Annals of Tropical Research 46 (1):37-47(2024)
https://doi.org/10.32945/atr4614.2024

Reaction of Abaca Hybrid BC2-7 (BANDALA) to Bunchy Top Virus Isolates in Eastern Visayas, Philippines

Jofil A. Mati-om[1*], Ruben M. Gapasin[2] and Robelyn T. Piamonte[2]

ABSTRACT

The Institute of Plant Breeding at the University of the Philippines, Los Baños (IPB–UPLB) developed the abaca hybrid BC2–7 (BANDALA) to tolerate the BTVs that are prevalent throughout the Philippine archipelago. The reaction of this abaca hybrid BC2–7 (BANDALA) was tested with the different isolates of BTVs prevalent in the Eastern Visayas Region in the Philippines to confirm its resistance. In this study, the reaction of abaca hybrid BC2–7 (BANDALA) to four BTV isolates was evaluated and compared to the abaca varieties Inosa and Pacol. Based on disease incidence, all BTVs isolates infected 100% of Inosa plants but none of the abaca hybrid BC2–7 (BANDALA) and Pacol. The Leyte BTVs isolate produced symptoms in Inosa at 26–days after inoculation (DAI), Southern Leyte at 30–DAI, Biliran at 32–DAI and Samar at 38–DAI, while no symptoms were observed in abaca hybrid BC2–7 (BANDALA) and Pacol. Both the abaca hybrid BC2–7 (BANDALA) and Pacol showed high resistance to BTVs, whereas Inosa was highly susceptible. BTVs were detected from the plant samples using Polymerase Chain Reaction (PCR) with primers BBT–1 and BBT–2. Positive bands were detected from the Inosa inoculated with Leyte and Southern Leyte isolates at 5–DAI, Samar at 6–DAI, and Biliran at 7–DAI. The study confirms that abaca hybrid BC2–7 (BANDALA) and Pacol are highly resistant to all BTV isolates found in the Eastern Visayas Region of the Philippines.

Received: 10 August 2023
Accepted: 28 February 2024
Published: 29 June 2024

[1]School of Agri-Fisheries, Biliran Province State University-Biliran Campus, Biliran, Biliran
[2]Department of Pest Management, College of Agriculture and Food Sciences, Visayas State Univesity, Baybay City, Leyte, Philippines

***Corresponding Author.** Address: School of Agri-Fisheries, Biliran Province State University-Biliran Campus, Biliran, Biliran; Email: jofil.matiom@bipsu.edu.ph

Keywords: abaca hybrid, BC2–7 (BANDALA), bunchy top virus, PCR, resistance

INTRODUCTION

Abaca (*Musa textilis* Nee) is a native of the Philippines and grows well in many parts of the country due to favorable climatic conditions and volcanic soils. The crop is primarily grown for its strong and flexible fibers, which are three times stronger than cotton and sisal fibers (Armecin et al 2014) as well as for pulp used for a variety of applications, including specialty papers (Parac et al 2020). The Philippines is the world's leading abaca supplier, meeting 87.5% of global demand. Abaca fiber has great strength and there are many environmental benefits gained by interplanting crops with abaca (FAO 2021). However, meeting the increased demand for abaca fiber has been difficult because of declining abaca productivity, primarily due to virus diseases. Bunchy top virus (BTV) is the most serious virus disease affecting the abaca industry. Initially, the disease was understood to be caused by the *Banana bunchy top virus* (BBTV) (Bajet & Magnaye 2002). Later, a distinct virus species, the *Abaca bunchy top virus* (ABTV), was found to be linked to the disease (Sharman et al 2008). Both BBTV and ABTV are members of the *Nanoviridae* family, genus *Babuvirus*, with genome consisting of six single-stranded circular DNA components (DNA-R, -U3, -S, -M, -C and -N) of 1.0 -1.1kb (Vetten et al 2005, Sharman et al 2008). BBTV and ABTV are considered separate species because they have a mean of only 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). *Pentalonia nigronervosa* (banana aphid) transmits the virus in a persistent, circulative and non-propagative manner (Magee 1927).

In the 1940's, the onslaught of the disease resulted in thousands of abaca farms being abandoned, wiping out the abaca industry in the Southern Tagalog Region. The disease continues to devastate many abaca farms in the major growing areas in the country, such as Bicol and the Eastern Visayas regions (Raymundo et al 2002, PhilFida 2015). In 2003, the infection reached alarming rates in Eastern Visayas, the second–largest abaca–producing region, when two of its provinces (Leyte and Samar) were put in a state of calamity. The disease reportedly wiped out 16,737ha of a total of 26,374ha of abaca plantations (Nuñez 2013). The uncontrollable devastation caused by bunchy top disease still aggravates the abaca production problems of farmers (PCAARRD 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 the bunchy top disease through the elimination of diseased plants and through replanting using disease-free planting materials, or expansion of planting to disease-free areas. Breeding for virus resistance to the bunchy top virus is another program that aims to rehabilitate the abaca industry areas (PhilFIDA 2012).

The development of abaca resistant to viruses has long been the goal of the breeding program in the Philippines. This resulted in the discovery of wild banana (*Musa balbisinia*) var. Pacol that possesses high resistance to ABTV but has poor fiber yield and quality (Boguero et al 2016). A breeding program for abaca bunchy top resistance has produced hybrids derived from a cross between wild banana var. Pacol and the susceptible abaca var.

Abuab that possesses the high fiber quality trait (Lalusin & Villavencio 2014). Out of 63 lines tested, Hybrid 2 and Hybrid 7 did not express the bunchy top disease and tested negative for the presence of BBTV using Polymerase Chain Reaction (PCR) and Enzyme-Linked Immunosorbent Assay (ELISA). However, the resistance of these hybrids has only been investigated in the Caraga area of the Philippines (Parac et al 2020).

To address these concerns, the Philippine Government through the Department of Science and Technology – Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST–PCAARRD), funded certain science and technology interventions. One of these major initiatives is the promotion of improved Hybrid 7, now known as abaca hybrid BC2–7 (BANDALA), which is resistant to the BTVs and has high commercial fiber production (PCAARRD 2017). This abaca hybrid BC2–7 (BANDALA) is now being tested for its performance/stability in multilocational trials, one area of which is at the Visayas State University (VSU) in Leyte, Eastern Visayas. Although Parac et al (2020) reported on the resistance of BC2–7 (BANDALA) in Caraga Region, further investigation into the resistance and reaction of this abaca hybrid to the Eastern Visayas isolates of BTVs is needed to determine how this abaca hybrid BC2–7 (BANDALA) responds to BTVs in the region; hence, this study.

MATERIALS AND METHODS

Test Plant Preparations and Insect Transmission Assay

Thirty of each three–month–old tissue cultured virus-free plantlets of abaca var. Inosa, abaca hybrid BC2–7 (BANDALA), and Pacol were obtained from the Tissue Culture Laboratory of the National Abaca Research Center (NARC-VSU). Inosa was used as the susceptible test variety, abaca hybrid BC2–7 (BANDALA) and Pacol as the resistant test varieties. All the plants were maintained virus-free in the screenhouse before aphid inoculation. Major abaca growing areas in Eastern Visayas with documented BTV infections were surveyed for BTVs. Abaca plants with typical bunchy top symptoms (chlorotic, thinning and curving leaf margins, stiff and upright bunchy leaf appearance) were identified and its vector, black aphids (*P. nigronervosa*), were collected. Four abaca plantations were sampled: VSU (Leyte isolate); Sogod (Southern Leyte isolate); Catarman, Northern Samar (Samar Isolate); and Naval (Biliran isolate). The collected samples were designated as the isolate based on these sources.

Transmission assay was carried out using the viruliferous aphids (*P. nigronervosa*), which were collected from bunchy top infected abaca plants using the protocol described by Parac et al (2020) and Mati-om et al (2022). A portion of the leaf petiole carrying *P. nigronervosa* was detached from the infected plant and colonies of 20 insects were transferred using a camel-hair brush to healthy test plants of Inosa, abaca hybrid BC2–7 (BANDALA) and Pacol at the 3–5 leaf stage for a 4-day inoculation access period (IAP). The test plants were immediately covered by 2.43mx1.21m nylon mesh. At the end of the IAP, test plants were sprayed with insecticides, (Cypermethrin, 5 EC) in accordance with the manufacturer's recommendations. Following the IAP, leaf tissues were collected each day for 14 days at random from each of the test plants, specifically the second and third shoots that were not too young or too old for the early detection of BTVs. Plants were visually

inspected daily for the development of BTVs symptoms for 54 days after inoculation (DAI).

Disease Assessment

At 54 DAI, disease incidence was calculated as the percentage of inoculated plants that were visibly infected. Virus infection was confirmed by detecting the presence of BTVs from DAI-1 to DAI-14 using PCR analysis. The disease incubation period was determined by recording the number of days between the time of inoculation and the appearance of visible symptoms of BTVs. The severity of the disease was determined using the 1–5 rating scale (Niyongere et al 2011), which was as follows: 1–dark green streaks on the leaf veins, 2–dark green streaks on leaf midribs and petioles, 3–marginal leaf chlorosis, 4–dwarfing of leaves and 5–bunchy top aspect: upright, crowded and brittle leaves at the plant apex/death of plants. Based on the rating scale, the disease reaction was categorized as 0 for highly resistant, 1 for resistant, 2 for intermediate, 3 for susceptible, and 4 and 5 for highly susceptible.

DNA Extraction and Virus Detection

The Dellaporta extraction method was used to extract total DNA from abaca plants (Dellaporta et al 1983). The presence of bunchy top virus was detected using the primer pairs BBT–1 and BBT–2 (Thomson & Dietzgen 1995) which were designed to amplify the 349–bp fragment of the DNA–R (replicase gene of the virus). The PCR procedure was carried out using the optimized protocol of Mati-om et al (2022).

RESULTS AND DISCUSSION

Disease Incidence

Inosa showed 100% incidence of disease with all the inoculated BTVs (Table 1). The Leyte isolate was the most virulent of the BTVs tested. The inoculated plants exhibited disease with stiff, upright, crowded, brittle leaves, dwarfed and bunched leaves at the plant's apex, and eventually the plants died. Symptoms caused by the Samar and Biliran isolates included severe bunching/crowding and dwarfing of leaves, stiff leaves, dark green streaks on the veins with marginal leaf chlorosis. The bunchy top symptoms of the Southern Leyte isolate was dark green streaks of variable length in the leaves, midribs and petioles; stiff upright and crowded leaves of the plant's apex. As the disease progressed, infected plants became stunted and malformed with more upright leaves that resulted in a bunchy appearance before dying. On the other hand, no disease incidence was observed on abaca hybrid BC2–7 (BANDALA) and Pacol. Lalusin and Villavencio (2014) manually inoculated and re-inoculated 300 Abaca hybrid BC2–7 BANDALA seedlings with viruliferous aphids. After two months, there was no evidence of bunchy top virus in any of the inoculated seedlings. Parac et al (2020) also reported no BTV incidence on the same hybrid at 1, 3 and 6 months following inoculation, which was confirmed by ELISA and PCR.

Table 1. Disease incidence of BTV isolates on abaca genotypes at 54 DAI

Genotypes	Disease Incidence			
	Biliran	Leyte	Samar	Southern Leyte
Abaca hybrid BC2–7 (BANDALA)	0	0	0	0
Pacol	0	0	0	0
Inosa	100%	100%	100%	100%

Incubation Period

The minimum incubation period of the disease in the Inosa variety ranged from 26–38 days. The Leyte isolate had the minimum incubation period of 26 days, followed by Southern Leyte (30 days), Biliran (32 days), and Samar at 38 days (Table 2). Earlier studies on the incubation period or the disease onset of BBTV was similar ranging from 25–37 days (Wu and Su 1990), 21 days (Hafner et al 1995) 25–85 days (Hooks et al 2008), and 35–42 days (Hooks et al 2009). The shorter incubation period of Inosa variety could be due to genetic differences in BTVs strains present across the region.

Table 2. Incubation period of BTVs isolates in abaca genotypes

Genotypes	Incubation period			
	Biliran	Leyte	Samar	Southern Leyte
Abaca hybrid BC2–7 (BANDALA)	—	—	—	—
Pacol	—	—	—	—
Inosa	32	26	38	30

No BTV symptoms were observed in abaca hybrid BC2–7 (BANDALA) and Pacol plants. However, a mosaic pattern in the leaves of one or two individual plants was observed. Yellowing and mosaic discoloration patterns in the leaves were observed in abaca hybrid BC2–7 (BANDALA), possibly because the aphids were introduced directly into the test plants from the field. Sta Cruz et al (2016) recently demonstrated that disease symptoms were caused by a mixed infection of two or more BTV viruses. Mixed infected plants may show symptoms of a single infection only or a combination of symptoms because of other viruses. For instance, some plants infected with *Banana bunchy top virus* (BBTV) and *Banana bract mosaic virus* (BBrMV), *Sugarcane mosaic virus* (SCMV), or *Cucumber mosaic virus* (CMV) had bunchy top symptoms alone or in combination with mosaic disease symptoms. In PCR analysis up to 14 days after innoculation, no BTVs were detected in abaca hybrid BC2–7 (BANDALA) and Pacol, confirming that the yellowing and mosaic pattern was caused by another virus that the aphid vector may have carried aside from BTV. Kenyon et al (1997) found that *P. nigronervosa* was the more efficient than the other aphid species (*Aphis gossypii* and *Rhopalosiphum maydis*) that transmit the mosaic virus of banana.

Severity of BTVs in Abaca hybrid BC2-7 (BANDALA), Pacol and Inosa

Disease severity of infected plantlets was assessed using a rating scale of 0–5. The Inosa was rated on a scale between 4 and5, indicating dwarfing of leaves up to

bunchy top aspect with upright, crowded and brittle leaves at the abaca plant's apex (Table 3). In addition, Inosa was found to be highly susceptible since it developed the typical symptoms starting at 26–38 DAI. The severity score from Biliran isolate was 4.57, 4.67 from Southern Leyte, 4.73 from Leyte, and 4.77 from Samar. BTVs infection of Inosa was confirmed by PCR analysis, which revealed that the virus was present in both asymptomatic and symptomatic samples from all Inosa test plants. Because Inosa developed severe disease symptoms during the evaluation, the disease pressure was favorable for disease development. The inoculated plants of abaca hybrid BC2–7 (BANDALA) and Pacol did not exhibit typical disease symptoms.The virus was not detectable in any of the abaca hybrid BC2–7 (BANDALA) and Pacol test plants tested by PCR analysis or observed until the end of the 54-day period.

Table 3. Disease severity rating and reaction of genotypes to BTV isolates

Genotypes	Disease severity				Disease reaction
	Biliran	Leyte	Samar	Southern Leyte	
Abaca hybrid BC2–7 (BANDALA)	0	0	0	0	Highly Resistant
Pacol	0	0	0	0	Highly Resistant
Inosa	4.57	4.73	4.77	4.67	Highly Susceptible

General Response of Abaca hybrid BC2-7 (BANDALA), Pacol and Inosa to BTVs in Eastern Visayas

In Inosa, BTVs were detected as early as 5–DAI. The results of PCR analysis were used to compare the development of BTVs (Figure 1). The virus was detected at 5–DAI in Leyte isolate (Figure 1(B): Lane 5) and Southern Leyte isolate (Figure 1(D): Lane 5). These two isolates had the shortest incubation period of 26 and 30 DAI, respectively. BTVs were detected at 6–DAI in Samar isolate (Figure 1(C): Lane 6) and 7–DAI in Biliran isolate (Figure 1(A): Lane 7) with 38–DAI and 32–DAI of incubation period, respectively. Likewise, Inosa variety is highly susceptible based on the response of BTVs. In the screenhouse, Inosa was favorable to BTVs since it develops severe symptoms as well as high disease incidence and severity. The virus was not detectable by PCR and no visible infection developed in all abaca hybrid Bc2–7 (BANDALA) and its parental Pacol test plants, which remained symptomless until 54–DAI (Figure 2).

In this study, the abaca hybrid BC2–7 (BANDALA) was rated as highly resistant, with a response similar to the Pacol (resistant test varieties). Since abaca hybrid BC2–7 (BANDALA) and Pacol did not develop the disease, no incidence, no symptoms observed, and zero disease severity were recorded. This parameter is thought to be useful for determining the response of varieties or lines to virus disease. Furthermore, the absence of BTVs in abaca hybrid BC2–7 (BANDALA) and Pacol indicates a resistant response to BTVs. Using the BBT–1 and BBT–2 primer pairs, abaca hybrid BC2–7 (BANDALA) and Pacol were found to be BTV-free in PCR. Likewise, by amplifying 349–bp (Lane +), the presence of BTVs in all Inosa test plants were detected. This primer pair was designed for the Australian isolate and is used to amplify the DNA–1 (also referred to as DNA–R, replicase) component of all BBTV isolates, regardless of origin (Thomson & Dietzgen 1995, Sta Cruz et al 2016).

Reaction of abaca hybrid BC2-7

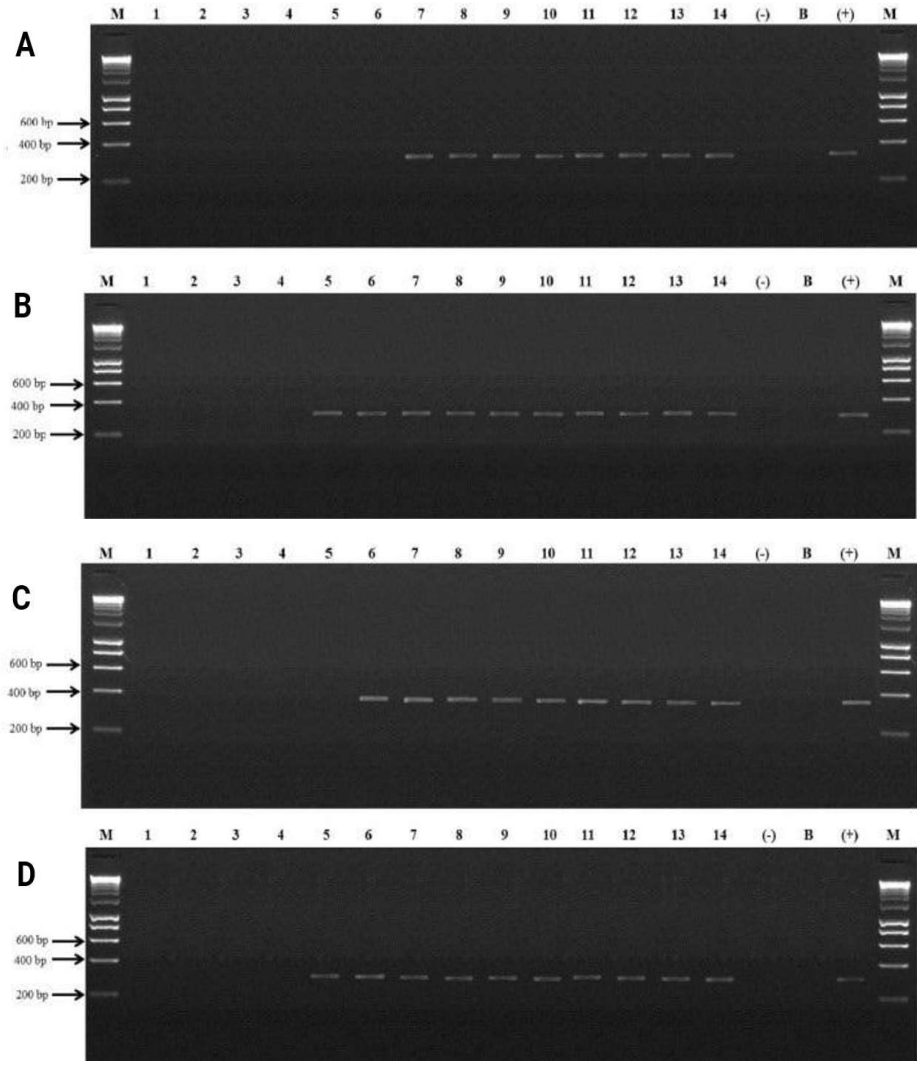

Figure 1. PCR amplified DNAs (349–bp) of BTV isolates using the primer pairs BBT–1 and BBT–2 in Inosa: (A) Biliran isolate (Lane 1–6, BTVs negative, Lane 7–14, BTVs positive) (B) Leyte isolate (Lane 1–4, BTVs negative, Lane 5–14, BTVs positive) (C) Samar isolate (Lane 1–5, BTVs negative, Lane 6–14, BTVs positive) and (D) Southern Leyte isolate (Lane 1–4, BTVs negative, Lane 5–14, BTVs positive). Lane M 1kb DNA ladder (HyperLadder™, Bioline, USA); Lane (–), negative control of BTVs, Lane B– Blank (water only), Lane (+), positive control of BTVs

The findings of this study confirmed the findings of Parac et al (2020), who found that in the screenhouse evaluation, all inoculated abaca Hybrid 2, abaca hybrid BC2–7 (BANDALA) previously known as Hybrid 7 and Pacol test plants were symptomless even when the conditions for disease development were highly favorable. As symptoms as a basis for virus disease diagnosis are generally presumptive or insufficient, early detection at the molecular level is critical for disease management. The abaca hybrid BC2–7 (BANDALA) has been proven to be

highly resistant in the field based on the experimental set-up at NARC-VSU for six years. Parac et al (2020) characterized the resistance of abaca hybrid BC2–7 (BANDALA) and selected hybrids of abaca for bunchy top diseases using ELISA and PCR. They did not detect BTVs in any hybrid test plants which were all symptomless throughout the six–months post inoculation (mpi). Further to that, Piamonte and Sta Cruz (2018) did not detect the virus from field samples using their sensitive and reliable BTVs detection by PCR. The response of the abaca hybrid BC2–7 (BANDALA) can be attributed to resistance itself and it was not due to having escaped infection. The conditions during this evaluation was favorable for the development of the disease because the infection of the susceptible check (Inosa) was severe, where the plant developed severe disease.

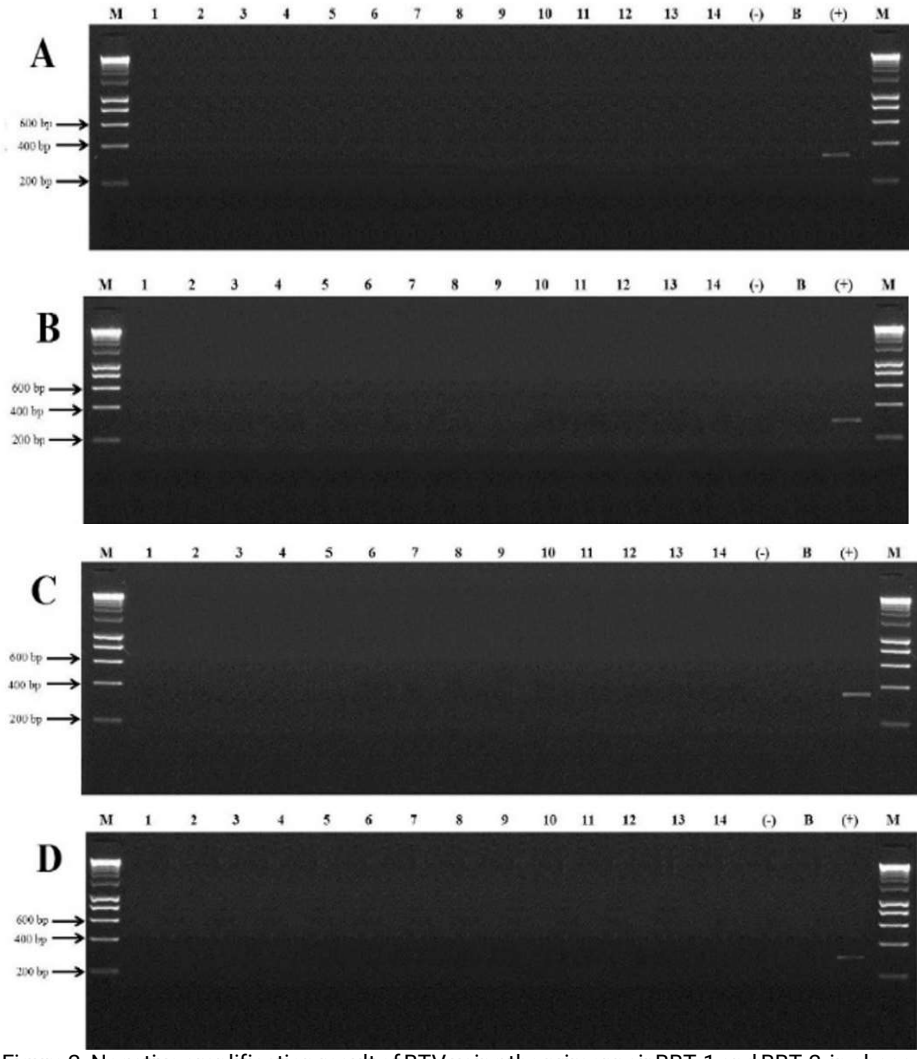

Figure 2. Negative amplification result of BTV using the primer pair BBT-1 and BBT-2, in abaca hybrid BC2-7 (BANDALA): (A) Biliran isolate, (B) Leyte isolate, (C) Samar isolate and (D) Southern Leyte isolate. Lane M 1kb HyperLadderTM (Bioline, USA); Lane 1 (1DAI) - 14 (14 DAI) negative of BTV. Lane (–) negative control of BTVs, Lane B Blank (water only), Lane (+) positive control of BTV

CONCLUSION

The response of abaca hybrid BC2–7 (BANDALA) and Pacol were highly resistant to different BTVs isolates infecting abaca in the Eastern Visayas region of the Philippines, while the Inosa was highly susceptible to the BTVs, based on the disease incidence, incubation period and severity assessment. The resistant response of the abaca hybrid BC2–7 (BANDALA) was similar to Pacol, which did not acquire the disease in the screenhouse experimental conditions. In terms of disease incidence, BTVs were transmitted to 100% of the Inosa test plants by its vector *P. nigronervosa*, and no infection was recorded in both the abaca hybrid BC2–7 (BANDALA) and Pacol. The results also suggest that the shortest incubation period was 26–DAI and the longest at 38–DAI for the susceptible variety (Inosa). The virus was detectable at 5–7 DAI in Inosa by PCR analysis. These were evaluated under the conditions of high disease pressure, indicating that the observed responses of BC2–7 (BANDALA) and Pacol were due to resistance, and not due to escape from infection. The absence of virus infection and bunchy top disease symptoms in these plants is also indicative of disease resistance. PCR analysis revealed that until 54–DAI, all test plants of abaca hybrid BC2–7 (BANDALA) and Pacol were BTVs free.

The reaction of this abaca hybrid BC2–7 (BANDALA) to the *Abaca mosaic virus* (AMV) must be evaluated. In general, the use of abaca hybrid BC2–7 (BANDALA) which is highly resistant to BTVs, will undoubtedly contribute to the long-term viability of the Philippine abaca industry. This will also aid in proper field deployment of other promising abaca resistant lines in the region as well as the development of screening and selection strategies. To summarize, abaca hybrid BC2–7 (BANDALA) can be planted for pulp production in the Philippines' Eastern Visayas region.

ACKNOWLEDGMENT

We thank the National Abaca Research Center Biotechnology Laboratory for providing the facilities used in conducting the experiments, and we also appreciate the support of the Graduate Research and Education Assistantship for Technology (GREAT) and the Thesis Dissertation Program of the Department of Science and Technology – Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development (DOST-PCAARRD).

AUTHOR CONTRIBUTIONS

JAM conducted the study, performed the analysis, curated the data, and wrote the original draft. RTP handled the review and editing, while RMG contributed to the study's conceptualization and provided the final review and editing.

FUNDING SOURCE

This work was funded by DOST-PCAARRD under the program "Revitalizing the Abaca Industry through S&T Interventions for Higher Crop Productivity Using High-Yielding and Bunchy Top-Resistant Abaca Hybrids”.

AVAILABILITY OF DATA AND MATERIALS

The data required are presented in the paper and supplementary materials. For any questions regarding the data, interested readers should contact directly the corresponding author.

ETHICAL CONSIDERATION

This article does not contain any studies with human participants or animals performed by any of the authors.

COMPETING INTEREST

The authors declare that they have no conflict of interests.

REFERENCES

Armecin RB, Sinon FG & Moreno LO. 2014. Biomass and Bioenergy—Applications. Springer. Chapter 6. Abaca Fiber: A renewable bio-resource for industrial uses and other applications (pp343–352). ISBN: 978-3-319-07577-8

Bajet NB and Magnaye LV. 2002. Virus diseases of banana and abaca in the Philippines. Philippine Agriculture and Resource Research Foundation, Inc. (pp82). Los Baños, Laguna

Boguero APB, Parducho MAL, Mendoza MR, Abustan MAM & Lalusin AG. 2016. Molecular screening of abaca (*Musa textilis* Nee) accessions using microsatellite markers associated with resistance to bunchy top virus. *Philippine Journal of Crop Sciences* 41(2):13–19

Dellaporta SL, Wood J & Hicks JB. 1983. A plant DNA minipreparation: Version II. Plant *Molecular Biology Reporter* 1(4):19–21

Food and Agriculture Organization (FAO). 2021. Future fibers: Abaca. [accessed 2022 July 15] https//www.fao.org/economic/futuresfibres/fibres/abaca0/en/

Hafner GJ, Harding RM & Dale JL. 1995. Movement and transmission of *Banana bunchy top virus* DNA component one in bananas. *Journal of General Virology* 76:2279–2285

Hooks CRR, Wright MG, Kabasawa R & Almeida RPP. 2008. Effect of *Banana bunchy top virus* infection on morphology and growth characteristics of banana. *Annals on Applied Biology* 153(1):1–9

Hooks CRR, Manandhar R, Perez EP, Wang KH & Almieda, RPP. 2009. Comparative susceptibility of two banana cultivars to *Banana bunchy top virus* under laboratory and field environments. *Journal of Economic Entomology* 102(3):897–904

Hull R and Davis JW. 1992. Approaches to nonconventional control of plant virus diseases. *Critical Reviews in Plant Sciences*. 11:17–33

Kenyon L, Brown M & Khonje P. 1997. First report of *Banana bunchy top virus* in Malawi. *Plant Disease* 81(9):1096

Lalusin AG and Villavencio ML. 2014. Industrial Crops. Handbook of Plant Breeding Industrial Crops. Springer. Chapter 9. Abaca (*Musa textilis* Nee) Breeding in the Philippines (pp265-289). ISBN: 978-1-4939-1446-3

Magee CJ. 1927. Investigation on the bunchy top disease of the banana. *Council for Scientific and Industrial Research* (pp87). Melbourne, Australia

Mati-om JA, Mati-om MB & Piamonte RT. 2022. PCR optimization for the detection of bunchy top virus of abaca (*Musa textilis* Nee) in Eastern Visayas Philippines. *Annals of Tropical Research* 44(1):61–73

Niyongere C, Ateka E, Losenge T, Blomme G & Lepoint P. 2011. Screening *Musa* genotypes for banana bunchy top disease resistance in Burundi. *Acta Horticulturae* 897:439–448

Nuñez JMC. 2013. Social Impacts of the Abaca Bunchy Top Disease and Adaptive Strategies of Farm Households: A case in Leyte, Philippines. *Annals of Tropical Research* 35(2):69–87

Parac EP, Lalusin AG, Pangga IB & Sta Cruz FC. 2020. Characterization of selected Hybrids of Abaca (*Musa textilis* Nee) with resistance to bunchy top and mosaic diseases. *Philippine Agricultural Scientist* 103(1):1–12

Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD).2017. Annual Report. Los Baños, Laguna: DOST-PCAARRD. (pp117)

Philippine Fiber Industry Development Authority (PhilFIDA). 2012. PhilFida; Abaca plantation in Bicol Region up for rehab. [accessed 2017 March 12]. http://www.philfida.da.gov.ph/images/Publications/ABACA.pdf

Philippine Fiber Industry Development Authority (PhilFIDA). 2015. PhilFida; Abaca Technoguide. [accessed 2015 January 15] http://www.philfida.da.gov.ph/images/Publications/Technoguides/ABACA.pdf

Piamonte RT, Sta Cruz FC. 2018. Development of sensitive and reliable detection of Banana bunchy top virus in abaca (*Musa textilis* Nee) by Polymerase Chain Reaction. *Phil J of Crop Sci*. 43(2):12–19

Raymundo AD, Cipriano BP, Garcia SI, Boromeo RT, Tapalla PB. 2002. Economic losses in abaca due to bunchy top and mosaic virus diseases in the Bicol and Eastern Visayas regions. *J of Trop Plant Patho*. 38:31–34

Sharman M, Thomas JE, Holton TA. 2008. *Abaca bunchy top virus*, a new member of the genus *Babuvirus* (family *Nanoviridae*). *Archives of Virology* 153(1):135–147

Sta Cruz FC, Glenrose JB & Alviar AN. 2016. Serological and molecular detection of mixed bunchy top and mosaic virus infection in Abaca (*Musa textiles* Nee). *Philippine Agricultural Sciences* 99:(1)88–98

Thomson JE and Dietzgen RG. 1995. Detection of DNA and RNA plant viruses by PCR and RT-PCR using a rapid virus release protocol without tissue homogenization. *Journal of Virology Methods* 54(2-3):85–95

Vetten HJ, Chu PWG, Dale JL, Harding R, Hu J, Katul L, Kojima M, Randles JW, Sano Y & Thomas JE. 2005. Virus Taxonomy. Eighth report of the International Committee on Taxonomy of Viruses. Elsevier. (*Nanoviridae)* (pp343–352) ISBN:9780122499517

Wu RY and Su HJ. 1990. Transmission of *Banana bunchy top virus* by aphids to banana plantlets from tissue culture. *Botanical Bulletin of Academia Sinica* 31:7–10