Resistance of *Musa balbisiana* accessions of the Philippines to *banana bunchy top virus*

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Abstract

Mitigation of banana bunchy top disease (BBTD) is still a challenge worldwide. BBTD is caused by banana bunchy top virus (BBTV), the most important virus affecting banana. Currently, no cultivar or accession of banana has complete resistance to BBTD. A total of 36 wild *Musa* spp. accessions, including 34 *Musa balbisiana* and two *M. acuminata* subsp. errans ('Agutay'), were screened for resistance against BBTV. In greenhouse tests using viruliferous banana aphids *(Pentalonia nigronervosa*), all *M. balbisiana* accessions remained symptomless and BBTV was not detected in any of these plants by PCR at three- and six-months post inoculation. In contrast, 100% disease incidence was recorded in *M. acuminata* subsp. *errans* and in cv. Lakatan susceptible control plants. The PCR-negative *M. balbisiana* plants were then transferred to a field with high BBTV inoculum pressure where they remained symptomless and PCR-negative for up to five years, while all cv. Lakatan developed BBTD. This study confirmed the resistance of wild *M. balbisiana* accessions to BBTV. It is therefore expected to provide a resource for conventional and marker assisted breeding.

Introduction

Banana bunchy top disease (BBTD) continues to spread and affect banana production worldwide. Recent records include the first reports of BBTV from Tanzania (Mpoki et al., 2021; and Uganda (Ocimati et al., 2021). The occurrence of this disease in banana cropping areas planted with susceptible varieties can result in a total yield loss (Qazi, 2015). Once infected with BBTV, plants rarely recover. Banana bunchy top virus (BBTV; genus *Babuvirus*, family *Nanoviridae*) is the causal agent of BBTD and its genome comprises six circular ssDNA components (Harding et al., 1991; Xie & Hu, 1995). The major vector is the banana aphid or *Pentalonia nigronervosa* (Dela Cueva et al., 2015; Thomas, 2008). Long-distance transmission is attributed to the movement of infected planting materials (Thomas et al., 1994).

BBTV occurs widely in Africa, Asia, and the Pacific and is difficult to control. Common strategies against the disease are quarantine, eradication of infected plants, and the use of clean planting material (Thomas, 2019). Widely used control practices in the Philippines is eradication of infected plants using bamboo sticks dipped in herbicide, and the use of clean planting materials (Aguilar et al., 2010; Molina et al., 2009). A number of studies have explored the potential of transgenic resistance to BBTV in bananas (Borth et al., 2011; Shekhawat et al., 2012; Elayabalan et al., 2013; Mware et al 2016) but to date, these advances have not been successfully transferred to a field situation. Biopriming with endophytic and rhizosphere bacteria (Kavino et. al., 2007; Harish et al., 2009; Jebakumar and Selvarajan 2018) has shown some promise for BBTD control when applied prior to infection, resulting in reduced incidence of infection and a delay in symptom expression under greenhouse conditions. Screening and

67 breeding of new *Musa* spp. cultivars have been done in attempts to improve resistance
68 to important banana pests and diseases (Ortiz, 2013), but BBTV still poses a challenge
69 as there are still no known natural sources of resistance against this virus (Shekhawat et
70 al., 2012).

71 The wild species of banana, *M. acuminata* (AAw) and *M. balbisiana* (BBw), are
72 the major sources of the genomes of edible bananas cultivated today, contributing "A"
73 genome and "B" genome components, respectively (Valmayor et al., 2002;
74 Venkataramana et al., 2015). Though ultimately susceptible, banana cultivars can differ
75 markedly in the ease of infection with BBTV and with the intensity of symptoms
76 expressed and it has often been observed that banana cultivars with a B genome
77 component tend to display a level of resistance to BBTV (Ngatat et al., 2017; Jose, 1981;
78 Muharam; 1984, Espino et al., 1993; Niyongere et al., 2011; Sachter-Smith, 2015;
79 Hapsari and Masrum; 2012, Latifah et al., 2021). Resistance against important diseases
80 has been sought in genotypes of banana from the wild (Jenny et al., 2002) though none
81 have been conclusively established for BBTV. The Philippines has a diverse collection
82 of species of banana from the wild (Valmayor et al., 2002). Recognizing this potential,
83 this study screened Philippines wild-type banana species, predominantly *M. balbisiana*,
84 from the germplasm collection of National Plant Genetic Resources Laboratory, Institute
85 of Plant Breeding (NPGRL, IPB) for resistance against BBTV.

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Materials and methods

Study Site. The screening trial of wild banana was conducted in a greenhouse (14º9'19"N, 121º15'40"E) and field research site (14º8'60"N, 121º16'20"E) at the Institute of Plant Breeding, College of Agriculture and Food Science, University of the Philippines-Los Baños (IPB, CAFS, UPLB). The study started in November 2015 and the experimental field was selected due to the prevalence of BBTV and banana aphid in the area. The field trial site occupied 7,950 m^2 with nine experimental plots. Plant spacing was 1m within rows and 2m between. The area had an average rainfall of 1700 mm and annual mean temperature of 23.8°C (minimum) and 31.7°C (maximum) from 2016-2021.

Preparation of Musa accessions. Thirty-six wild banana accessions of the Philippines were screened in the study. It includes 34 Musa balbisiana accessions and two M. acuminata subsp. errans. The wild banana progenitors were collected from different regions of the Philippines as illustrated in figure 1. The collection site and identification of each accession are specified in table 1. The two *M. acuminata* subsp. *errans* and 32 *M. balbisiana* accessions were derived from seeds. The remaining two *M. balbisiana* are tissue-cultured. Test plants from seeds and tissue culture were transplanted to pots with sterilized soil and coir dust at a ratio of 1:3. Test plants were incubated under greenhouse conditions. Two-month-old seedlings were used in artificial inoculation of BBTV.

Collection of BBTV inoculum and aphid vector. Reference BBTV isolate REF2-LAG was obtained from banana cv. Lakatan with typical BBTV symptoms from Los Baños, Laguna, Philippines. An 861 nt fragment of the DNA-R segment of the isolate was

obtained using primer pair BBTVREP-F and BBTVREP-R (Islam et al. 2010). The resulting sequence was deposited in GenBank with accession code ON038409. For the banana aphid vector (*Pentalonia nigronervosa*), a colony was derived from a virus-free wingless nymph reared on virus-free banana cv. Lakatan plants (Parna & Agarwala, 2010). Modified Dellaporta minipreparation procedure was used in the extraction of aphid DNA (Dellaporta et al., 1983). PCR Primers LepF and LepR (Hebert et al. 2004) were used to amplify the mitochondrial cytochrome c oxidase I gene. The PCR conditions used are as follows: 94ºC for 2 min, 35 cycle of 94ºC for 1 min, 56ºC for 30 s and 72ºC for 1 min, and final extension at 72ºC for 5 min. The amplicon was sequenced, and the sequence deposited in GenBank under accession number ON077361. The BBTV isolate and aphid colony were kept in 32-mesh insect-proof screen cages under greenhouse conditions.

Screening of wild Musa test plants for BBTV resistance. Accessions from seeds had a total of 60 replicates while tissue cultured accessions had 25 replicates. A standard transmission protocol of BBTV was performed using aphids from a single colony and the BBTV reference isolate. Aphid transmission of BBTV followed the protocol of Thomas and Dietzgen (1991) with a few modifications (Dijkstra and Jager, 1998; Anhalt and Almeida, 2008). Wingless aphids were transferred into a sterilized closed container and starved for four hours. Starved aphids were then transferred to a banana cv. Lakatan plant infected with the BBTV reference isolate for an 18 to 24-hour acquisition access period (AAP). Twenty viruliferous aphids were then transferred to each test plant and allowed to feed for a 24 to 30-hour inoculation access period (IAP). Ten banana cv. Lakatan plants (dela Cruz et al., 2008) were used as positive controls per batch of

inoculations. Inoculated test plants were sprayed with insecticide to eliminate the aphids after the IAP. Samples were maintained inside an insect-proof or 32 mesh screen cage in greenhouse conditions and regularly observed for symptom appearance.

Confirmation of resistance of wild banana accessions from greenhouse trial. Wild banana accessions with 0% BBTD infection six months post-inoculation in greenhouse were then transferred to a field with high incidence of BBTD for further evaluation. Basic land preparation was performed such as mowing, plowing, rotavating and furrowing prior to transplanting. Wild *Musa* accessions were transplanted to the field per batch of screening. As a susceptible check, six-month-old virus-free banana cv. Lakatan plants were also planted parallel to test plants with the same number of replicates.

Screened *M. balbisiana* accessions in the field with suckers and producing bunches with viable seeds were subjected to re-screening against BBTV. The corms of the suckers of accessions 2016-018, 1998-087, 2016-013, 2013-155, 2016-051, 2016-003, and 2013-101 were macropropagated in large drums in an insect-proof cage and a total of four replicates per accession were screened against BBTV. On the other hand, seeds from accessions 2016-003, 2013-155, 2013-156, 1998-078, 1997-276 were harvested and grown in seedbeds. Fifteen seedlings per accession served as replicates. Test plants were inoculated with BBTV using the standard transmission protocol and monitored as outlined above. The trial was performed under greenhouse conditions.

Detection of BBTV. The presence of BBTV in test plants in the greenhouse was determined by PCR. Detection was conducted at three and six months after inoculation before proceeding to the field trial. Then, for accessions in the field, samples were taken from 10 randomly selected plants per *Musa* accession every six months from

158 transplanting. Leaf samples were taken from the second youngest leaf with portions of
159 the midrib (Thomas et al., 1994). About 0.5g per sample was homogenized in a 100 ×
160 150 mm polyethylene bag with a 3 mL extraction buffer (0.05 M Tris-HCl, 0.5% sodium
161 sulfite, and 2.5% non-fat milk). One ml of leaf extract was transferred into a sterile 1.5 ml
162 tube and centrifuged for 5 min at 10,000 rpm. The supernatant was used as a template
163 for PCR assay targeting the DNA-R of BBTV using BBT1 and BBT2 primer pair
164 (Thomson and Dietzgen 1991). Exactly 2 µl of template was added into a PCR cocktail
165 containing 1x PCR buffer, 1.76 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of forward BBT1 (3'
166 CTCGTCATGTGCAAGGTTATGTCG 5') and reverse primer BBT2, (5'
167 GAAGTTCTCCAGCTATTCATCGCC 3'), 1 U Taq DNA polymerase and DEPC water to
168 attain a final volume of 15 µl. Samples were run in a Mycycler thermal cycler (Bio-
169 Rad, USA) using the following PCR conditions: 94ºC for 3 min, 35 cycles of 94ºC for 30
170 s, 56ºC for 1 min and 72ºC for 1 min, and a final extension of 3 min at 72ºC. The
171 presence of BBTV was indicated by the amplification of the 349 bp band visualized by
172 gel electrophoresis with ethidium bromide staining. PCR assays were duplicated.

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Results

Screening of wild *Musa* test plant accessions

The positive control banana cv. Lakatan was the first to show symptoms of BBTD. Marginal chlorosis and dark green streaks along midrib were observed between 15-21 days after inoculation (DAI). Among wild banana accessions, only *M. acuminata* subsp. *errans* accessions, 2017-042 and 2021-029, expressed initial symptoms of BBTD at 21-28 DAI as shown in figure 2. By six weeks after inoculation, 100% disease incidence was recorded in *M. acuminata subsp. errans* showing confirmatory symptoms of BBTD such as dot and dash flecks, narrow and erect emerging leaves with marginal chlorosis and bunchy top. Meanwhile, none of the 34 *M. balbisiana* accessions showed any symptoms of the disease even at six-month post-inoculation (Figure 3). At three months post-inoculation, *M. acuminata* subsp. *errans* accessions and cv. Lakatan recorded a 100% BBTV incidence based in PCR assays. All *M. balbisiana* entries were negative after repeated PCR assays as indicated in Table 2.

Confirmation of resistance of wild M. balbisiana accessions (Field Trial). All 34 Wild *M. balbisiana* accessions from seeds and tissue culture proceeded to the field trial. Healthy tissue-cultured banana cv. Lakatan plants were also planted along the rows of *M. balbisiana* accessions. As early as one month after transplanting, initial symptoms such as j-hook, chlorosis and dark green streaks along the midrib started to appear in cv. Lakatan plants. Symptoms of BBTD became severe with stunting, leaf narrowing, and bunchy top exhibited. Incidence of BBTV in control cv. Lakatan plants per batch of inoculation were indicated in table 2. *M. balbisiana* test plants did not show any

symptoms and continued to develop and produce suckers. Vigorous growth was noted even under high disease pressure as shown in in figure 4. Macropropagated plants (figure 5) and seedlings derived from field trial plants were also symptomless.

BBTV was not detected by PCR in any replicates of all *M. balbisiana* accessions from seeds (n=60) and tissue culture (n=25) that were collected from the field trial while all samples of cv. Lakatan test plants were positive for BBTV infection with 100% incidence at 3 months after inoculation based in PCR. Macropropagated suckers and seedling derivatives of accessions from field trial plants also tested negative for BBTV by PCR.

Discussion

BBTV causes the most destructive virus disease of bananas. The disease virtually eliminated smallholdings of banana cv. Lakatan in the Luzon region of the Philippines (Molina et al. 2009) and thus most banana produce of the Philippines now comes from the Mindanao region. The most common practice in controlling BBTV in Mindanao is with the use of clean, tissue-cultured plants (Molina et al. 2009). However, the use of virus-free planting materials alone does not guarantee total protection against BBTD, and on-going inspection and eradication programs are challenging to enact and expensive to run. An efficient and sustainable strategy for controlling virus diseases involves the use of resistant varieties. However, there are still no reported varieties of banana resistant to BBTD (Hooks et al. 2009). In this study, the potential of wild *Musa* spp. as natural sources of resistance against BBTV was assessed.

Although *M. acuminata* subsp. *errans* accessions were susceptible to BBTV infection with a 100% infection rate and the control banana cv. 'Lakatan' showed strong BBTD symptoms and a 100% incidence of BBTV by PCR, all 34 *M. balbisiana* accessions showed no symptoms under greenhouse and field conditions. The virus was also not detected in *M. balbisiana* accessions by PCR after multiple rounds of screening. This study has revealed that wild *M. balbisiana* accessions from the Philippines are likely sources of resistance to BBTV. The lack of infection of the *M. balbisiana* accessions is unlikely to be due to lack of aphid feeding, as aphid survival numbers on the inoculated plants was high, and indeed some of these *M. balbisiana* accessions were used as maintenance hosts for the non-viruliferous aphid colonies.

Susceptibility of *Musa* cultivars has often been found to be related to genomic grouping, with cultivars containing a B genome component derived from *M. balbisiana* (BB) often showing a level of tolerance or resistance to BBTV (Ngatat et al., 2017; Jose; 1981, Muharam, 1984; Espino et al., 1993; Niyongere et al., 2011; Sachter-Smith, 2015; Hapsari and Masrum, 2012; Latifah et al., 2021). This association is not always consistent, however, and some A genome cultivars such as Gros Michel (AAA) show resistance to infection by BBTV while B genome-containing cultivars can vary markedly in their reaction (Niyongere et al., 2011; Ngatat et al., 2017; Magee, 1948). Thus, there may be several sources of resistance or tolerance to BBTV in *Musa* germplasm. The results presented here support the hypothesis that a source of resistance to BBTV is present in the B genome of wild *M. balbisiana* and that it has been transferred to many edible cultivars that contain a proportion of B genome. However, chromosome structural changes including translocations are known within wild banana species and in edible interspecific hybrids (Šimoníková et al 2022), so it is likely that resistance genes present on a chromosome in one cultivar may not be present in the same chromosome of another. The source of resistance describe in this work appears to be widely distributed in *M. balbisiana* from the Philippines.

The centre of origin for *M. balbisiana* is thought to range from north-east India, the northern limits of south-east Asia, southern China, to the Philippines (Perrier et al, 2009), though presence in the Philippines may be as feral populations introduced by humans (De Langhe et al 2015). Interestingly the centre of origin for the BBTV pathosystem where the maximum degree of BBTV diversity is found (Rao et al., 2017; Stainton et al., 2015) also coincides

263 with this region. One would expect that resistance to BBTV may have also
264 evolved here.

265 Although the BBTV-resistant wild *M. balbisiana* from the Philippines are
266 seeded, and thus not directly suitable as an edible banana, they have
267 alternative uses. Filipinos prefer the flower bud of wild *M. balbisiana* rather
268 than cultivated bananas in cooking. Its thick and green leaves are also
269 marketed in the region for culinary purposes.

270 Breeding of banana continues with the goal of incorporating resistance
271 to diseases, abiotic stresses and other desirable morphological traits (Wilson
272 et al. 2020). A widely used scheme in banana breeding today is the crossing
273 of triploid and diploid banana (3×/2×) followed by tetraploid and diploid or
274 4×/2× (Jenny 2002; Vezina 2014). *M. balbisiana* accessions can be used as
275 parents for conventional breeding and hand pollinated bunches can produce
276 over 40,000 seeds per bunch. *M. balbisiana* is recognized as a source of
277 useful traits within *Musa* including vigorous growth, strong suckering, drought
278 tolerance and resistance to abiotic stresses (Bakry et al 2021).

279 With the use of current molecular tools, investigation on molecular
280 mechanisms of resistance in wild *M. balbisana* accessions can be conducted
281 (Solomon-Blackburn & Barker 2001; Sharma et al. 2018). The findings of the
282 present study have led to the implementation of a local research project on
283 fast-tracking the development of BBTV-resistant banana cultivars using
284 modern biotechnology tools. A next-generation sequencing strategy (RNA-seq)
285 is currently being performed to find alleles related to BBTD resistance.

Discoveries will be further applied in assisting DNA marker development to aid the ultimate development of genetically modified resistant bananas.

Conclusion

Thirty four wild *M. balbisiana* (BBw) accessions exhibited total resistance to BBTV as shown by the absence of symptoms and negative molecular detection of BBTV under greenhouse and field evaluation. The study confirms that resistance against BBTD exists in the wild ecosystem and provides a solid base for future efforts to produce BBTV-resistant banana cultivars. These accessions could be used as a source of resistance for conventional breeding of BBTV-resistant hybrids. The use of molecular tools to investigate the genetic aspect of resistance in these accessions could lead to applications such as marker assisted breeding and incorporation of resistance genes through transgenic approaches.

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References

Aguilar, E.A., Dela Cruz, Jr., F.S., Dizon, T.O., Artes, L.A., Gueco, L.S., Calara, M.C.J., and Cañas, J.A.F., 2010. Mga Rekomendadong Teknolohiya sa Pag-aalaga ng Saging na Saba, Lakatan at Latundan. University of the Philippines, Los Baños, Laguna, Philippines.

Anhalt, M.D., and Almeida, R.P.P., 2008. Effect of Temperature, Vector Life Stage, and Plant Access Period on Transmission of Banana bunchy top virus to Banana. Phytopathol. 98, 743–748. 10.1094/phyto-98-6-0743.

Bakry F., Horry J-P., Jenny C., 2021. Making banana breeding more effective. In: Kema G, Drenth A, eds. Achieving sustainable cultivation of bananas; Volume 2 Germplasm and genetic improvement. Cambridge, UK: Burleigh Dodds Science Publishing, pp. 217-55. 10.19103/AS.2020.0070

Borth, W., Perez, E., Cheah, K., Chen, Y., Xie, W. S., Gaskill, D., Khalil, S., Sether, D., Melzer, M., Wang, M., Manshardt, R., Gonsalves, D., and Hu, J. S, 2011. Transgenic banana plants resistant to banana bunchy top virus infection. Acta Hortic. 449–457. https://doi.org/10.17660/actahortic.2011.897.61.

De Langhe E., Perrier X., Donohue M., Denham T., 2015. The Original Banana Split: Multi-disciplinary implications of the generation of African and

333 Pacific Plantains in Island Southeast Asia. Ethnobot Res Appl. 14, 299-312.
334 10.17348/era.14.0.299-312

335 Dela Cruz Jr., F.S., Gueco, L.S., Damasco, O.P., Huelgas, V.C., dela Cueva,
336 F.M., Dizon, T.O., Sison, M.L.J., Banasihan, I.G., Sinohin, V.O., and Molina Jr.,
337 A.B., 2008. Farmer's handbook on introduced and local banana cultivars in
338 the Philippines. Bioversity International. p 67.

339 Dela Cueva, F. M., Silva, F.F.M.A., Molina Jr., A.B., 2015. Banana bunchy top.
340 In: Dela Cueva, F. M., Pascual, C.B., Bajet, C.M., Dalisay, T. U. (ed). Pests
341 and Diseases of Economically Important Crops in the Philippines. Pest
342 Management Council of the Philippines, Inc. University of the Philippines Los
343 Baños, College, Laguna.

344 Dellaporta,S. L., Wood,J., Hicks, J.B., 1983. A plant DNA minipreparation:
345 Version II. Plant Mol Biol Rep. 1: 19-21.

346 Dijkstra, J., and Jager, C. D., 1998. Virus Transmission by Aphids. In:
347 Practical Plant Virology Protocols and Exercises. Springer Berlin.

348 Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small
349 quantities of fresh leaf tissue. Phytochem Bullet. 19, 11-15.

350 Elayabalan, S., Kalaiponmani, K., Subramaniam, S., Selvarajan, R.,
351 Panchanathan, R., Muthuvelayoutham, R., Kumar, K. & Balasubramanian, P.,
352 2013. Development of Agrobacterium-mediated transformation of highly
353 valued hill banana cultivar Virupakshi (AAB) for resistance to BBTV disease.
354 World J Microbiol Biotechnol. 29, 589-596. 10.4172/2169-0111.1000114

355 Espino, R.C., Magnaye, L.V., Johns, A.P., Juanillo, C. 1993. Evaluation of
356 Philippine banana cultivars for resistance to bunchy-top and fusarium wilt. pp.

89-102. In: Valmayor RV, Hwang SC, Ploetz R, Lee SC, Roa NV (eds.) Proceedings: International Symposium on Recent Developments in Banana Cultivation Technology, 14-18 December 1992, Chiuju, Pingtung, Taiwan. Taiwan Banana Research Institute, INIBAP/ASPNET, Los Banos, Philippines.

Hapsari, L. & Masrum, A., 2012. Preliminary screening resistance of Musa germplasms for Banana Bunchy Top Disease in Purwodadi Botanic Garden, Pasuruan, East Java. Buletin Kebun Raya, 15, 57-70.

Harding, R.M., Burns. T.M., and Dale, J.L., 1991. Virus-like particles associated with banana bunchy top disease contain small single-stranded DNA. J Gen Virol. 72: 225–230. 10.1099/0022-1317-72-2-225.

Harish, S., Kavino, M., Kumar, N., and Samiyappan, R., 2009. Biopriming banana with plant growth-promoting endophytic bacteria induces systemic resistance against banana bunchy top virus. Acta Hortic. 828: 295-302. 10.17660/actahortic.2009.828.30.

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H., Hallwachs, W., 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences of the United States of America 101: 14812-14817.

Hooks, C. R. R., Manandhar, R., Perez, E. P., Wang, K.-H., and Almeida, R. P. P., 2009. Comparative Susceptibility of Two Banana Cultivars to Banana Bunchy Top Virus Under Laboratory and Field Environments. J Econ Entomol. 102, 897-904. 10.1603/029.102.0306.

379 Islam, N., Naqvi, A., Jan, A. T. Haq, Q. 2010. Genetic diversity and possible
380 evidence of recombination among Banana Bunchy Top Virus (BBTV) isolates.
381 Int Res J Microbiol. 1, 1-12.

382 Jose, P. C., 1981. Reaction of different varieties of banana against bunchy top
383 disease. Agric Res J Kerala. 19, 108-110.

384 Jebakumar, R. M. & Selvarajan, R., 2018. Biopriming of micropropagated
385 banana plants at pre- or post-BBTV inoculation stage with rhizosphere and
386 endophytic bacteria determines their ability to induce systemic resistance
387 against BBTV in cultivar Grand Naine. Biocontrol Sci Technol. 28, 1074-1090.
388 10.1080/09583157.2018.1514583

389 Jenny, C., Tomekpe, K., Bakry, F., and Escalant, J. V., 2002. Conventional
390 Breeding of Bananas. In: Jacome, L., Lepoivre, P., Marin, D., Ortiz, R.,
391 Romero R., and Escalant J.V. (Eds.). Mycosphaerella leaf spot diseases of
392 bananas: Present status and outlook. essay, International Network for the
393 Improvement of Banana and Plantain, pp. 199-208.

394 Kavino, M., Harish, S., Kumar, N., Saravanakumar, D., and Samiyappan, R.,
395 2007. Induction of systemic resistance in banana (Musa spp.) against banana
396 bunchy top virus (BBTV) by combining chitin with root-colonizing
397 Pseudomonas fluorescens strain CHA0. Eur J Plant Pathol. 120, 353-362.
398 10.1007/s10658-007-9223-8.

399 Latifah, Hidayat, S. H., Mutaqin, K. H., Widodo & Sutanto, A. 2021. Survey of
400 Banana bunchy top virus on non-cultivated bananas in West Java. IOP
401 Conference Series: Earth and Environmental Science. 694, 012044.
402 10.1088/1755-1315/694/1/012044.

403 Magee, C.J.P., 1948. Transmission of bunchy top to banana varieties. J Aust
404 Inst Agric Sci. 14, 18-24.

405 Molina A. B., Sinohin V. G. O., dela Cueva F. M., Dela Esguerra A. V., Crucido
406 S. S., Vida E., Temanel B.E., Anit E.A., Eusebio J.E., 2009. Managing banana
407 bunchy top virus in smallholdings in the Philippines. Acta Hortic. 828, 383-387.
408 10.17660/ActaHortic.2009.828.39.

409 Mpoki, S., Mahuku, G., Mbanzibwa, D.R., et al., 2021. First report of banana
410 bunchy top virus in banana and plantain (Musa spp.) in Tanzania. Plant Dis in
411 press. 10.1094/pdis-07-21-1387-pdn

412 Muharam, A., 1984. Test for resistance of some banana cultivars to banana
413 bunchy-top disease. Bulletin Penelitian Hortikultura. 11, 16-19.

414 Mware, B. O., 2016. Development of banana bunchy top virus resistance in
415 bananas: Rnai approach. PhD, Queensland University of Technology.

416 Niyongere, C., Ateka, E., Losenge, T., Blomme, G., and Lepoint, P. 2011.
417 Screening Musa Genotypes for Banana Bunchy Top Disease Resistance In
418 Burundi. Acta Hortic. 897, 439-447. 10.17660/actahortic.2011.897.60.

419 Ngatat, S., Hanna, R., Kumar, P. L., Gray, S. M., Cilia, M., Ghogomu, R. T. &
420 Fontem, D. A., 2017. Relative susceptibility of Musa genotypes to banana
421 bunchy top disease in Cameroon and implication for disease management.
422 Crop Prot. 101, 116-122. 10.1016/j.cropro.2017.07.018

423 Ocimati, W., Tazuba, A.F., Tushemereirwe, W.K., Tugume, J., Omondi, B.A.,
424 Acema, D.et al. 2021. First report of banana bunchy top disease caused by

425 Banana bunchy top virus in Uganda. New Dis Rep. 44, e12052.
426 10.1002/ndr2.12052

427 Ortiz R., 2013. Conventional Banana and Plantain Breeding. Acta Hortic. 986,
428 177–194. 10.17660/actahortic.2013.986.19.

429 Parna Bhadra, B. K. Agarwala, A comparison of fitness characters of two host
430 plant-based congeneric species of the banana aphid, Pentalonia nigronervosa
431 and P. caladii. J Insect Sci. 10, 140. https://doi.org/10.1673/031.010.14001.

432 Perrier, X., 2009. Combining biological approaches to shed light on the
433 evolution of edible bananas. Ethnobot Res and Appl. 7, 199-216.

434 Qazi, J., 2015. Banana bunchy top virus and the bunchy top disease. J Gen
435 Plant Pathol. 82, 2–11. 10.1007/s10327-015-0642-7.

436 Rao, X. Q., Wu, Z. L., Zhou, L., Sun, J. & Li, H. P., 2017. Genetic diversity of
437 banana bunchy top virus isolates from China. Acta Virol. 61, 217-222.
438 10.4149/av_2017_02_13.

439 Sachter-Smith, G. L., 2015. In search of naturally occurring host resistance to
440 Banana bunchy top virus. Master of Science Master of Science, University of
441 Hawai'i at Mānoa.

442 Sharma, M., Tarafdar, A., Chandran, S., Chobe, D., and Ghosh, R., 2018.
443 Molecular markers as tools for identification and introgression of virus-
444 resistant genes. 10.21775/9781910190814.04.

445 Shekhawat, U. K., Ganapathi, T. R., and Hadapad, A. B., 2012. Transgenic
446 banana plants expressing small interfering RNAs targeted against viral

447 replication initiation gene display high-level resistance to banana bunchy top
448 virus infection. J Gen Virol. 93, 1804-1813. 10.1099/vir.0.041871-0.

449 Šimoníková, D.; Cížková, J.; ˇ Zoulová, V.; Christelová, P.; Hˇribová, E.
450 Advances in the Molecular Cytogenetics of Bananas, Family Musaceae Plants.
451 2022. 11, 482. https://doi.org/10.3390/ plants11040482.

452 Solomon-Blackburn, R., and Barker, H., 2001. Breeding virus resistant
453 potatoes (Solanum tuberosum): a review of traditional and molecular
454 approaches. Heredity. 86, 17-35. https://doi.org/10.1046/j.1365-
455 2540.2001.00799.x.

456 Stainton, D., Kraberger, S., Walters, M., Wiltshire, E. J., Rosario, K., Halafihi,
457 M. I., Lolohea, S., Katoa, I., Faitua, T. A. H., Aholelei, W., Taufa, L., Thomas, J.
458 E., Collings, D. A., Martin, D. P. & Varsani, A. 2012. Evidence of inter-
459 component recombination, intra-component recombination and reassortment
460 in banana bunchy top virus. J Gen Virol. 93, 1103-1119.
461 10.1099/vir.0.040337-0.

462 Thomas, J. E., and Dietzgen, R. G., 1991. Purification, characterization, and
463 serological detection of virus-like particles associated with banana bunchy top
464 disease in Australia. J Gen Virol. 72, 217-224. 10.1099/0022-1317-72-2-217.

465 Thomas, J.E., Iskra-Caruana, M.L., and Jones, D.R., 1994. Banana bunchy
466 top disease. Musa Disease Fact Sheet No. 4. International Network for the
467 Improvement of banana and plantain (INIBAP). Montpellier, pp 2.

468 Thomas, J.E., 2008. Banana bunchy top virus. In: Mahy BWJ, Regenmortel
469 MHVV (eds) Encyclopedia of Virology. Elsevier, Oxford, pp 272-279Thomas,
470 J.E. and Iskra-Caruana, M.L., 2000. Diseases caused by viruses. In: Jones,

471 D.R. (ed.). Diseases of banana, Abacá and Enset. CABI, Wallingford (GBR).
472 pp. 241-293.

473 Thomas, J.E., 2019. Banana bunchy top. In: Jones D, ed. Handbook of
474 Diseases of Banana, Abacá and Enset. Wallingford, Oxfordshire, UK: CAB
475 International, pp 362-76.

476 Thomson, D., and Dietzgen, R. G., 1995. Detection of DNA and RNA plant
477 viruses by PCR and RT-PCR using a rapid virus release protocol without
478 tissue homogenization. J Virol Methods. 54, 85-95.

479 Valmayor, R.V., Espino, R.R.C., Pascua, O.C., 2002. The wilds and cultivated
480 bananas of the Philippines. Los Baños, Laguna: PARFI and BAR.

481 Venkataramana, R.K., Sampangi-Ramaiah, M.H., Ajitha, R., Khadke, G.N.,
482 Chellam, V., 2015. Insights into Musa balbisiana and Musa acuminata species
483 divergence and development of genic microsatellites by transcriptomics
484 approach. Plant Gene. 4: 78-82. 10.1016/j.plgene.2015.09.007

485 Vézina, A. (2014, August 7). Who's breeding bananas? Retrieved from
486 https://www.promusa.org/blogpost363-Who-s-breeding-bananas.

487 Wilson, V., Tenkouano, A., Swennen, R., Crouch, H., Gauhl, F., Gauhl, P.,
488 Ortiz, R., Vuylsteke, D., Crouch, J., and Austin, P., 2020. Ten Year
489 Progression of Musa Breeding from 1987 to 1997: 1. Pollination Success and
490 Seed Production (Fecundity) Patterns among Multiple Ploidy Crosses. Asian
491 Journal of Research in Botany 4, 53.

492 Xie, W., Hu, J.S. 1995. Molecular Cloning, Sequence Analysis, and Detection
493 of Banana Bunchy Top Virus in Hawaii. Phytopathol. 85, 339. 10.1094/phyto-
494 85-33

Conflict of Interests

The authors declare that they have no conflict of interests.

Ethical Approval

This article does not contain any studies with human and animal participations performed by the authors

502 Table 1. List of wild Musa accessions screened from germplasm collection of NPGRL-IPB

Plant Source Type	GB/ PHL Number	Collection Number	Local name	Scientific name	Collection Site
Seeds	GB24350	1997-276	Butuhan	*Musa balbisiana*	Zambales
Seeds	GB24364	1998-078	Butuhan	*Musa balbisiana*	Quirino
Seeds	GB24366	1998-085	Butuhan	*Musa balbisiana*	Quirino
Seeds	GB24376	1998-315	Pik-iw	*Musa balbisiana*	Marinduque
Seeds	GB24376	1998-315 H2	Pik-iw	*Musa balbisiana*	Marinduque
Seeds	GB24405	1998-453	Butuhan	*Musa balbisiana*	Ilocos Norte
Seeds		1998-456	Balibisiana	*Musa balbisiana*	Ilocos Sur
Seeds	GB24421	1998-575	Butuhan	*Musa balbisiana*	Leyte
Seeds	GB24442	1998-633	Pik-iw	*Musa balbisiana*	Quezon
Seeds	PHL32673	2010-047	Balbisiana	*Musa balbisiana*	Oriental Mindoro
Seeds	GB61941	2013-100	Pakol	*Musa balbisiana*	Catanduanes

503 Table 1. List of wild Musa accessions screened from germplasm collection of NPGRL-IPB

Plant Type	GB/ PHL Number	Collection Number	Local name	Scientific name	Collection Site
Seeds	GB61941	2013-100 H2	Moko	*Musa balbisiana*	Catanduanes
Seeds	GB61942	2013-101	Butuhan	*Musa balbisiana*	Catanduanes
Seeds	GB61996	2013-155	Moko-Bulo	*Musa balbisiana*	Camarines Sur
Seeds	GB61996	2013-155 H2	Moko	*Musa balbisiana*	Camarines Sur
Seeds	GB61997	2013-156	Butuhan	*Musa balbisiana*	Camarines Sur
Seeds	GB62000	2013-159	Moko	*Musa balbisiana*	Camarines Norte
Seeds	GB66554	2016-003	Pik-ew/ Butuhan	*Musa balbisiana*	Agusan del Norte
Seeds	GB66560	2016-013	Balbisiana	*Musa balbisiana*	Oriental Mindoro
Seeds	GB66565	2016-018	Balbisiana	*Musa balbisiana*	Agusan del Norte
Seeds	GB66565	2016-018	Balbisiana	*Musa balbisiana*	Agusan del Norte
Seeds	GB66583	2016-048 H1	Pakol	*Musa balbisiana*	Albay

504 Table 1. List of wild Musa accessions screened from germplasm collection of NPGRL-IPB

Plant Source Type	GB/ PHL Number	Collection Number	Local name	Scientific name	Collection Site
Seeds	GB66586	2016-051	Butuhan	*Musa balbisiana*	Camarines Sur
Seeds	GB66586	2016-051 H1	Butuhan	*Musa balbisiana*	Camarines Sur
Seeds	GB66586	2016-051 H2	Butuhan	*Musa balbisiana*	Camarines Sur
Seeds	GB66587	2016-052	Balibisiana	*Musa balbisiana*	Camarines Sur
Seeds	GB66588	2016-053	Balibisiana	*Musa balbisiana*	Camarines Sur
Seeds	GB66591	2016-056 H3	Balbisiana	*Musa balbisiana*	Camarines Norte
Seeds	GB66592	2016-057	Balibisiana	*Musa balbisiana*	Camarines Norte
Seeds	GB66592	2016-057 H2	Moko	*Musa balbisiana*	Camarines Norte
Seeds	GB66648	2017-040	Balbisiana	*Musa balbisiana*	Quezon
Seeds	GB67532	2017-042	Agutay	*M. acuminata subsp. errans*	Laguna
Seeds	GB67541	2017-048	Pik-iw	*Musa balbisiana*	Quezon

505 Table 1. List of wild Musa accessions screened from germplasm collection of NPGRL-IPB

Plant Source Type	GB/ PHL Number	Collection Number	Local name	Scientific name	Collection Site
Seeds	GB67548	2017-055	Balbisiana	*Musa balbisiana*	Quezon
Seeds	GB 71332	2021-029	Agutay	*M. acuminata subsp. errans*	Laguna
Tissue culture		1999-058	Butuhan	*Musa balbisiana*	Laguna
Tissue culture		1999-088	Butuhan	*Musa balbisiana*	Davao Del Sur

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514 Table 2. Result of greenhouse and field screening of wild banana against BBTV

Collection Number	Scientific name	Source	Greenhouse BBTV % Infection	Field BBTV % Infection	Location in the field
Batch 1 and 2					
2013-155	*Musa balbisiana*	Seeds	0	0	Plot 1
2016-003	*Musa balbisiana*	Seeds	0	0	Plot 1
2013-159	*Musa balbisiana*	Seeds	0	0	Plot 2
2016-051	*Musa balbisiana*	Seeds	0	0	Plot 2
Positive Control Lakatan	*M. acuminata*	Tissue Cultured	100%	100%	n/a (100%*)
Batch 3					
2013-101	*Musa balbisiana*	Seeds	0	0	Plot 3
2013-156	*Musa balbisiana*	Seeds	0	0	Plot 3
2017-040	*Musa balbisiana*	Seeds	0	0	Plot 3
2017-042	***M. acuminata subsp. errans***	**Seeds**	**100%**	**NT****	**Greenhouse Trial only**
Positive Control Lakatan	*M. acuminata*	Tissue Cultured	100%	100%	n/a (100%*)
Batch 4					
1998-085	*Musa balbisiana*	Seeds	0	0	Plot 4
1998-315	*Musa balbisiana*	Seeds	0	0	Plot 4
Positive Control Lakatan	*M. acuminata*	Tissue Cultured	100%	100%	n/a (100%*)

515 *% Mortality rate **NT- Not tested

516 Table 2. Result of greenhouse and field screening of wild banana test plants against BBTV (Continuation...)

Collection Number	Scientific name	Source	Greenhouse BBTV % Infection	Field BBTV % Infection	Location in the field
Batch 5					
1999-088	*Musa balbisiana*	Tissue Cultured	0	0	Plot 5
1998-058	*Musa balbisiana*	Tissue Cultured	0	0	Plot 5
2010-047	*Musa balbisiana*	Seeds	0	0	Plot 5
2016-013	*Musa balbisiana*	Seeds	0	0	Plot 5
2016-018	*Musa balbisiana*	Seeds	0	0	Plot 5
1998-078	*Musa balbisiana*	Seeds	0	0	Plot 5
Positive Control Lakatan	*M. acuminata*	Tissue Cultured	100%	100%	n/a (100%*)
Batch 6					
2016-052	*Musa balbisiana*	Seeds	0	0	Plot 6
2016-018	*Musa balbisiana*	Seeds	0	0	Plot 6
1997-276	*Musa balbisiana*	Seeds	0	0	Plot 6
Positive Control Lakatan	*M. acuminata*	Tissue Cultured	100%	100%	n/a (100%*)
Batch 7					
2016-048 H1	*Musa balbisiana*	Seeds	0	0	Plot 7
2016-051 H1	*Musa balbisiana*	Seeds	0	0	Plot 7
1998-633	*Musa balbisiana*	Seeds	0	0	Plot 7
Positive Control Lakatan	*M. acuminata*	Tissue Cultured	100%	100%	n/a (43%*)

517 *% Mortality rate

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Table 2. Result of greenhouse and field screening of wild banana test plants against BBTV (Continuation...)

Collection Number	Scientific name	Source	Greenhouse BBTV % Infection	Field BBTV % Infection	Location in the field
Batch 8					
2013-155 H2	*Musa balbisiana*	Seeds	0	0	Plot 8
2016-056 H3	*Musa balbisiana*	Seeds	0	0	Plot 8
1998-315 H2	*Musa balbisiana*	Seeds	0	0	Plot 8
1998-575	*Musa balbisiana*	Seeds	0	0	Plot 8
2013-100	*Musa balbisiana*	Seeds	0	0	Plot 8
2016-051 H2	*Musa balbisiana*	Seeds	0	0	Plot 8
Positive Control Lakatan	*M. acuminata*	Tissue Cultured	100%	100%	Plot 8 (50%*)

*% Mortality rate

536 Table 2. Result of greenhouse and field screening of wild banana test plants against BBTV (Continuation...)

Collection Number	Scientific name	Source	Greenhouse BBTV % Infection	Field BBTV % Infection	Location in the field
Batch 9					
2017-055	*Musa balbisiana*	Seeds	0	0	Plot 9
2013-100 H2	*Musa balbisiana*	Seeds	0	0	Plot 9
2016-057 H2	*Musa balbisiana*	Seeds	0	0	Plot 9
1998-453	*Musa balbisiana*	Seeds	0	0	Plot 9
2016-053	*Musa balbisiana*	Seeds	0	0	Plot 9
2017-048	*Musa balbisiana*	Seeds	0	0	Plot 9
1998-456	*Musa balbisiana*	Seeds	0	0	Plot 9
2021-029	***M. acuminata subsp. errans***	**Seeds**	**100%**	**NT****	**Greenhouse Trial only**
Positive Control Lakatan	*M. acuminata*	Tissue Cultured	100%	100%	Plot 9

537 *% Mortality rate **NT- Not tested

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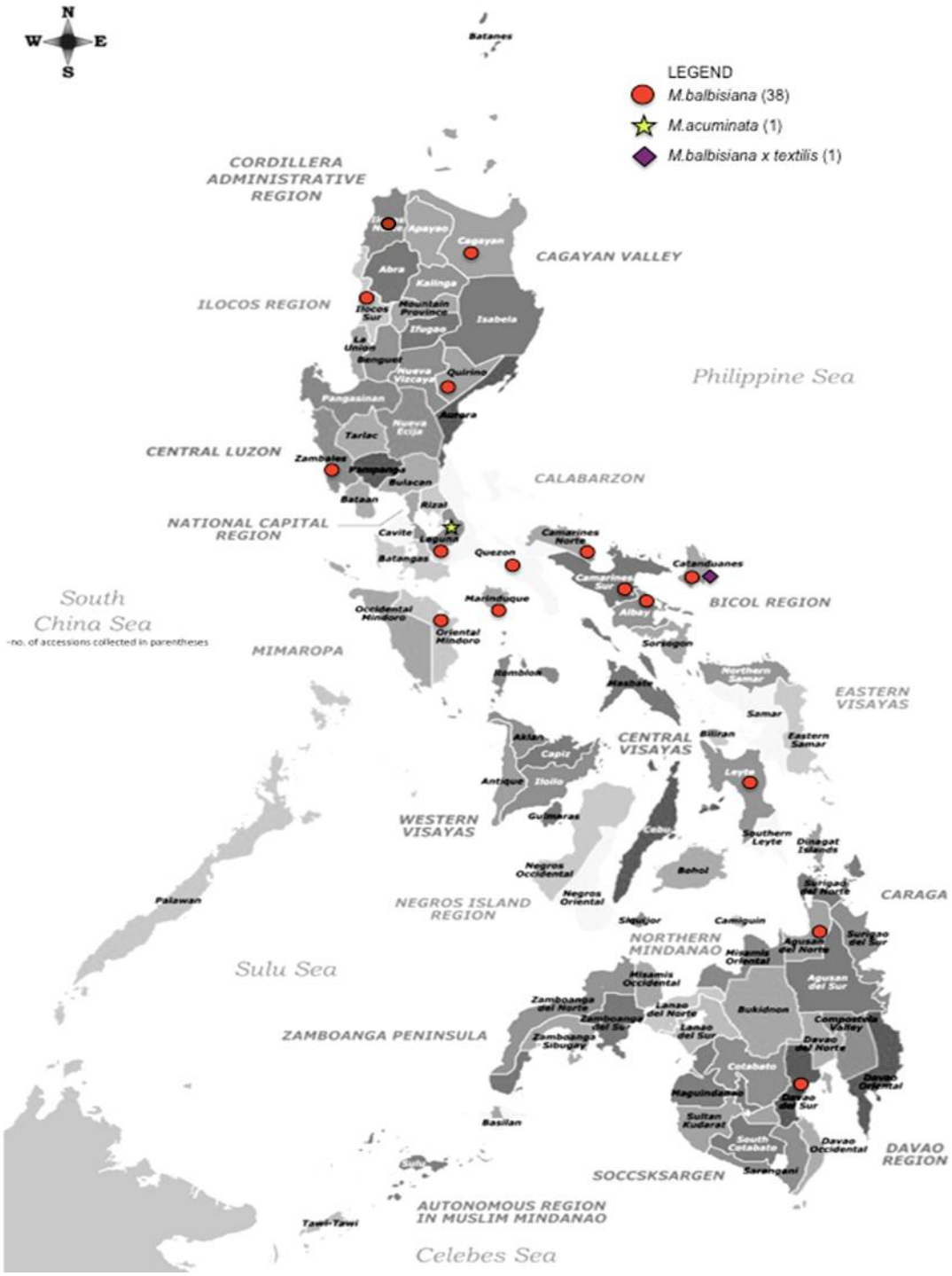

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541 Figure 1. Philippine map showing the places of collection (in red dots) of

542 different *Musa balbisiana* accessions.

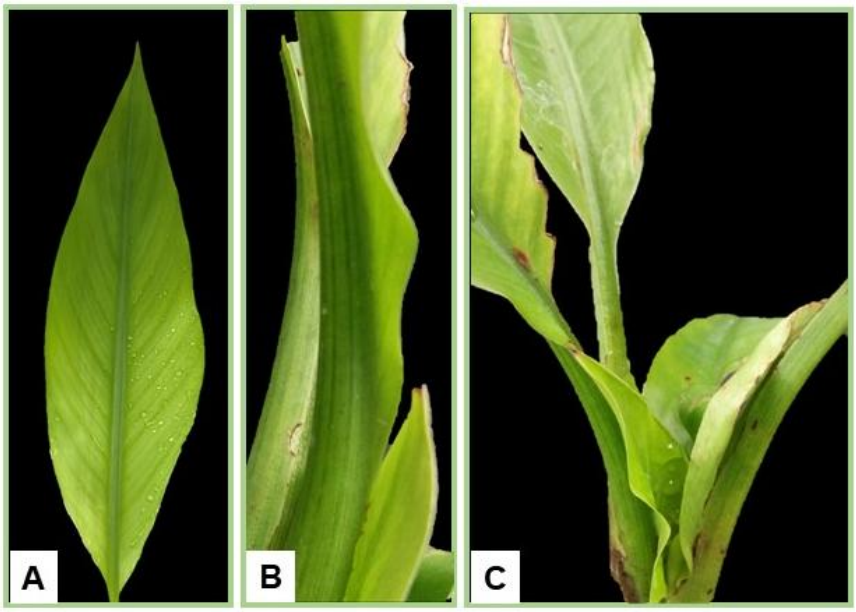

Figure 2. BBTV symptoms on 'Agutay' (*M. acuminata* ssp. *errans*): (a) chlorosis on young leaves, (b) dot and dash flecks from midrib to petiole (c) bunchy top

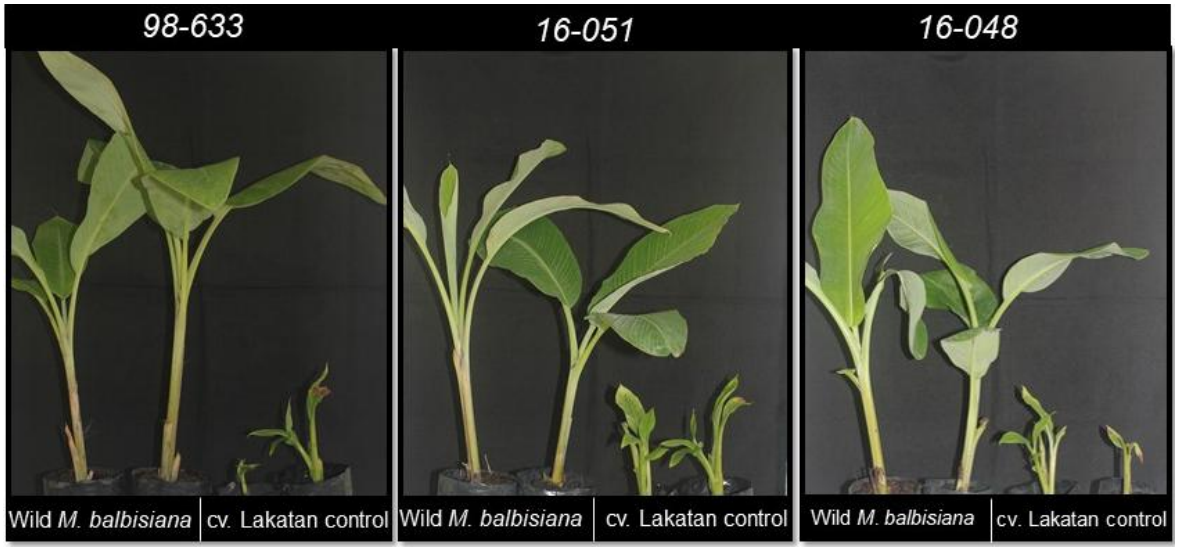

Figure 3. Asymptomatic *M. balbisiana* (MB) accessions 1998-633 (a), 2016-051 (b), and 2016-048 (c) in comparison with the susceptible control banana cv. Lakatan (right).

Figure 4. *Musa balbisiana* accessions screening under field trial: (A) oldest standing plot (B) field screening set-up with severely infected cv. Lakatan control. Red line represents susceptible control banana cv. Lakatan row. Blue line represents *M. balbisiana* row.

Figure 5. Post screening result: (a-b) Suckers emerging from corms of wild M. balbisiana; (c)suckers developed and remained negative against BBTV