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Characteristics of Selected Hybrids of Abaca (*Musa textilis* Nee) with Resistance to Bunchy Top

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Two abaca hybrids namely, Hybrid 2 and Hybrid 7, which were derived from a cross between the resistant wild banana (*Musa balbisiana*) var. Pacol and the susceptible abaca var. Abuab possessing the high fiber quality trait, have been previously selected with promising resistance to bunchy top disease. In this study, the responses of these hybrids to virus inoculation by the aphid (*Pentalonia nigronervosa*) under screenhouse condition and to natural infection in the field were characterized. Under screenhouse condition, Hybrid 7 did not show the bunchy top disease symptoms of dark green streaks on veins and midribs, marginal leaf chlorosis, narrow and stiff leaves or upright and crowding of leaves at the apex of the plant, while Hybrid 2 expressed the disease in only 1 of 15 (7%) plants tested over the 6-mo observation period. The virus was not detectable by enzyme-linked immunosorbent assay (ELISA) using polyclonal antibody against *Banana bunchy top virus* (BBTV) in all asymptomatic Hybrid 2, Hybrid 7 and 'Pacol'. Plants were confirmed negative for BBTV when tested by polymerase chain reaction (PCR) using the primer pair BBT1 and BBT2 that amplifies the 349-bp fragment of viral DNA-R component. The response was observed under condition of high disease pressure wherein the susceptible 'Inosa' and 'Abuab' developed severe disease characterized by high disease incidence, high amount of disease (measured by the Area Under Disease Progress Curve), and severe symptoms. The results observed under screenhouse condition were consistent with the response to natural infection involving plants that had been grown for 5 yr (2012–2017) in the field located at the Caraga State University, Ampayon, Butuan City, Philippines. Disease index was 4% for Hybrid 2 and 0% for Hybrid 7, indicating a resistant response to bunchy top. Knowledge on the resistance characteristics would be useful information for proper field deployment of these hybrids, and for breeding varieties with resistance to bunchy top.Key Words: abaca hybrids, *Banana bunchy top virus*, bunchy top resistance, *Musa textilis* Nee

Abbreviations: AUDPC – Area Under the Disease Progress Curve, BBTV – *Banana bunchy top virus*, DI – disease index, ELISA – enzyme-linked immunosorbent assay, mpi – months post inoculation, PCR – polymerase chain reaction

INTRODUCTION

Abaca (*Musa textilis* Nee), also known as Manila hemp, is an economically important crop in the Philippines, the country that is recognized as the biggest supplier of abaca products worldwide. The crop is primarily grown for its strong and flexible fiber which is three times stronger than cotton and twice as strong as sisal fibers (Armecin et al. 2014), and also for pulp with variety of uses including specialty papers. Abaca fiber as the country's longstanding export commodity also serves as basic material for a variety of fabrics and yarns, and for many other uses (PCAARRD 2013; PhilFIDA 2015).

The Philippine abaca industry has maintained its status as the world's largest producer of abaca fiber and it continues to maintain a strong position in both international and domestic markets, generating US$ 113.33M annually (PhilFIDA 2016, unpublished). However, the abaca industry is faced with various constraints including damage due to virus diseases.

For more than a century, the bunchy top disease has considerably affected the production of abaca in the Bicol Region, Eastern Visayas and Mindanao where the crop is widely grown in the country (Calinisan 1934; Raymundo and Bajet 2000; Bajet and Magnaye 2002; PhilFIDA 2015; Sta. Cruz et al. 2016). Plants affected by bunchy top disease are typically stunted, and produce undersized suckers with short, narrow, stiff and upcurled leaves, and chlorotic to necrotic leaf margins (Ocfemia 1926; Ocfemia 1930; Raymundo 2000; Bajet and Magnaye 2002). Infected

plants ultimately become unproductive, and have to be eliminated to prevent further disease spread. The abaca bunchy top in the country was first reported to occur in Silang, Cavite in 1915 (Ocfemia 1926). The disease is caused by the *Banana bunchy top virus* (BBTV) (Magee 1953; Bajet and Magnaye 2002; Vetten et al. 2005; Furuya et al. 2006; Natsuaki and Furuya 2007). Later, a distinct virus species, the *Abaca bunchy top virus* (ABTV), was found to be associated with the disease (Sharman et al. 2008; Sta. Cruz et al. 2018 unpublished). Both BBTV and ABTV belong to the family *Nanoviridae*, genus *Babuvirus*, with genome consisting of six single-stranded circular DNA components (DNA-R, -U3, -S, -M, -C and –N) of 1.0–1.1 kb (Karan et al. 1997; Vetten et al. 2005; Sharman et al. 2008). BBTV and ABTV are considered as separate species having a mean of 63% overall nucleotide sequence identity across all six DNA components (Sharman et al. 2008), which is less than the species demarcation of 85% identity for nanoviruses (Vetten et al. 2005). ABTV has been detected in the Bicol Region but not in Visayas and Mindanao (Sharman et al. 2008; Sta. Cruz et al. 2018). The virus is transmitted by *P. nigronervosa* in a persistent, circulative and non-propagative manner (Magee 1927).

The uncontrollable devastation caused by bunchy top aggravates the abaca production problems of farmers (DOST-PCAARRD 2015; DA-PRDP 2017). Since 1992, the Fiber Development Authority (FIDA), now known as PhilFIDA, has increased efforts to manage the disease through the abaca rehabilitation program that aims to rehabilitate abaca plantations severely affected by bunchy top through elimination of diseased plants and through replanting using disease-free planting materials, or expansion of planting to disease-free areas (PhilFIDA 2012). Breeding for virus resistance to bunchy top is another program that aims to rehabilitate the abaca industry. Attempts to produce BBTV-resistant abaca have been conducted through mutation breeding as reported by Dizon et al. (2012) who have identified some putative resistant lines, but the resistance needs further evaluation in the field.

Breeding program for abaca bunchy top resistance has produced hybrids derived from a cross between the resistant wild banana (*Musa balbisiana*) var. Pacol, and the susceptible abaca var. Abuab that possesses the high fiber quality trait (Lalusin et al. 2006, as cited by Lalusin and Villavencio 2014). Two lines namely, Hybrid 2 and Hybrid 7, out of 63 did not express the bunchy top disease, and were found negative for the presence of BBTV by PCR and ELISA analyses. However, the resistance of these hybrids has not been well characterized. Resistance to a virus can be characterized based on the property of the plant that reduces virus multiplication, reduces or prevents virus spread within the plant, or reduces symptom expression (Hull and Davis 1992).

In this study, the response of Hybrid 2 and Hybrid 7 to bunchy top disease was characterized by comparing the development of the disease in these hybrids with their parentals, 'Pacol' and 'Abuab', and the susceptible control varieties 'Tinawagan Pula' and 'Inosa' over a 6-mo period under screenhouse condition, and compared with natural infection in the field. The response was characterized based on reduction of virus multiplication or reduction of symptom expression, which were measured as absence of infection, or presence of infection but with delayed disease onset, reduced disease incidence, or reduced symptom severity. Understanding the response of these hybrids to bunchy top disease will be useful in the proper deployment of the hybrids in the field and also in designing methods for proper screening and selection of other promising abaca-resistant lines. The findings of this study would contribute to the development of appropriate breeding strategies for the rehabilitation of abaca plantations and better management of the abaca bunchy top disease.

MATERIALS AND METHODS

Abaca Hybrids, Their Parentals and Susceptible Control Varieties

Abaca Hybrid 2 and Hybrid 7 is a cross between the resistant wild banana ('Pacol') and susceptible abaca ('Abuab'), which had been developed by researchers from the Institute of Plant Breeding of the University of the Philippines (IPB-UPLB) headed by Dr. Antonio Lalusin. The genes that are linked to resistance in 'Pacol' and fiber quality in 'Abuab' had been identified through the use of simple sequence repeat markers (Lalusin et al. 2006, as cited by Lalusin and Villavencio 2014). Tissue-culture-derived plantlets (2-mo-old) that had been tested virus-free were used in the characterization of bunchy top resistance in screenhouse evaluation. Plantlets of Hybrid 2 and Hybrid 7 were obtained from the Tissue Culture Laboratory of Caraga State University, Ampayon, Butuan City, Philippines while 'Abuab', 'Pacol' and 'Tinawagan Pula' from the Tissue Culture Laboratory of the National Abaca Research Center (NARC) and 'Inosa' from the Tissue Culture Laboratory of IPB-UPLB. 'Tinawagan Pula' and 'Inosa' were used as the susceptible control varieties. The test plants were maintained virus-free in the screenhouse before virus inoculation.

The resistance of abaca hybrids under natural infection in the field was characterized from the experimental trial site that had been previously established by another

research group since June 2012 in an area of 2500 m2 at the Caraga State University Experimental Farm. 'Tinawagan Pula' and 'Inosa' were used as the susceptible control varieties. The authors were given permission to conduct the study in their experimental trial site.

Virus Inoculation

For the screenhouse evaluation, virus inoculation was conducted using colonies of *Pentalonia nigronervosa* that were collected in BBTV-infected fields following the protocol of Niyongere et al. (2011). In our study, colonies of *P. nigronervosa* collected from field-infected abaca plants var. Inosa, which had been previously tested as BBTV positive in ELISA, were used for virus inoculation. Portion of the leaf petiole carrying about 20-30 colonies of *P. nigronervosa* was detached from the infected plant, and then placed near the base of the test plants. The viruliferous insects were allowed to move to the test plants by instinctive natural migration to ensure that the aphid's insect stylet was not damaged. The experiment was laid out in Randomized Complete Block Design (RCBD) with 12 treatments, namely: Treatment 1 (T1), Inoculated Hybrid 2; T2, Uninoculated Hybrid 2; T3, Inoculated Hybrid 7; T4, Uninoculated Hybrid 7; T5, Inoculated 'Pacol': T6, Uninoculated 'Pacol; T7, Inoculated 'Abuab'; T8, Uninoculated 'Abuab'; T9, Inoculated 'Tinawagan Pula'; T10, Uninoculated 'Tinawagan Pula'; T11, Inoculated 'Inosa'; and T12, Uninoculated 'Inosa'. Each treatment was replicated three times with five plants per replication. After a 2-wk inoculation period, the plants were sprayed with insecticide (Carbofuran).

As earlier mentioned, the response of abaca hybrids to natural infection in the field was characterized from the experimental trial site that had been set up by another group of researchersTinawagan Pula' and 'Inosa' were used as susceptible control varieties but the resistant parental 'Pacol' and susceptible 'Abuab' were not included in their set-up. The experiment was laid out in RCBD with four treatments, namely, Treatment 1 (T1), Hybrid 2; T2, Hybrid 7; T3, Tinawagan Pula; and T4, Inosa. Each treatment was replicated three times with six plants per replication.

Disease Assessment

In the screenhouse evaluation, test plants were monitored for the development of bunchy top disease which was measured using various parameters (disease onset, disease incidence, disease progress and symptom severity) at different months post inoculation (mpi) starting at 1 mpi, 3 mpi, and 6 mpi. The response to virus inoculation was determined by computing the disease index (DI) compared with the susceptible varieties. In the field evaluation, the disease was assessed on the fifth year of the experimental field trial.

Disease Onset and Disease Incidence

Disease onset was determined based on the time when the early disease symptom of dark green streaks appeared on the veins and midribs of the inoculated plants. The presence of disease symptoms at different times of disease assessment was determined by visual observation of any of the typical bunchy top symptoms such as dark green streaks on veins and midribs, marginal leaf chlorosis, narrow and stiff leaves, and upright and crowding of leaves at the apex of the plant. Virus infection was then confirmed by detecting the presence of BBTV by ELISA and PCR analysis. Disease incidence was the proportion of plants that became infected to the total number of inoculated plants.

Disease Progress

The progress of the disease was determined by computing the area under the disease progress curve (AUDPC) based on the formula

$$AUDPC=\sum(X_{i+1}+X_i)/2)\times(t_{i+1}-t_i)$$

where X_i is the disease incidence at i^{th} observation, and t_i is time (months after disease occurrence) at i^{th} observation (Campbell and Madden 1990).

Symptom Severity

The severity of bunchy top was determined using the severity rating scale developed by Parac and Sta. Cruz (2018, unpublished). The rating scale from 1 to 9 indicated symptom severity as follows: 1- no visible symptom; 3- dark green streaks developed on leaf veins and midribs; 5- progressive dark green streaks on leaf veins and midribs, marginal leaf chlorosis; 7- severe chlorosis, narrow and stiff leaves; and 9- severe bunchy-top, upright and crowding of leaves at the apex of the plant, stunted growth.

Disease Index

Disease index (DI) was computed based on the formula for tungro resistance evaluation (Standard Evaluation System) (INGER, IRRI) with some modifications as shown below:

Disease index = [3(n) + 5(n) + 7(n) + 9 (n)/ total number of plants scored x highest severity score at the rating period] x 100

where 3, 5, 7 and 9 = symptom severity rating; and n = number of plants for each symptom rating. The response was determined based on DI value: 0–30%, resistant; 31–60%, moderately susceptible; and 61–100%, susceptible.

Virus Detection by Enzyme-Linked Immunosorbent Assay

Analysis for BBTV infection was conducted following the compound indirect ELISA method using commercial polyclonal BBTV antibody from Agdia (Agdia, Elkhart, Inc., USA) and according to the manufacturer's protocol. Leaf sample (0.1 g) collected from each test plant was homogenized using mortar and pestle in 0.05 M carbonate buffer, pH 9.6, at 1:10 dilution. One hundred microliters (100 µL) of the homogenized sap was dispensed using a micropipette to each well of microtiter ELISA plate. The BBTV positive sample was taken from BBTV-infected plant that has been maintained in the greenhouse, and from the Agdia virus positive sample. The healthy control was taken from virus-free tissue cultured plantlet, and from Agdia virus negative sample. The sample which showed two times higher ELISA absorbance value at 405 nm than the threshold value computed as mean of four healthy control samples was considered as virus positive.

Virus Detection by Polymerase Chain Reaction

The method by Piamonte and Sta. Cruz (2018) was used to extract the total nucleic acid from the abaca leaf samples. The presence of BBTV was detected in 50 ng DNA template by PCR using the primer pair BBT-1 and BBT-2 designed to amplify a 349-bp fragment of the partial BBTV replicase gene (DNA-R) and following the conditions specified in the published literature (Thomson and Dietzgen 1995). The positive control was obtained in sample from symptomatic plant which had been previously tested by PCR to be BBTV-infected. The negative control was obtained from healthy tissue culture plantlets. The samples were also tested by PCR for the presence of *Musa* sequence using an internal control primer pair AGMI 025 and AGMI 026 to confirm that the negative reaction was due to the absence of the virus, and that there was no inhibitory compound that may have prevented DNA amplification. The primer pair AGMI 025 and AGMI 026 (5'-TTA AAG GTG GGT TAG CAT TAG G -3' and 5'-TTT GAT GTC ACA ATG GTG TTC C-3') amplifies a product size of 248 bp (Lagoda et al. 1998). The presence of PCR-amplified DNA was analyzed by electrophoresis, and the gel was stained with gel red (Biotum), and then visualized using the Alpha Imager Mini Analysis System (Alpha Innotech).

RESULTS AND DISCUSSION

The response of abaca Hybrid 2 and Hybrid 7 to bunchy top disease was characterized under screenhouse condition. Development of the disease based on disease incidence and symptom severity of these hybrids over the 6-mo period was compared with that of the susceptible varieties 'Tinawagan Pula' and 'Inosa', and with their parentals 'Pacol' and 'Abuab'. 'Pacol' is the source of bunchy top resistance while 'Abuab' is susceptible but possesses the high fiber quality trait. The response of these hyrids was also characterized under field condition.

Disease Onset and Disease Incidence

Incidence of bunchy top was determined by visual observation of symptom, which was confirmed by detecting the presence of BBTV by ELISA and PCR. Under screenhouse condition, Hybrid 7 did not develop the disease throughout the 6-mo period based on visual observation of symptom (Table 1). The inoculated plants did not show any disease symptom of dark green streaks on veins and midribs, marginal leaf chlorosis, narrow and stiff leaves or upright and crowding of leaves at the apex of the plant when observed at 1, 3 or 6 mpi. The absence of bunchy top disease on Hybrid 7 was confirmed by ELISA and PCR analysis. The virus was not detectable in all the plants tested until the end of the observation

Table 1. Incidence of bunchy top disease in abaca hybrids, their parentals and susceptible control varieties at 6 months post inoculation (mpi).

Abaca Hybrid/ Variety	Disease Incidence (%) [1]					
	Screenhouse Evaluation			Field Evaluation		
	Visual Observation	Enzyme-linked immunosorbent assay	Polymerase chain reaction	Visual observation	Enzyme-linked immunosorbent assay	Polymerase chain reaction
Hybrid 2	7	7	7	0	0	0
Hybrid 7	0	0	0	0	0	0
Pacol	0	0	0	Not tested	Not tested	Not tested
Abuab	100	73	100	Not tested	Not tested	Not tested
Inosa	100	80	100	100	80	95
Tinawagan Pula	67	48	73	90	70	85

[1] Mean percentage of bunchy top incidence in three replications with five plants per replication

period at 6 mpi (Table 1). On the other hand, although Hybrid 2 developed the disease, only one of 15 test plants (7%) showed symptom, and onset of infection was delayed until 6 mpi. Infection of Hybrid 2 was confirmed by ELISA and PCR analysis, wherein the virus was detectable in the symptomatic plant. The disease pressure was high wherein the susceptible 'Inosa' developed 100% infection, although 'Tinawagan Pula' had relatively lower infection.

The development of bunchy top disease was compared using the results of PCR analysis (Fig. 1A). Infection did not develop at all in Hybrid 7 starting at 1 mpi until the end of the observation period at 6 mpi. Consistent with visual observation, Hybrid 2 developed the disease but in only 7% of the plants, and infection was delayed until 6 mpi. 'Inosa' and 'Tinawagan Pula' developed the disease early at 1 mpi with 27% and 13% of the plants, respectively, being positive for BBTV. The disease progressed rapidly at 3 mpi wherein all (100%) 'Inosa' plants had fully developed the disease, while 'Tinawagan Pula' had 67% and 73% infection at 3 mpi and 6 mpi, respectively. The resistant parental 'Pacol' did not develop the disease until 6 mpi. At 1 mpi, infection was not yet detected in 'Abuab', but the disease developed rapidly thereafter with 73% infection at 3 mpi, which fully developed (100%) at 6 mpi. The incidence of infection between the hybrids and susceptible varieties were significantly different at each observation period from 3 mpi to 6 mpi (Fig. 1A).

Since the susceptible control varieties developed high incidence of infection, the absence of disease in Hybrid 7 and the lower incidence of infection in Hybrid 2 indicate a resistant response to bunchy top, and that the response was not due to escape of infection. 'Inosa' had AUDPC values of 427%-months, indicating high amount of disease during the observation period (Fig. 1B). 'Tinawagan Pula' had AUDPC value of 280%-months. Thus, the low AUDPC values of Hybrid 2 (10%-months) and Hybrid 7 (0%-months) further indicate a resistant response to bunchy top disease. The resistant parent, 'Pacol', had no value for AUDPC while 'Abuab' had 333% -months (Fig. 1B).

Delayed disease onset is a resistance characteristic of virus diseases including abaca bunchy top. The putative abaca bunchy top resistant lines obtained by induced mutation had delayed disease at 3 mo after inoculation (Dizon et al. 2012). In our study, the development of the disease was studied wherein the response was determined at various stages of infection following the method used by Sta. Cruz et al. (2003) for the characterization of rice tungro resistance. Analysis by ELISA showed that rice tungro bacilliform virus (RTBV)

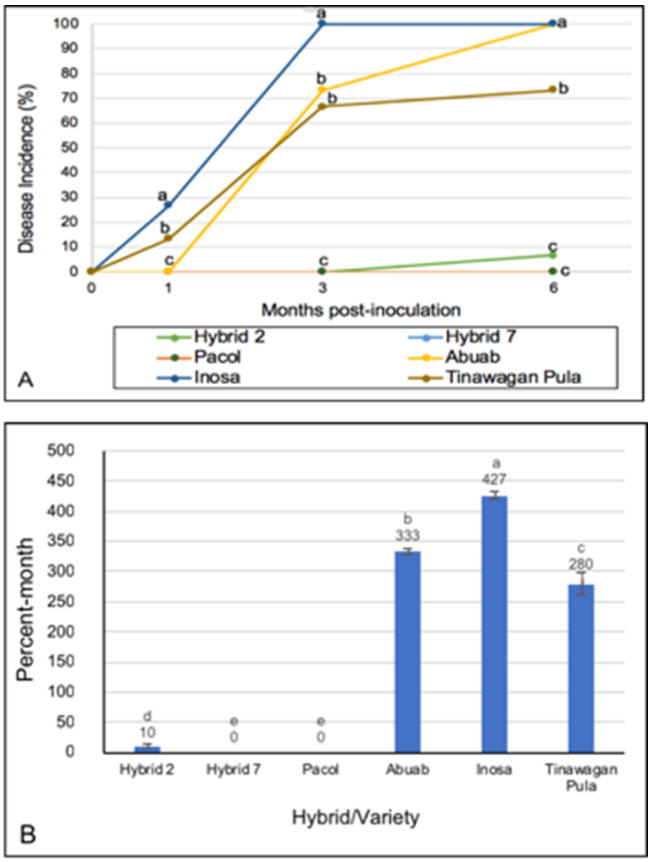

Fig. 1. Development of bunchy top disease in inoculated abaca hybrids, parentals and susceptible control varieties in screenhouse evaluation. A) Disease incidence; and B) Area Under the Disease Progress Curve (AUDPC). Uninoculated control for each hybrid and variety remained uninfected and not shown in the figure. Means with the same letter are not significantly different at 5% Tukey's HSD.

accumulated in a cyclic pattern from early to late stages of infection in the tungro-susceptible variety 'Taichung Native' 1 (TN1) and the tolerant variety 'Balimau Putih'. These changes in virus accumulation resulted in differences in RTBV levels and incidence of infection when analyzed at different stages of infection. Similar method was used for the characterization of papaya ringspot virus resistance. Alviar and co-workers (2012) also observed delayed onset and development of papaya ringspot virus infection of tolerant papaya varieties 'Sinta' and 'Cariflora'. Both varieties have delayed disease development which appeared at 2 wk post inoculation (wpi) compared to earlier disease onset at 1 wpi in the susceptible 'Solo' variety.

Symptomless Abaca Hybrids Even Under Conditions of High Disease Pressure

In the screenhouse evaluation, all inoculated Hybrid 7 plants were symptomless even when conditions for disease development were highly favorable (Fig. 2 and 3).

Since Hybrid 7 and 'Pacol' did not develop the disease, all test plants (100%) had severity score (ss) = 1 (Fig. 2A). At 6 mpi, 93% of Hybrid 2 had no disease symptom (severity score of 1) (Fig. 2A), while only 1 of 15 test plants (7%) was symptomatic with ss = 5 (Fig. 2B). 'Abuab', on the other hand, had 53% of plants with ss = 5, 20% with ss = 7 and 27% with ss = 9. The condition for development of the disease was favorable since the susceptible varieties developed severe disease symptoms. The susceptible 'Inosa' had 20% of plants with ss = 7 and 80% with ss = 9. Plant with severity score of 7 had symptoms of severe chlorosis, narrow and stiff leaves and plants with ss = 9 had severe bunchy-top, upright and crowding of leaves at the shoot apex of the plant, and stunted growth. 'Tinawagan Pula' had 20% of plants with ss = 5, 13% with ss = 7 and 33% with ss = 9. Plants with severity score of 5 had symptoms of progressive dark green streaks on leaf veins and midribs, marginal leaf chlorosis. Infected 'Abuab', 'Inosa' and 'Tinawagan Pula' exhibited severe bunchy top symptom of stunting and crowding of leaves at the apex (Fig. 3D-F).

Consistent Resistant Response of Hybrid 7 in Field Evaluation

The resistant response of abaca hybrids under screenhouse condition was confirmed in field evaluation (Table 1). Hybrid 7 did not develop the disease during the 5-yr period under field condition, which was consistent with results of screenhouse evaluation. Hybrid 2 did not develop the disease in field evaluation while one out of 15 plants became infected in the screenhouse evaluation (Table 1). Possibly, this single infected plant may not be Hybrid 2, probably a contaminant from any of the susceptible controls used in the test. However, the development of infection in Hybrid 2 was not typical of a susceptible response, as infection was delayed at 6 mpi. In a previous field evaluation conducted by Lalusin and co-workers (unpublished), Hybrid 2 has been also found to be infected but at a very low incidence. The reaction of Hybrid 2 to bunchy top under screenhouse condition conforms to the findings of Lalusin et al. (2017, unpublished) that Hybrid 2 can be infectible.

The response of the two hybrids can be attributed to resistance itself, and it was not due to escape of infection. The field evaluation had been set up in the field for 5 yr and yet the hybrids remained uninfected (Fig. 4A-B). The condition during the evaluation was favorable for the

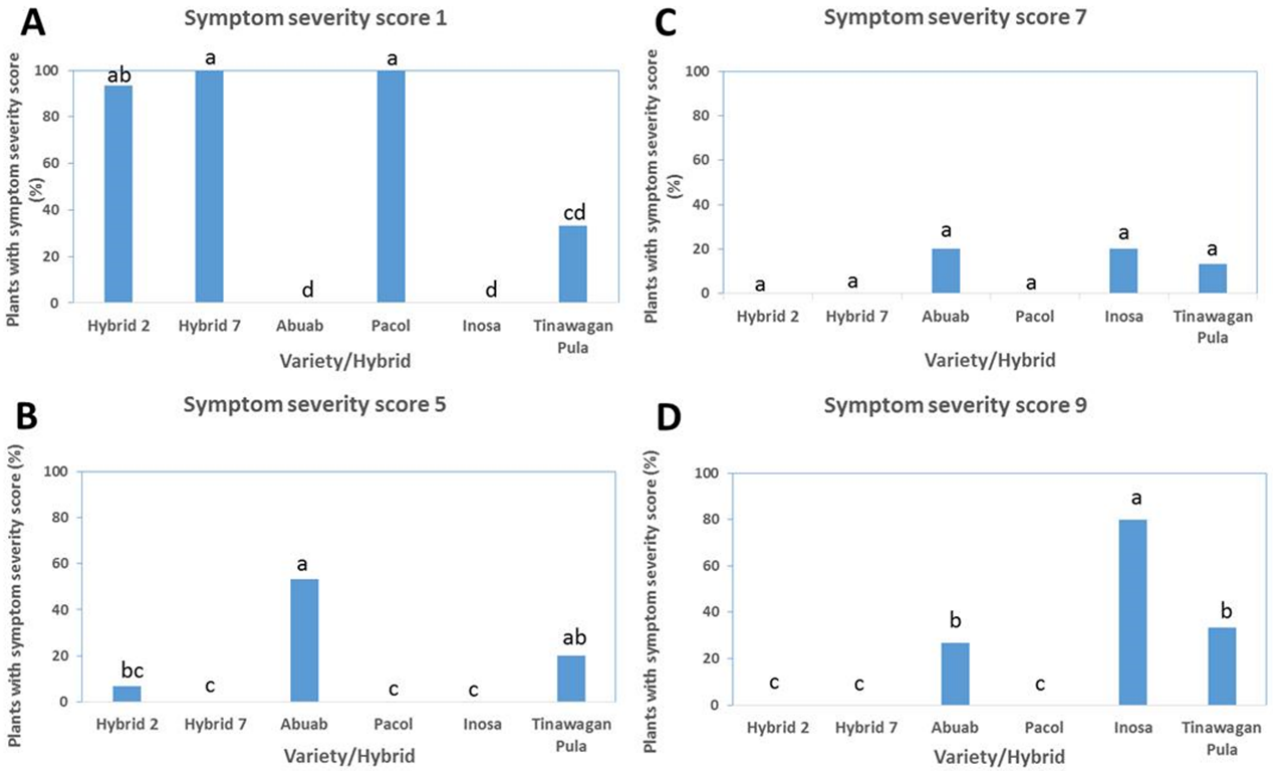

Fig. 2. Number of plants with symptom severity score (%) of abaca hybrids, parentals and susceptible varieties at 6 months post inoculation (mpi). Symptom severity score: A) 1- no visible symptom; B) 5- progressive dark green streaks on leaf veins and midribs, marginal leaf chlorosis; C) 7- severe chlorosis, narrow and stiff leaves and D) 9- severe bunchy-top, upright and crowding of leaves at the apex of the plant, stunted growth. Means with the same letter are not significantly different at 5% Tukey's HSD. Plants with symptom severity scores 5 and 7 were arcsin and square root transformed before analysis, respectively.

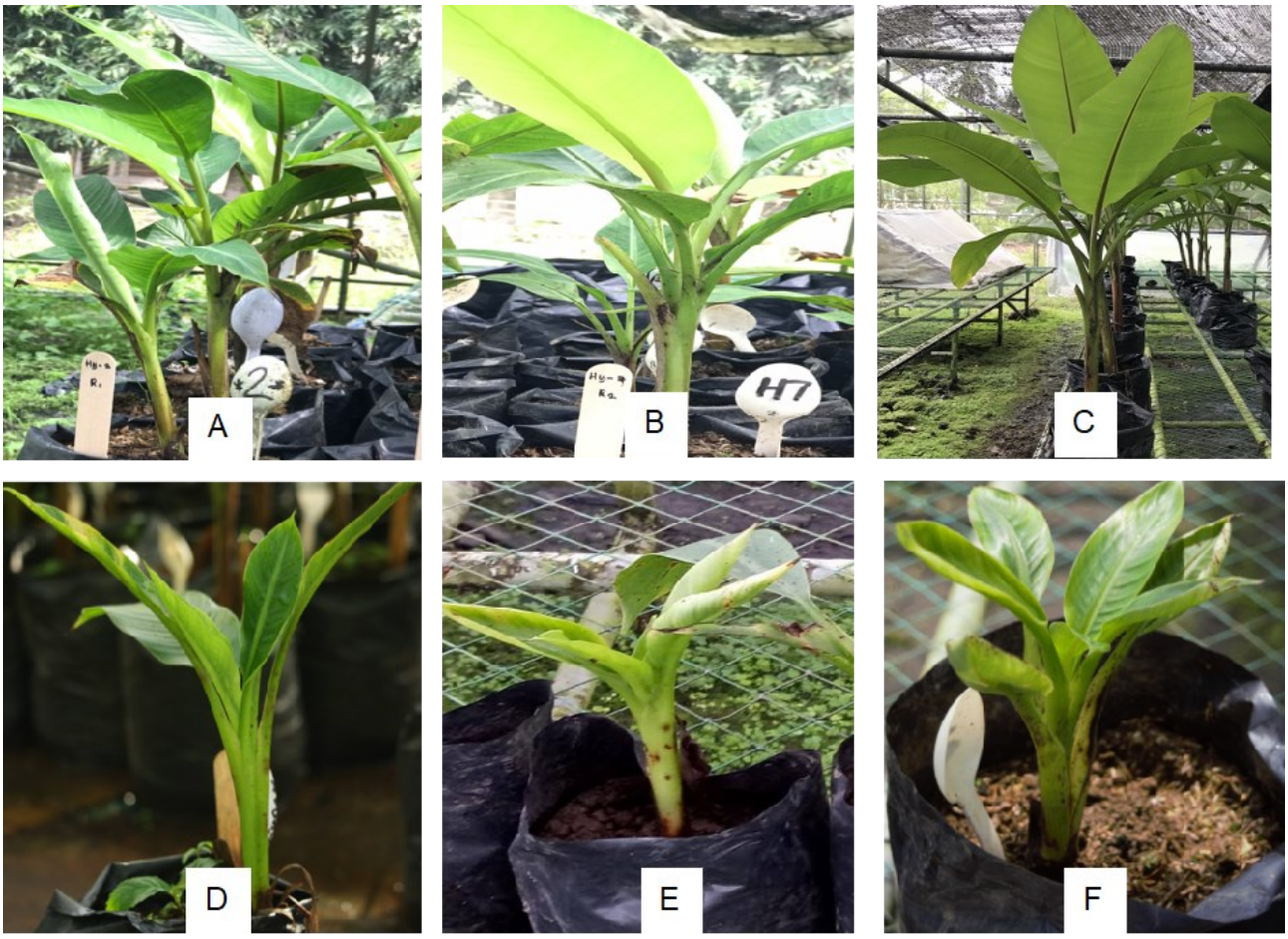

Fig. 3. Symptom of abaca bunchy top disease at 6 mo after inoculation in screenhouse evaluation: A–C) Hybrid 2, Hybrid 7 and 'Pacol', respectively, with no visible disease symptom; D–F) 'Abuab', 'Inosa' and 'Tinawagan Pula', respectively, with severe bunchy top symptom of stunting and crowding of leaves at the shoot apex.

development of the disease. Infection of the susceptible control particularly 'Inosa' was severe wherein the plant developed severe disease with symptoms of dark green streak on the leaf veins, midribs and petioles which later showed severe marginal chlorosis to severe bunchy top with upright, crowded and brittle leaves at the apex of the plant (Fig. 4C-D).

Absence of Infection in Abaca Hybrids as Confirmed by PCR Analysis

The absence of infection in abaca hybrids under screenhouse and field conditions was confirmed by PCR analysis using the primer pair BBT-1 and BBT-2 that amplifies the 349-bp fragment of the BBTV DNA-R component. The virus was not detectable in all Hybrid 7 and its parental 'Pacol' test plants which remained symptomless until 6 mpi in the screenhouse evaluation, and in Hybrid 2 except one positive sample (Fig. 5). The parental 'Abuab' and the susceptible control 'Inosa' and Tinawagan Pula' were confirmed positive by PCR for BBTV infection. Likewise, absence of infection in field-grown hybrids was confirmed by PCR analysis. Both 'Inosa' and 'Tinawagan Pula' were infected (Fig. 6).

Analysis by PCR using an internal control confirmed that the negative reaction to BBTV was due to absence of the virus and not the presence of PCR inhibitory compounds. Abaca DNA extracts contain high amount of inhibitory compounds which interfere with DNA amplification by PCR (Piamonte and Sta. Cruz, 2018). The primer pair, AGMI 025 and AGMI 026 designed to amplify the *Musa* sequence (Lagoda et al. 1998) with an expected product size of 248 bp, was used as an internal control. For screenhouse evaluation, the BBTV negative samples of Hybrid 2, Hybrid 7 and 'Pacol' were positive for the *Musa* sequence (Fig. 5). Likewise, the *Musa* sequence was detected in both hybrids from field samples. The results indicated that the template DNA did not contain PCR inhibitory substances that may give false negative results. Thus, the negative reactions of Hybrid 2 (except sample 5), Hybrid 7 and 'Pacol' were confirmed to be due to absence of the virus (Fig. 6).

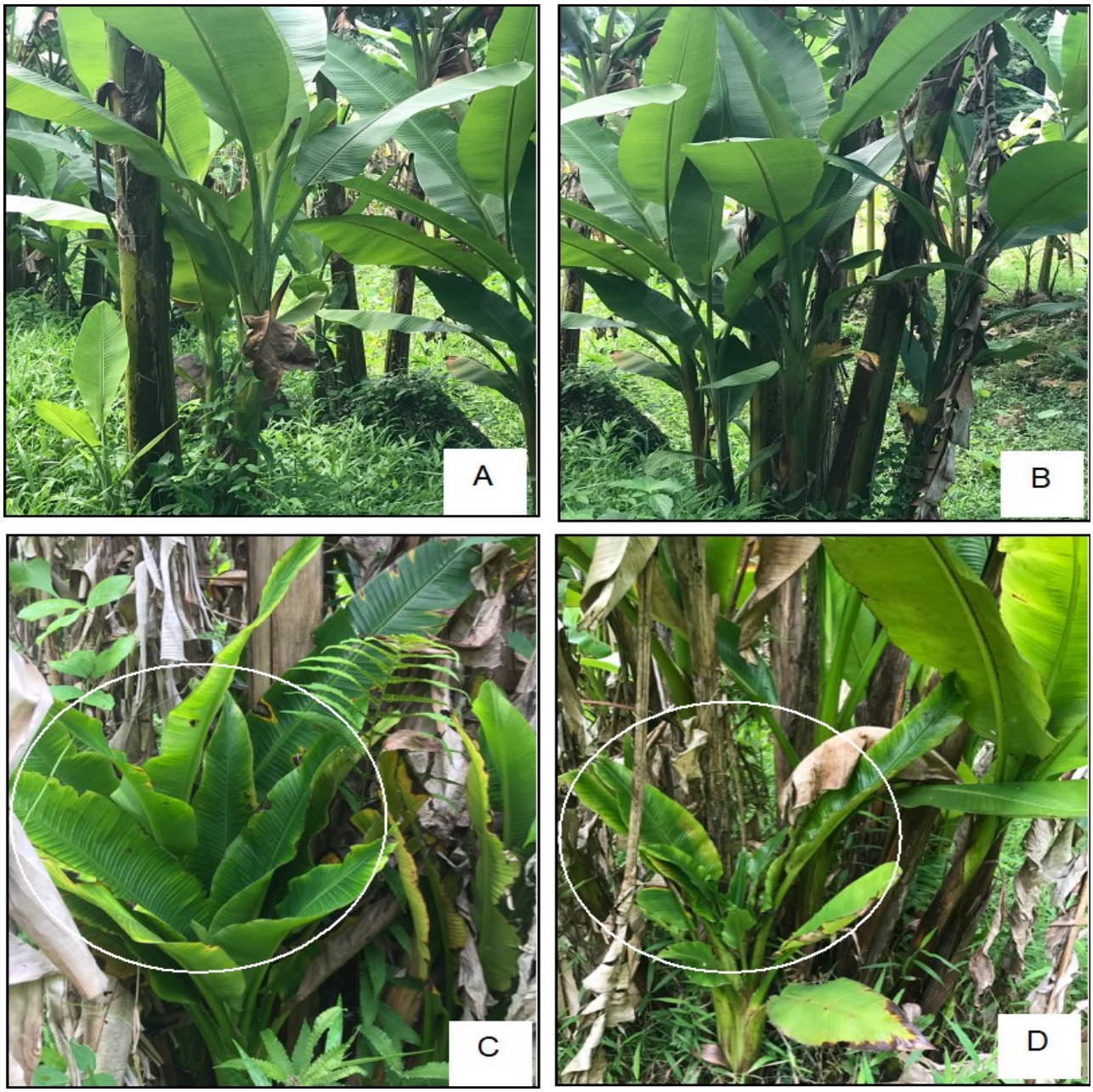

Fig. 4. Bunchy top disease symptom on selected abaca hybrids and susceptible varieties in field evaluation (2013–2017): A) Hybrid 2; B) Hybrid 7, showing no visible symptoms of bunchy top; and C–D Severe bunchy top with upright, crowded leaves at the shoot apex of follower plant of 'Inosa' and 'Tinawagan Pula', respectively.

Low Disease Index Indicates Resistant Response of Hybrids to Bunchy Top Disease

The disease index (DI) which measures both incidence and severity showed the response to virus inoculation of abaca hybrids. In this study, both Hybrid 2 and Hybrid 7 were rated as resistant with similar response as the parental 'Pacol' (Table 2). Since Hybrid 7 did not develop the disease, it had a DI of 0% similar to the resistant parental 'Pacol', while Hybrid 2 had 4% DI. On the other hand, the parental 'Abuab' was susceptible with DI of 72%. The susceptible control 'Inosa' had DI of 96%. Since 'Tinawagan Pula' has DI of 55%, the response was moderately susceptible. Disease index is considered as a good parameter for determining the response of varieties or lines to virus disease.

CONCLUSION

The responses of abaca Hybrid 2 and Hybrid 7 to virus inoculation by the aphid vector *P. nigronervosa* under screenhouse condition, and natural infection in the field

Table 2. Response based on disease index of abaca hybrids, parentals and susceptible control varieties to bunchy top disease.

Abaca Hybrid/ Variety	Disease Index (%)	Response [1]
Hybrid 2	4	Resistant
Hybrid 7	0	Resistant
Pacol	0	Resistant
Abuab	72	Susceptible
Inosa	96	Susceptible
Tinawagan Pula	55	Moderately susceptible

[1]Response based on DI value: 0–30%, resistant; 31–60%, moderately susceptible; and 61–100%, susceptible.

were characterized in this study. Hybrid 7 is considered resistant wherein inoculated plants did not develop the disease during the 6-mo observation period under screenhouse condition, and until the 5th year of field trial conducted at Ampayon, Butuan City. Disease resistance is characterized by the absence of virus infection wherein the plants did not express any bunchy top disease symptom, and were negative for the presence of BBTV as tested by ELISA and PCR. The resistant response of Hybrid 7 was similar to that of the parental 'Pacol'. Hybrid 2 is also resistant as most of the inoculated plants did not develop the disease except in one out of 15 plants tested under screenhouse condition, but no infection was detected in the field. The onset of disease in the infected plant was delayed, and the symptom was not severe compared with the susceptible control variety 'Inosa'. The hybrids were evaluated under conditions of high disease

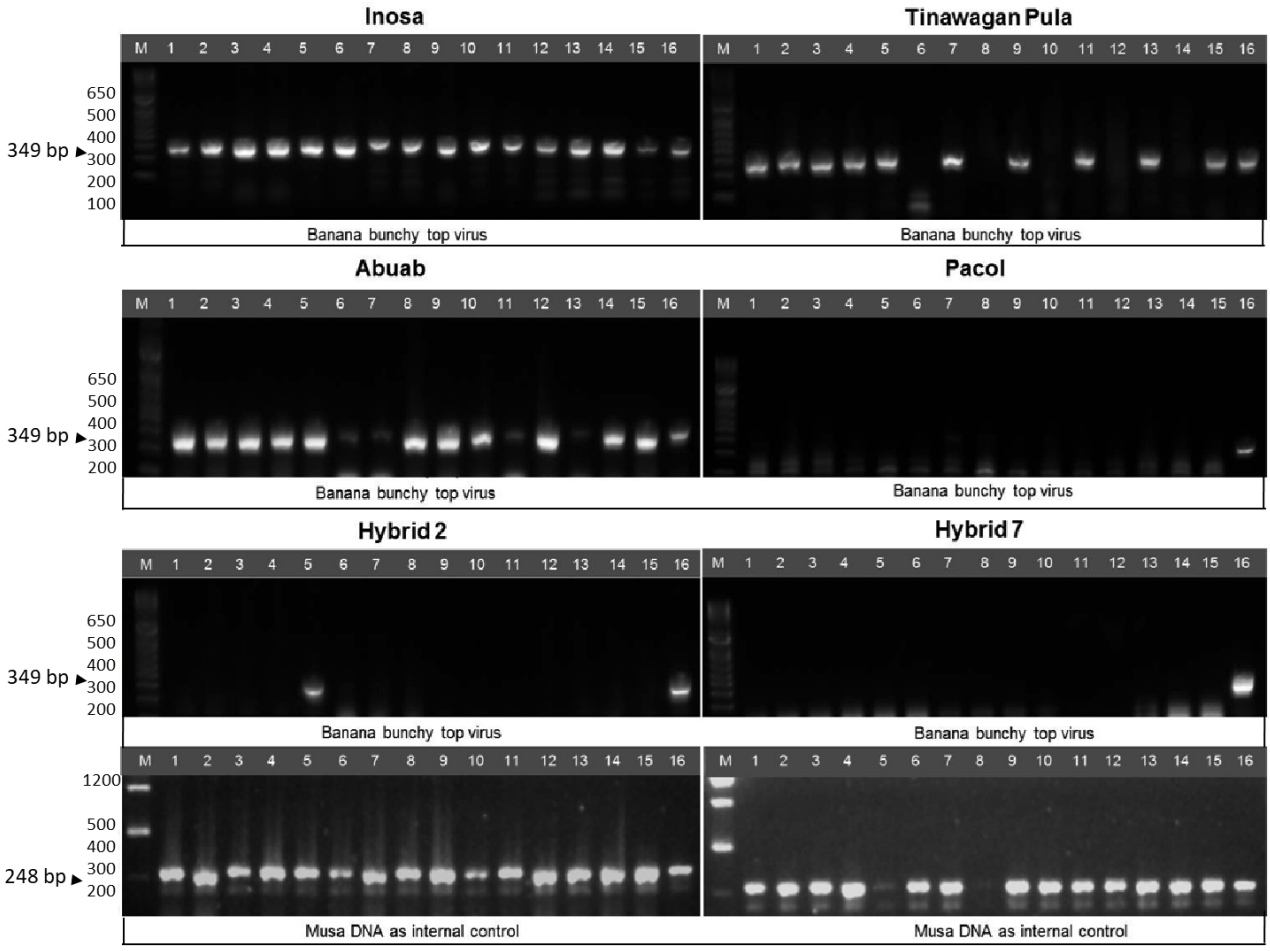

Fig. 5. Banana bunchy top virus DNA-R fragment (349 bp) and Musa sequence (248 bp) detected by polymerase chain reaction using the primer pairs BBT-1/ BBT-2 and AGM1 025/AGM1 026, respectively from screenhouse samples of susceptible control varieties ('Inosa' and 'Tinawagan Pula'), parental ('Abuab' and 'Pacol') but not in Hybrid 7 and Hybrid 2 (except for one sample). For each line or variety, For Banana bunchy top virus: Lane M, 1Kb plus DNA ladder (Invitrogen); Lanes 1–15, samples at 6 mo after inoculation; Lane 16, BBTV infected positive control; For Musa DNA: Lane M, 100 kb DNA ladder (Vivantis); Lanes 1–15, samples at 6 mo after inoculation; Lane 16, Musa DNA positive control.

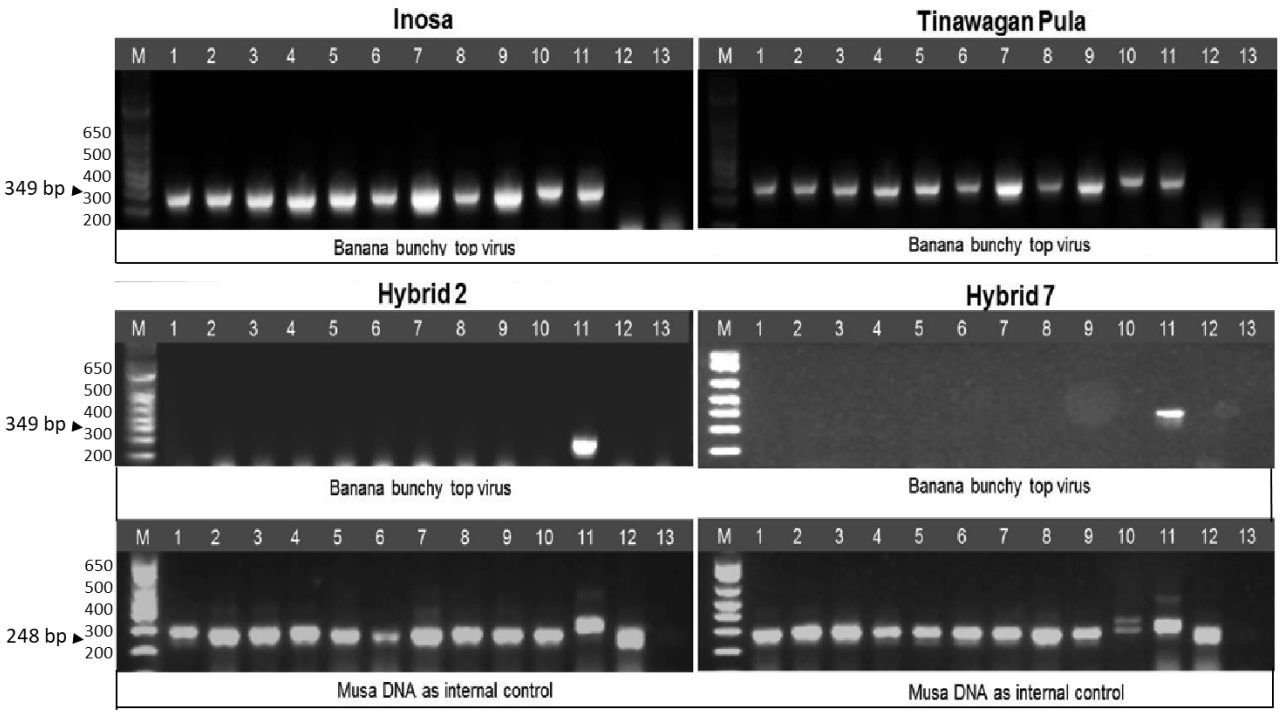

Fig. 6. Banana bunchy top virus DNA-R fragment (349 bp) and Musa sequence (248 bp) detected by polymerase chain reaction using the primer pair BBT-1/ BBT-2 and AGMI 025/AGMI 026, respectively from field samples of susceptible control varieties ('Inosa' and 'Tinawagan Pula') but not in Hybrid 2 and Hybrid 7. For each variety or hybrid, For Banana bunchy top virus: Lane M, 1Kb plus DNA ladder (Invitrogen); Lanes 1–10, test samples; Lane 11, BBTV infected positive control; Lane 12, Healthy negative control; Lane 13, Blank negative control. For Musa DNA: Lane M, 1 kb plus DNA ladder (Invitrogen); Lanes 1–10, test samples; Lane 11, Musa DNA positive control; Lane 12, Healthy plant, Lane 13, Blank negative control.

pressure, indicating that the observed response was due to resistance, and not to escape of infection. The susceptible control variety particularly 'Inosa' and the parental 'Abuab' developed severe disease characterized by early disease onset and high disease incidence, high amount of disease and severe symptoms. The presence of BBTV was detected in the susceptible 'Abuab', 'Inosa' and 'Tinawagan Pula' varieties by ELISA and PCR analysis, indicating that the virus present in the area was BBTV. Thus, the hybrids can be considered resistant to BBTV, and must also be tested for resistance to ABTV, the other virus species causing bunchy top. The antibody used in ELISA is produced against BBTV while the primer pair BBT1 and BBT2 can detect both BBTV and ABTV. In previous studies, the ABTV has been found present in the Bicol region, but not detected in Mindanao.

In this study, the methodology and parameters for characterization of resistance to bunchy top disease of abaca have been established. The resistance was characterized by comparing the disease development of abaca hybrids with their parental and susceptible control varieties using various parameters such as disease onset, disease incidence, amount of disease (area under the disease progress curve), and symptom severity. The response whether resistant or susceptible is determined based on the computed disease index which measures both disease incidence and symptom severity.

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