GATTTCTGCAGTTDATRTTHTCRCCATCCA	DNA-A	~ 1500

Gel Electrophoresis

Amplified PCR products (5 µL) mixed with 2 µL 6x

virus or begomovirus infections were collected and used in the study.

Symptoms of Begomovirus infection in tomato and pepper crops

Presence of begomovirus infection was detected in the field surveyed upon observation of its characteristic symptoms. In tomato, only begomovirus-like symptoms were noted. These symptoms observed included leaf curling and crinkling, interveinal and/or marginal yellowing of leaves, upward cupping of the leaves, and/or plant stunting (Table 2). All these symptoms were observed in two varieties of tomato grown and were noted to occur in all stages of crop development. Infected Diamante Max variety exhibited severe leaf curling and crinkling along with interveinal yellowing and plant stunting at early to late vegetative stage, while, moderate leaf curling, crinkling, and cupping without plant stunting were noted at flowering and fruiting stage (Figure 1). Similar begomovirus symptoms were observed in infected ‘Avatar’ variety but symptoms only ranged from mild apical leaf curling and interveinal yellowing at vegetative to early flowering stage to severe leaf crinkling and upward cupping at flowering to fruiting stage without growth stunting (Figure 1).

The begomoviral symptoms observed in this study were similar to the symptoms of begomovirus infections in previous reports (Tsai et al., 2011a; Retuerma et al., 1971). Typical symptoms of begomovirus reported in tomato ranged from severe to mild mosaic, leaf chlorosis, upward or downward curling of leaves, veinal chlorosis and/or plant stunting (Barboza et al., 2017; Inoue-Nagata et al., 2016; Navas-Castillo et al. 2011). The same symptoms such as leaf curling, yellowing, mosaic and blistering, and/or stunting of plants were reported in

(Tsai et al., 2011a).

In pepper crops, visual assessment of symptoms detected the presence of begomovirus infection. Pepper crops infected with begomovirus disease exhibited severe leaf deformations (upward curling/cupping, crinkling and puckering) in addition to leaf discoloration (interveinal yellowing/mosaic) (Table 2 and Figure 2). These symptoms were noted to occur at vegetative to early fruiting stage of bell peppers.



Figure 1. Tomato leaf curl disease-like symptoms on tomatoes. Mild to severe leaf curling and crinkling, interveinal and/or marginal chlorosis on ‘Diamante max’ (A-B) and on ‘Avatar’ (C-D) tomato variety.

The begomovirus-like symptoms observed in this study were similar to those previously described in

Table 2. Begomovirus-like symptoms associated with tomato and pepper crops grown in Cebu.

Disease	Specific symptoms	Crop Stage	Variety
Tomato			
Begomovirus-like	Leaf curling and crinkling; Interveinal and/or marginal chlorosis of leaves; Upward cupping of the leaves; Plant stunting	Early to late vegetative, Flowering, Fruiting stage	Diamante Max Avatar
Pepper			
Begomovirus-like	Upward leaf curling; Interveinal leaf chlorosis; Yellowing and crinkling of apical leaves	Bell pepper Chilli pepper	Vegetative Flowering Fruiting
Other virus-like	Leaf mosaic or mottling; Leaf distortion and/or shoestring	Chilli pepper	Flowering Fruiting

tomato crops infected with tomato leaf curl disease in the country (Tsai et al., 2011a; Retuerma et al., 1971). The high resemblance of begomoviral symptoms in this study with that of previous reports suggests the occurrence of begomovirus in Cebu, Philippines as previously reported

pepper plants infected with begomovirus. On chili peppers, typical leaf curl symptoms observed consisted of yellowing, leaf curling, reduction in leaf size, shortening of internodes and petioles, crowding of leaves and stunting (Hussain et al., 2004; Khan et al., 2007; Rezk, 2016) while sweet peppers infected with TYLCV

were observed to exhibit upward curling of leaf margins with interveinal and marginal chlorosis, thickening of veins, infected leaves were often found thicker than the normal ones and yellowing of the leaves in severe infection (Khan et al., 2007). Recently, occurrence of yellow leaf curl disease on pepper was reported from almost all pepper growing areas in Indonesia (Koeda et al., 2016). In the Philippines, association of begomoviruses with pepper was reported by Tsai et al. (2011) which noted that symptomatic pepper plants exhibited similar symptoms of leaf curling, yellowing, mosaic and blistering and/or stunting.

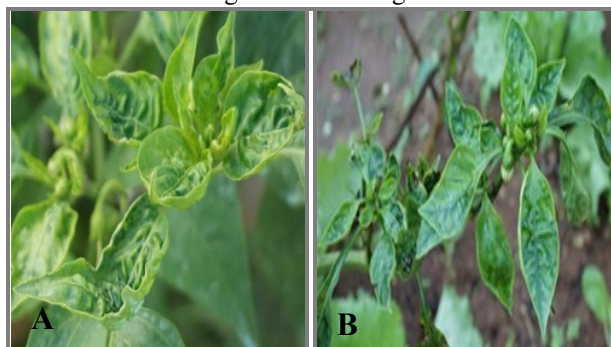


Figure 2. Begomovirus-like symptoms on peppers. A-B) upward leaf curling and crinkling, interveinal chlorosis; and puckering of leaves.

Visual observation was found helpful in detecting begomovirus through the presence of characteristic leaf curling/cupping/crinkling symptoms along with interveinal yellowing. However, it was used only for initial detection of begomovirus in the field and samples were further subjected to molecular detection.

Incidence of begomoviral disease in tomato

The begomovirus-like disease was detected in all the tomato farms visited through visual assessment of symptoms. Other virus-like symptoms were not observed. The presence of begomoviruses on symptomatic tomato samples was confirmed by PCR using TY1F/TY2R (Acotto et al., 1993) and PAL1v1978/PAR1c715H (Gilbertson et al., 1991) as

samples (Table 3, Figure 3). While, using PAL1v1978/PAR1c715H primers, which amplify DNA-A component of the viral genome (including the 5' region of C1 gene, the intergenic region (IR), the V2 gene and the 5' region of the CP gene), ~1.5 kb fragment size was generated in 88% (22/25) of the total samples (Table 3, Figure 3). The same sizes of amplified DNA products (~585 bp for TY1F/TY2R; ~1.5 kb for PAL1v1978/PAR1c715H) were also present in positive control sample (DNA from leaf curl virus-infected tomato) for both primer pairs but none in negative control samples (blank sample and DNA from healthy plant) (Figures 3) indicating that the product obtained in the samples was that of begomovirus. The rest of the tomato samples did not produce any PCR product with both sets of degenerate primers, suggesting the absence of begomovirus in these samples.

The PCR detection of begomovirus using degenerate primers confirmed the association of begomoviruses with tomato that showed symptoms of leaf curling, upward leaf cupping, interveinal and marginal leaf chlorosis and plant stunting. Degenerate primers have been used extensively for the detection of begomoviruses in a wide range of crop plants particularly in tomatoes (Tsai et al., 2011). In the Philippines, begomoviruses infecting tomatoes were mostly detected using degenerate primers TY1F/TY2R. The PAL1v1978/PAR1c715H pair has been used to amplify ~1.6 kb genomic DNA fragments from tomatoes infected with geminiviruses from India and Taiwan (Rojas et al., 1993) as well as from tomato and squash samples from Philippines (Bela-ong and Bajet, 2007). It also amplified ~1.5 kb of the DNA-A of begomovirus-infected tomatoes from the Philippines (Tsai et al. 2011).

Begomovirus infections were present all over tomato-growing farms in Cebu indicating its widespread occurrence across Cebu province. Similar observations on the distribution of begomovirus in the country have been reported previously (Tsai et al., 2011a). This widespread occurrence of the begomovirus may reflect both a very high inoculum reservoir and the prevalence and efficiency of its whitefly vectors. Results presented

Table 3. Occurrence of begomovirus disease from symptomatic tomato samples as detected by visual observation and polymerase chain reaction.

Location (No. of Farms)	No. of Samples	Disease/symptom observed		No. of Positive Samples	
		Begomovirus-like symptoms	Other virus-like symptoms	TY-1F/ TY-2R	PAL1v1978/ PAR1c715H
Cebu City (1)	5	5	0	5	5
Barili (3)	15	15	0	15	13
Dalaguete (1)	5	5	0	5	4
Total	25	100%	0%	25 (100%)	22 (88%)

degenerate primers for begomoviruses. In tomato, the coat protein primers (TY1F/TY2R) successfully amplified expected DNA fragments of ~585 bp in all

provide useful information on the current distribution and complexity of begomovirus infection in

tomatoes in the Philippines. This situation is expected to be quite dynamic through new virus variants arising and the increased movement of plant materials by man exporting farm produce to distant markets also altering the distribution of virus species and mixed infections thereof.

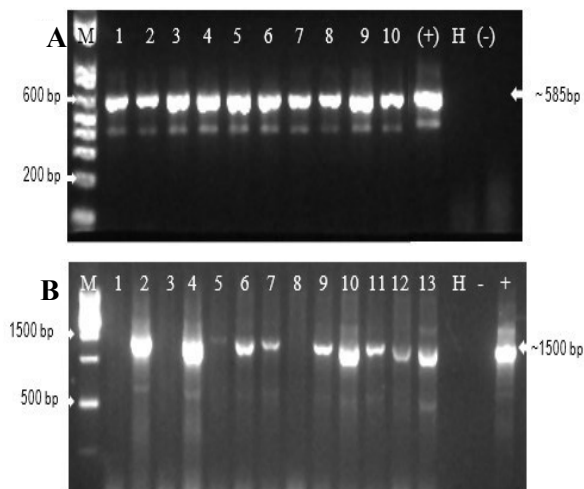


Figure 3. Begomovirus coat protein (~585 bp) (A) and DNA-A (~1,500 bp) (B) detected by polymerase chain reaction using primer pair TY1F/TY2R and PAL1v1979/PAR1c715H, respectively, in tomato begomovirus infected samples from Cebu. Lanes: M, 1 Kb plus DNA ladder (Invitrogen); 1-10 infected samples; H, healthy plant as negative control; (-), water as PCR negative control; and (+), as positive control

Incidence of begomoviral disease in pepper

Begomovirus-like disease occurred in 100% (40/40 samples) of the pepper samples as detected by visual assessment of symptoms (Table 4). The other virus-like diseases were not detected to occur in any of the farms surveyed in Cebu. Presence of begomovirus on these symptomatic plants was confirmed by PCR using TY1F/TY2R and PAL1v1978/PAR1c715H primers.

Table 4. Occurrence of begomovirus disease from symptomatic pepper samples as detected by visual observation and polymerase chain reaction.

Location (No. of Farms)	No. of Samples	Disease/symptom observed		No. of Positive Samples		
		Begomovirus-like symptoms	Other virus-like symptoms	TY1F/ TY2R	TY1F/ TY2R ^b	PAL1v1978/ PAR1c715H ^b
Cebu City (1)	5	5	0	2	2/2	2/2
Barili (5)	25	25	0	9	-	-
Dalaguete (2)	10	10	0	6	1/2	0/2
Total	40	40	0	17 (42.5%)	3/4 (75%)	2/4 (50%)

^a gDNA as template

^b RCA-amplified begomoviral DNA as template; no. of positive samples/No. of samples

Begomoviral coat protein (~585 bp) was detected

in 42.5% (17/40) of the samples using TY1F/TY2R primers and gDNA as template (Table 4, Figure 4A). However, the bands produced were faint or light in all positive samples. No amplicon was produced in both healthy pepper samples and in the reaction in which distilled water was substituted for the template, indicating that the primers were specific for the DNA of the begomovirus being detected. On the other hand, using PAL1v1978/PAR1c715H primers failed to generate a DNA product visible with gel electrophoresis in all pepper samples, only positive control (ToLCPV infected plant) produced ~1.5 kb DNA-A fragment as expected (results not shown here).

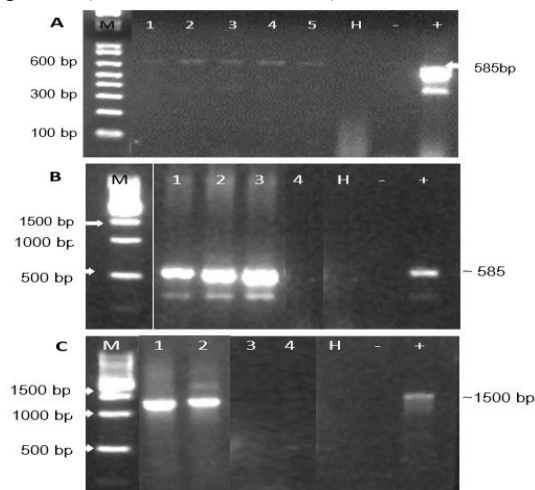


Figure 4. Begomovirus detection by PCR in representative pepper infected samples using gDNA (A) and RCA-amplified begomoviral DNA as template. A-B) Begomovirus coat protein (~585 bp) detected using the 0TY1F/TY2R primer pair; and B) DNA-A (~1,500 bp) using primer pair PAL1v1979/PAR1c715H primers. Lanes: M, 1 Kb plus DNA ladder (Invitrogen); 1-5 (A), 1-4 (B) pepper samples; H, healthy plant as negative control; (-), water as PCR negative control; and (+), as positive control.

The faint bands obtained using coat protein primers and the non-amplification using DNA-A primers could be due to low concentration of the viral DNA in the total plant DNA which was insufficient for amplification

using PCR. The same argument was presented in other reports in that amplification of a begomovirus was very faint or absent in samples that have very low virus titre (Jeevalatha et al., 2014; Karlsson, 2012). In this situation, a more sensitive technique such as RCA-PCR is essential to detect the virus at low titre (Jeevalatha et al., 2014). The RCA-PCR was 109 times more sensitive than PCR assay in that it enables to detect the ToLCNDV-potato using RCA-PCR in samples which were found negative in PCR avoiding false negative results during virus indexing (Jeevalatha et al., 2014). Rocha et al. (2010) have also reported that they could detect the begomovirus in a higher percentage of samples using RCA-PCR than PCR.

In this study, representative samples were subjected to RCA in order to increase the amount of circular DNA in the extracts and further PCR analysis was done after RCA. With RCA-amplified DNA as template, 75% (3/4) and 50% (2/4) of the RCA-amplified samples were positive of begomovirus using the same primers (Table 4). All begomovirus-positive samples produced the expected PCR products of ~585 bp and ~1.5 kb using TY1F/TY2R and PAL1v1978/PAR1c715H primers, respectively (Figure 4B and 4C). This confirmed the presence of begomovirus in some of the pepper samples and that its amount was successfully enriched by RCA that resulted in the detection of begomovirus in these samples. This also confirmed the non-detectable amounts of viral DNA in the extracts as PCR was successful after prior amplification with RCA. The rest of the samples (25% or 1/4) were still negative implying that these samples indeed were not infected by begomovirus. Results further implied that RCA-PCR assay was of great help to detect begomovirus in pepper samples with very low titre.

There are a few reports on the incidence of begomovirus infecting pepper especially in the Philippines. Report from Spain indicated that *C. annuum* is a host of TYLCV (Reina et al., 1999). In other reports, PCR followed by restriction enzyme analysis and nucleic acid hybridization were successfully used to detect field-infected plants of *C. annuum* and *C. chinense* in the Dominican Republic and Cuba (Quinones et al., 2001) and Jamaica (Roye et al., 1999), respectively. This result supported the previous reports that referred to *Capsicum* spp. as a host for TYLCV. The first evidence for the susceptibility of *C. frutescens*, *C. baccatum* and *C. chinense* was reported by Polston et al. (2006). Recently, association of begomoviruses on pepper plants were also reported in Costa Rica (Barboza et al., 2017) and in Indonesia (Koeda et al., 2016). In the Philippines, pepper-infecting begomoviruses (ToLCPV and ToLCCeV) were previously detected in 3 of 18 pepper samples from Laguna (Los Baños), Cebu and Bukidnon (Tsai et al., 2011a). In the present work, occurrences of begomovirus infection on pepper crops were noted in

Cebu particularly in Cebu City (Sudlon 1), Barili (Tal-ot, Mayana & Cagay) and Dalaguete (Mantalongon).

4 CONCLUSIONS

Begomovirus infections are detected in tomato and pepper crops in Cebu by visual and PCR techniques. Characteristic symptoms of infected tomato and pepper plants include leaf curling/cupping/crinkling along with interveinal and/or marginal chlorosis of leaves. Begomovirus infections were detected in 100% and 75% of tomato and pepper samples, respectively, through PCR assay. Present data provides valuable information on the occurrence of begomovirus in the area which can greatly help in coping up disease threats so as to mitigate yield losses. However, further sequencing and phylogenetic relationship based on full length genome sequences are necessary to identify begomovirus at species level. Species level identification of the virus would provide valuable information in designing effective and efficient virus management options.

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