



Resistance Reaction of Abaca (*Musa textilis* Nee) Hybrids to Bunchy Top and Establishment of Disease Severity Rating Scale for Screenhouse Screening

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Received: September 23, 2021

Revised: December 12, 2021

Accepted: December 30, 2021

Available Online: December 31, 2021

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Cite this article: Parac, E.P., Sta. Cruz, F.C. & Lalusin, A.G. (2021). Resistance Reaction of Abaca (*Musa textilis* Nee) Hybrids to Bunchy Top and Establishment of Disease Severity Rating Scale for Screenhouse Screening. *Journal of Ecosystem Science and Eco-Governance*, 3(2):18-26.

ABSTRACT

Two abaca hybrids, namely, Hybrid 2 and Hybrid 7, which were derived from a cross between the resistant wild banana ‘Pacol’ and the susceptible ‘Abuab’ abaca, were evaluated for resistance to bunchy top disease. The response of these hybrids to bunchy top on abaca in the screenhouse was assessed and compared with the parental varieties, Pacol (*Musa balbisiana*) and Abuab, and susceptible varieties, ‘Inosa’ and ‘Tinawagang Pula’. Two-month-old tissue-culture-derived plantlets that had been tested to be virus-free were mechanically inoculated with viruliferous aphid *P. nigronervosa*, and the infection was observed for six months. Hybrid 7 did not develop the bunchy top disease throughout the six-month observation period, while Hybrid 2 developed the disease in only 1 of 15 test plants over the six-month observation period. Hybrid 2 did not show any symptom of bunchy top from 1 - 3 months of post-inoculation (mpi), but symptoms of dark green streak on the leaf veins were observed at six mpi, and later the leaves became narrow. Both susceptible and parental (Abuab) varieties showed typical symptoms of bunchy top at 1-month post-inoculation, while Hybrid 2 had delayed symptom development of bunchy top at 6-month post-inoculation. The resistance was observed under conditions of high disease pressure wherein the susceptible control varieties, “Tinawagang Pula” and “Inosa,” and the parental Abuab developed severe disease characterized by early disease onset and high disease incidence. A symptom severity rating scale of 1-9 was established in this study. The rating scale represented the pattern on infected plants starting with the development of dark green streaks on the leaf veins and midribs followed by marginal chlorosis and narrowing of leaves, and then the plants became bunchy in appearance characterized by crowding of leaves at the apex and stunting. Knowledge of the response of these hybrids would be useful in designing for the development and proper deployment of virus-resistant plants in the field.

Keywords: *Bunchy top, severity rating scale, abaca hybrids, bunchy top resistance*

1 Introduction

Musa textilis Nee, also known as the ‘Manila hemp’, is a succulent annual plant similar to banana and an extremely important crop in the Philippines-

a country that is recognized as the biggest supplier of abaca products worldwide. It is primarily grown for its strong and flexible fiber, three times stronger

than cotton and twice as strong as sisal fibers (Armeccin et al. 2014). As the country's longstanding export commodity, abaca fiber also serves as a basic material for a variety of fabrics and yarns and many other uses, including banknotes, lens-cleaning tissue, tea bags, meat casing, vacuum cleaner, brushes, and capacitor (PCAARRD 2013; PhilFIDA 2015). Due to the current interest in biodegradable products and forest conservation, it is expected that the abaca industry will continue to flourish in both domestic and international markets.

The abaca industry has maintained its status as the world's largest producer accounting for 85% share of world exports, and it continues to maintain a strong position in both international and domestic markets, generating 113.33 M US \$ annually (PhilFIDA 2016). About 85% of the total world fiber comes from the Philippines, while 15% was supplied by South America (PCARRD 2013). It is exported raw, manufactured, or as finished fiber craft. Thus, it becomes one of the priority agricultural commodities of the Department of Agriculture (PhilFIDA 2015).

Despite its high market and being the prime export commodity, abaca experiences a decline in production. Constraints that bring to the industry's declining productivity include poor technology adoption of farmers, low input production practices, lack of clean, high-yielding planting materials, and the prevalence of diseases primarily by virus diseases, particularly bunchy top (Raymundo and Bajet 2000). The abaca bunchy top wiped out several abaca plantations in the Bicol Region, Mindanao, and Eastern Visayas Region. Plants infected with this virus ultimately become unproductive and have to be disposed of to eliminate the virus and prevent further infection.

The bunchy top disease of abaca, in particular, showing similar symptoms as the banana bunchy top disease (BBTD), is prevalent and restricted to the Philippines (Sharman et al. 2008; Dizon et al. 2012). The disease is caused by the *Banana bunchy top virus* (BBTV) and is transmitted persistently by the banana aphid, *Pentalonia nigronervosa* Coq. (Magee 1953). The occurrence of abaca bunchy top in the country was first reported in 1915 (Ocfemia 1926; Bajet and Magnaye 2002) and was also observed in abaca plantations in Silang, Cavite in 1915. However, it was not a serious threat to abaca cultivation until 1923 (Ocfemia 1934). Since that time, increased virulence caused

the abandonment of plantations in the provinces of Cavite and Laguna (Paete), where they are no longer grown today. In 1928, the disease wiped out 12,000 ha in Cavite, Batangas, Laguna (Calinisan 1938) and spread towards the Bicol Region, shutting down the abaca industry in Southern Tagalog in the 1940s (Magnaye 1989).

A bunchy-top is currently widespread in abaca-growing areas and is considered the most destructive of abaca disease because the infected plants are severely stunted and are unproductive. Nationwide, an estimated 22.5 ha abaca area is affected by the disease out of 121.8 ha (PhilFIDA 2016; DA-PRDP 2017). The biggest areas affected are in Eastern Visayas, with 10.1 ha. Production of abaca has been significantly reduced due largely to the use of infected suckers and corm pieces. The estimated loss in fiber yield in the Bicol region as a result of the widespread occurrence of bunchy-top is enormous. On a region-wide mean disease incidence of 5.19%, the estimated yield loss in fiber yield is 833.5 kg valued at Php18.3M (Raymundo 2002). The province of Sorsogon appeared to be the hardest hit with an equivalent loss of Php9.4M. Although Catanduanes has an average disease incidence of only 2.83%, lower than those observed in Albay and Camarines Sur, it came in second in terms of Php5.8M loss because of the large hectareage planted to abaca. In Eastern Visayas, the estimated fiber loss is 312 kg with a value of Php8.4M. Northern Samar and Northern Leyte sustained the highest losses, with estimated fiber losses of 153.1M and 116.2 kg valued at Php3.8M and Php3.4M, respectively. Southern Leyte has the lowest estimated loss due to the smaller area affected. The disease continues to be destructive to abaca plantations despite decades of research toward its management.

An early symptom of a bunchy top includes the indefinite yellowish-white area in the furled leaf. Yellowing also occurs in the margin and on the lamina of the youngest unfurled leaf (Sta. Cruz et al. 2016). The associated dark-green appearance of the infected leaves and the restricted growth of the youngest emerging leaf noticeable as early as 14-18 days after inoculation are the early diagnostic symptoms of the disease (Raymundo and Bajet 2000; Dizon et al. 2012). Advanced symptoms include severe stunting, dying of leaves, and death of the plant. Under field conditions, the severely infected plant rarely produced a meter-long pseudostem. Undersized suckers may be produced with small

leaves, narrow, stiff, curled up with chlorotic edges and arranged in rosette formation, and progressed to the advanced symptoms (Raymundo and Bajet 2000; Sta. Cruz et al. 2016). The disease spread through the movement of the aphid vector. The transmission of the virus by aphids is confined to a short distance, and the mean distance of new infections from their source of inoculum in an established plantation was estimated at 17.2 m (Robson et al. 2006). The movement of infected planting material is the major mechanism for disease spread over long distances across areas and countries (Thomas and Caruana 1994; PhilFIDA 2015).

Since 1992, the Fiber Development Authority (FIDA), now PhilFIDA, spent millions of pesos to control the disease and help farmers rehabilitate their farms. The rehabilitation program is part of the product support services by FIDA, which includes the development of new abaca areas, production of planting materials, facility upgrading, and abaca disease management project. The disease management project started in 2009 to eradicate abaca farms infected with abaca viral diseases. In support of the planting material requirements of abaca farmers for rehabilitation and expansion of their farms, the project also involves mass production of virus-free planting through tissue culture (FIDA 2012). Another effort done by the government in solving the problem was on the varietal improvement through plant breeding. Plant improvement, either by natural selection or through the efforts of breeders, has always relied upon creating, evaluating, and selecting the right combination of alleles. A number of abaca varieties with high fiber quality and virus resistance were developed.

Currently, the group of researchers from the Institute of Plant Breeding of the University of the Philippines (IPB-UPLB), headed by Dr. Antonio Lalusin, has successfully developed abaca hybrids (BC2) (Lalusin et al. 2006 as cited by Lalusin and Villavencio 2014). These hybrids (BC1) are a cross between Pacol (wild banana) x Abuab (abaca) (Umali and Fernando 1950). The genes that are linked to resistance in Pacol and fiber quality in Abuab have been identified through the use of molecular markers. The development of bunchy top disease on these hybrids, however, has not been fully studied, and the rating scale to assess the disease severity has not also fully developed. Hence, the study aimed to determine the

development of bunchy top disease on the two resistant abaca hybrids and establish a reliable disease rating for screening resistance to abaca bunchy top.

2 Materials and Methods

Plant Materials

Tissue-culture-derived plantlets were used in the greenhouse experiment. Two-month-old tissue-culture-derived plantlets of abaca hybrids (Hybrid 2 and Hybrid 7) were obtained from the Tissue Culture Laboratory of Caraga State University, Ampayon, Butuan City. Pacol (*M. balbasiana*) and Abuab (*M. textilis*), where the hybrids originated, were included as control. Tinawagang Pula and Inosa were also included as susceptible control. Inosa was obtained from the Tissue Culture Laboratory of the Institute of Plant Breeding of the University of the Philippines, Los Banos (IPB-UPLB), while Abuab, Pacol, and Tinawagang Puti from the Tissue Culture Laboratory of the National Abaca Research Center (NARC), respectively. These were maintained in the greenhouse prior to inoculating viruliferous aphid vectors.

Virus inoculation

Aphids feed on bunchy-top infected plants in the field were collected and used directly to inoculate trial plants (Niyongere 2012). Approximately 20 aphids were transferred to each test plant by removing leaf or petiole tissue from the bunchy-top infected plant on which aphids were feeding and transferring this tissue onto the test plant. Transmission assays were performed similarly as described by Lalusin et al. (2017).

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).

Disease assessment for bunched top development and rating scale establishment

In the screenhouse evaluation, test plants were monitored for the development of the bunched top disease, which was measured using various parameters (disease onset and disease incidence).

The development of bunched top disease on abaca hybrids was monitored at one month post-inoculation (mpi) until six mpi in the screenhouse. The response of the two hybrids was compared to the parental and susceptible varieties. The response of each variety was scored using the symptom severity rating scale that was established in this study. Virus infection was confirmed by detecting the presence of BBTv by Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) analysis.

3 Results and Discussion

Development of Bunched Top Disease in Abaca Disease Onset and Disease Incidence

The incidence of bunched top infection on inoculated plants was determined by visual observation of symptoms, which was confirmed by detecting the presence of BBTv by PCR. Using the results of PCR analysis, the development of bunched top disease was compared (Figure 1). The infection did not develop in Hybrid 7 starting at 1 mpi until the end of the observation period at 6 mpi. The development of the disease on inoculated abaca hybrids, their parentals, and the susceptible control followed a similar pattern but varied on symptom severity. Regardless of variety, each symptom expressed during the development of the disease indicated differences in symptom severity.

Generally, infected plants develop dark green streaks on the leaf veins and midribs followed by marginal chlorosis and narrowing of leaves, and then the plants become bunched in appearance characterized by crowding of leaves at the apex and stunting.

During the six-month observation period, Hybrid 7 did not show any symptoms of bunched top disease (Figure 2). Hybrid 2 did not show any symptoms of bunched top from 1 -3 months post-inoculation (mpi) but symptoms of dark green streak on the leaf veins at six mpi. Later, leaves became narrow. The expression of symptoms in Hybrid 2 was delayed compared to susceptible varieties (Figure 3).

On the other hand, the parental variety, Abuab, showed early disease symptom of dark green streaks on leaves at one mpi and then followed by moderate marginal chlorosis on leaves and formation of narrower and stiffer leaves at three mpi which later developed into an upright, crowded and brittle leaves at the apex of the plant at six mpi (Figure 4). The other parental, Pacol, did not show any symptom of bunched top for the six months of observation (Figure 5).

In the susceptible variety, Inosa, a dark green streak developed on leaf veins at one mpi. Later leaves became narrow and stiff at three mpi. At six mpi, severe stunting of growth was already observed, accompanied by crowding and dwarfing of leaves at the apex (Figure 6). At one mpi, dark green streaks on the leaf veins were first observed in Tinawagang Pula which later developed into severe marginal chlorosis accompanied by narrowing and stiffening of leaves at three mpi. At six mpi, severe stunting of growth was observed in most inoculated plants (Figure 7).

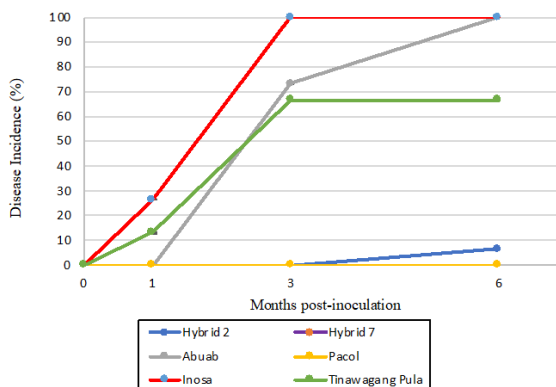


Figure 1. Development of bunched top disease in abaca hybrids, parentals, and susceptible control varieties in screenhouse evaluation.

During the disease development, the hybrids exhibited a varied response to the bunchy top, their parental and susceptible control varieties. As a result, a range of symptoms can occur in bunchy-top infected plants according to the time and the growth stage of the plant (Figures 8-10). Regardless of variety, each of the symptoms expressed during the development of the disease indicated differences in symptom severity. For example, during the 6-month observation period, Hybrid 2, unlike Inosa and Tinawagang Pula, did not show any advanced symptoms of severe marginal chlorosis and severe bunchy top appearance accompanied by dwarfing and crowding of leaves at the apex and stunted growth. The susceptible variety, Inosa, showed symptoms of dark green streaks on leaf veins and midrib at one mpi, and bunchy appearance with narrow and stiff leaves was observed at three mpi. Severe bunchy top characterized by stunted growth and dwarfing of leaves at the apex was already visible at six mpi. Tinawagang Pula, on the other hand, showed symptoms of dark green streak on leaf veins and midrib at one mpi and at three mpi, a bunchy top appearance with narrow, stiff, and crowded leaves appeared. At six mpi, bunchy top accompanied by stunting and dwarfing of leaves at the apex were most severe. Both Pacol and Hybrid 7 did not develop symptoms of bunchy top throughout the six months of observation.

The parental “Abuab” variety showed a dark green streak on leaves and leaf veins starting at one mpi until the leaves become narrow and stiff at three months post-inoculation. Severe bunchy top symptoms with an upright, crowded and brittle leaves at the apex of the plant were observed at six mpi. Thus, at any one time, the most severe symptom was expressed by either at 3 or 6 mpi, which was exhibited by the two susceptible varieties.

Sta. Cruz et al. (2012) reported the same observation on the pattern of symptom development in Papaya ringspot virus (PRSV) infected Sintia and Cariflora. The symptom development on Sintia and Cariflora infected with PRSV followed a similar pattern with the susceptible variety, Solo, wherein the early symptom of mottling was least severe while leaf deformation and shoe stringing were most severe.

Generally, Hybrid 2, the two susceptible varieties, Inosa and Tinawagang Pula, and one parental variety, Abuab, had almost the same severity of symptom development. The disease development of Hybrid 2 was delayed compared

to the susceptible and parental varieties. Both susceptible and parental varieties show typical symptoms of bunchy at 1-month post-inoculation, while Hybrid 2 showed symptom of bunchy top at 6-month post-inoculation. The Hybrid 7 and parental, Pacol showed complete resistance to bunchy top, which was observed throughout the trial. This conforms to the findings of Lalusin et al. (2014) that when Hybrid 7 was exposed to a bunchy top-infected field, it showed resistance to the disease. Pacol, on the other hand, has already been identified in the 1950s by the CSC-IPB, UPLB, as a source of resistance to bunchy top diseases. Moreover, the resistance of Hybrid 7 and Pacol was confirmed through PCR and ELISA analysis (Parac et al. 2020).

Development of Symptom Severity Rating Scale for Abaca Bunchy Top Resistance Screening

Currently, no available rating scale is used to assess the symptom severity for abaca bunchy top. However, in banana, Niyongere et al. (2011) and Boloy et al. (2014) established a disease severity score for banana bunchy top. Niyongere used a rating scale of 0 to 5 with the higher ratings indicating increasing severity of the disease such as: 0 – symptomless; 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; 5- “bunchy top, upright, crowded, brittle leaves at the apex of the plant. Boloy (2014), on the other hand, used a disease severity rating scale of 0 to 5 in assessing incidence, development, and distribution of banana bunchy top disease wherein 0- no symptoms, 1- dark green streaks on the leaf lamina, 2- dark green streaks on the leaf petiole, 3- chlorosis of the leaf margins, 4- reduction in leaf size and 5- bunchy top appearance.

Both rating scales were not used in this study because their rating scales were intended for the



Figure 2. Hybrid 7 at one month post-inoculation (mpi) without bunchy top symptoms.

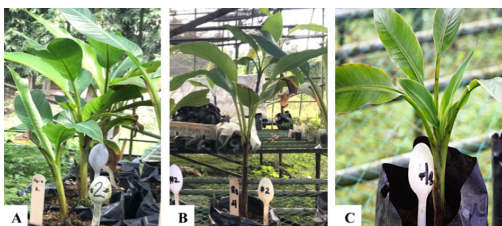


Figure 3. Symptom development of bunchy top disease in Hybrid 2: A-B) no visible symptom at one and three months post-inoculation (mpi); C) mild bunchy appearance with narrow and stiff leaves at six mpi.

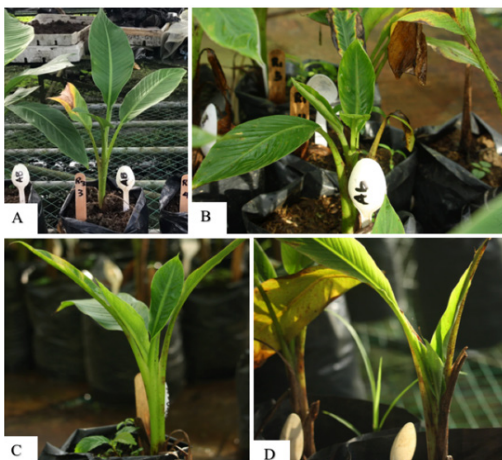


Figure 5. Symptom development of bunchy top disease in parental, Abuab variety: A-B) dark green streaks on leaf veins and midribs at one-month post inoculation (mpi); C) bunchy appearance with narrow and stiff leaves at three mpi; D- moderate bunchy top appearance with upright, crowded and brittle leaves at the apex at six mpi.

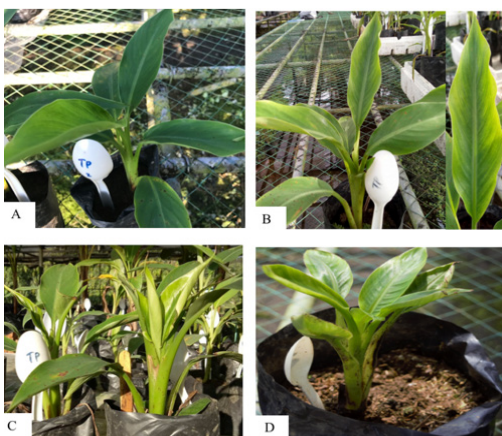


Figure 7. Symptom development of bunchy top disease on susceptible Tinawagang Pula: A-B) dark green streak on the leaf veins and midribs at one mpi; C) bunchy top with narrow, stiff, and crowded leaves at three mpi; D) severe bunchy top accompanied by stunting and dwarfing of leaves at the apex of the plant at six mpi.

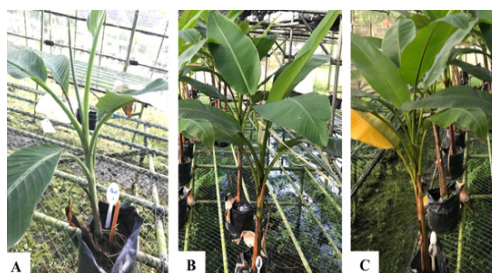


Figure 4. Pacol at one month post-inoculation (mpi) without bunchy top symptoms.

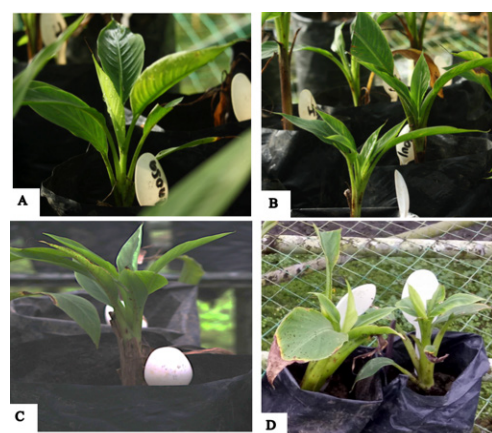


Figure 6. Symptom development of bunchy top disease on susceptible Inosa variety: A) dark green streaks on leaf veins and midribs at one month post-inoculation (mpi); B) bunchy appearance with narrow and stiff leaves at three mpi; C-D) severe bunchy top characterized by stunted growth and dwarfing of leaves at the apex of the plant at six mpi.

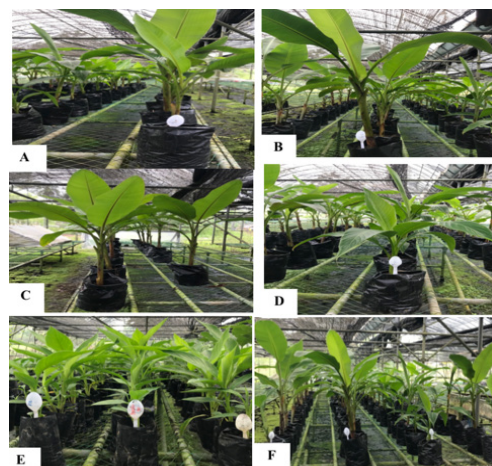


Figure 8. Symptom development of abaca hybrids and their parentals and susceptible control varieties at one month post-inoculation: (A-C) Hybrid 2, Hybrid 7 and resistant parental, Pacol showing no symptoms of bunchy top disease; D-F) Parental, Abuab, susceptible control varieties, Inosa and Tinawagang Pula.

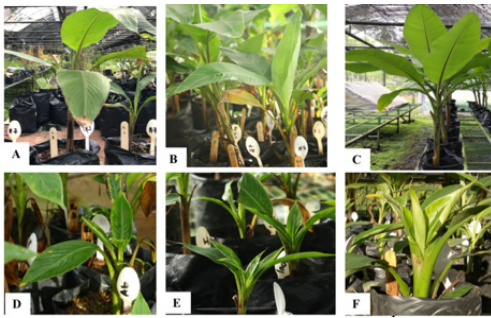


Figure 9. Symptom of abaca hybrids and their parentals and susceptible control varieties at three months posts inoculation: (A-C) Hybrid 2, Hybrid 7, and Pacol showing no symptoms of bunchy top disease; D) marginal chlorosis with moderate narrowing of leaves in Abuab; E) bunchy top showing erect and narrow leaves in Inosa; F) bunchy top symptoms accompanied by crowding of leaves at the apex of TP plants.

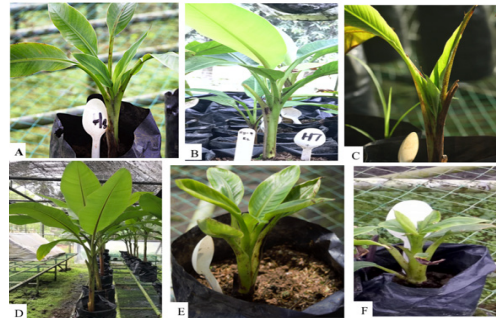


Figure 10. Symptom development of abaca hybrids and susceptible varieties at six months posts inoculation (mpi): A) Hybird 2 with bunchy top symptom such as narrowing of leaves at the apex of the plants; B) Hybrid 7 showed no development of bunchy top symptoms; C) Abuab with bunchy top symptom such as crowded of leaves at the apex; D) Pacol with no visible symptoms of bunchy top; E) severe bunchy top symptoms, stunted growth in TP variety; F) stunted and dwarf leaves in Inosa variety.

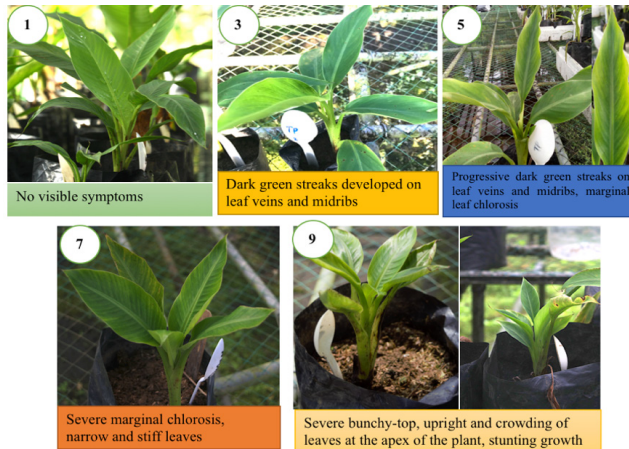


Figure 11. Representations of the rating scale (1-9) for assessing the symptom severity of abaca bunchy top under screenhouse condition.

Table 1. The symptom severity rating scale developed in this study based on the development of symptom severity of abaca bunchy top disease.

Score	Types and severity BBT symptoms
1	No visible symptoms
3	Dark green streaks on leaf veins
5	Progressive dark green streaks on leaf veins and midribs, marginal leaf chlorosis
7	Severe marginal chlorosis, narrow and stiff leaves
9	Severe bunchy-top, upright and crowding of leaves at the apex of the plant, stunted growth

development of bunchy top symptoms in banana under field conditions. Moreover, the rating scales established by Niyongere and Boloy had a range of symptoms on the leaf petioles, margins, and lamina, which were not observed in an infected abaca.

Hence, the symptom severity rating scale that was established in this study was patterned, with some modifications, from the rating scale developed by Niyongere et al. (2012) and Boloy et al. (2014).

The symptom severity was scored based on

bunchy top disease symptoms expressed by the plants during the course of disease development using a rating scale of 1-9 (Table 1). The severity scores 1, 3, 5, 7, and 9 were used in this study wherein ss = 1 (no visual symptom of BBTv), ss = 3 (dark green streaks on the leaf veins), ss = 5 (progressive dark green streaks in leaf veins and midribs, marginal leaf chlorosis), ss = 7 (severe marginal chlorosis, narrow and stiff leaves), and ss = 9 (bunchy-top with upright and crowding of leaves at the apex, stunted growth).

The plants were scored starting one month to six months of post-inoculation in order to know the right time to assess symptom severity rating. Generally, the scores of 7 to 9, which are characterized by severe marginal leaf chlorosis with narrowing of leaves to severe bunchy top appearance characterized by crowding of the leaves at the apex and stunted growth, were more frequent than the scores of 3 or 5, which represent the initial symptoms manifested by dark green streak on the leaf veins and midribs.

Moreover, the symptom severity on the leaves, which is easily observable, becomes the basis for assessing the disease (Figure 11). Each infected plant was assessed at 1, 3, and 6 mpi, respectively, for the severity of symptoms based on the severity rating scale developed. The rating scale developed in this study is the first rating scale for assessing symptom severity of bunchy top in abaca.

4 Conclusion and Recommendations

The resistance reaction of Hybrid 2 and Hybrid 7 to abaca bunchy top was characterized. During the six-month observation period, Hybrid 7 did not develop the bunchy top disease and was considered resistant. Hybrid 2 did not show any symptom of bunchy top from 1-3 months post-inoculation (mpi), but symptoms of dark green streak on the leaf veins were observed at 6 mpi, and later the leaves became narrow. The expression of symptoms in Hybrid 2 was delayed compared to susceptible varieties and parental, Abuab. Both susceptible and parental (Abuab) varieties showed typical symptoms of bunchy top at 1-month post-inoculation, while Hybrid 2 had developed symptom of bunchy top at 6-month post-inoculation. The Hybrid 7 and parental, Pacol showed complete resistance to bunchy top, which was observed throughout the evaluation. A variety can be considered resistant

when virus multiplication in plant is reduced or the spread of virus is prevented, resulting in reduction or absence of symptoms. Screenhouse evaluation showed that Hybrid 7 did not develop the bunchy top disease throughout the six-month observation period. Hybrid 2 developed mild disease characterized by delayed disease onset and lower percent disease incidence compared to the susceptible varieties, Inosa and Tinawagang Pula. These hybrids were found negative for the presence of the virus by polymerase chain reaction (PCR) analysis.

Symptom severity rating scale of 1-9 was also established in this study, starting from the development of dark green streaks on the leaf veins and midribs to the development of marginal chlorosis accompanied by narrowing and stiffening of leaves and severe bunchy top symptoms with upright and crowding of leaves at the apex and severe stunting as the highest rating score. The establishment of an appropriate symptom severity rating scale is crucial in screening for bunchy top resistance.

5 Acknowledgement

The authors would like to thank the DOST-SEI for the financial support and the Tissue Culture Laboratory of the National Abaca Research Center for providing the abaca varieties, Abuab and Tinawagang Pula.

Statement of Conflict of Interest

The authors declare no conflict of interest.

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