

Induced Resistance in Abaca (*Musa textilis* Nee) Against *Banana Bunchy Top Virus* through Exogenous Salicylic Acid Application

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ABSTRACT

Abaca (*Musa textilis* Nee) is one of the world's major sources of natural fiber. However, there is a significant decrease in production due to the bunchy top disease (BTD) caused by the bunchy top virus, and there is a need to apply necessary measures to avoid further economic losses in the fiber industry. The use of resistant varieties is the most effective control measure so far. However, resistance to the disease is absent in some important varieties with very high-quality fiber. Hence, plant immunity through systemic acquired resistance via elicitor application was employed in this study. To attain this, the effect of SA at 25, 50, 75, and 100 μ M in inducing systemic acquired resistance (SAR) in abaca against BTD infection was evaluated. Different concentrations of SA were sprayed on abaca seedlings divided into two setups (inoculated and uninoculated). SA at 75 μ M induced resistance in abaca by significantly delaying the disease onset, reducing the incidence, severity, and the rate of disease progress as plotted through area under the disease progress curve (AUDPC) of bunchy top disease. These results indicate that the application of 75 μ M SA can significantly lower disease incidence and progress on BTD-infected abaca seedlings. Using SA as an elicitor offers a safe, effective, affordable, and environmentally safe way of managing bunchy top diseases in abaca.

Keywords: abaca, bunchy top disease, salicylic acid, elicitor, systemic acquired resistance, AUDPC

INTRODUCTION

Abaca (*Musa textilis* Nee) is an important fiber crop that belongs to the Musaceae family. It is mainly produced in the Philippines, which is also the number one exporter of the fiber, hence it is also known as Manila hemp. Abaca fibers are raw materials in Philippine peso bills and car interiors (Lalusin 2018). The Philippines is the number one exporter of abaca fiber in the world market (FAO 2022). As of the last half of the previous year, the total abaca fiber production and area planted in the country is 16.03 thousand metric tons and 127.06 thousand hectares, respectively (PSA 2022). The country is the highest contributor to the global market of abaca fiber exports, distributing up to 86% in total or 72.8 thousand tons (FAO 2022).

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While the Philippines is enjoying the monopoly of the world's fiber demand, sustaining that supply is needed because of decreased productivity. There has been an average decrease of 1.16 thousand tons from 2015 until 2020 in abaca production in the country (FAO 2022). Climate, edaphic factors, pests, and diseases are some of the factors that could be involved in the decrease in production. In terms of diseases, the most common pathogens that attack abaca are viruses. One of the most serious viral diseases of abaca is the bunchy top disease (BTD), which wiped out 16,737 hectares from a total of 26,374 hectares of abaca plantations based on the last record (Nuñez 2013).

The bunchy top disease is caused by either *Abaca bunchy top virus* (Sharman 2008) or *the Banana bunchy top virus* (Piamonte & Sta. Cruz 2018), a *Babuvirus* from the *Nanoviridae* family with a single-stranded DNA genome structure (ICTV 2022). The disease has characteristic symptoms of stunting, bunching, and rosetting of leaves, appearance of dark green flecks or vein clearing of the minor leaf veins, and upcurling and chlorosis of leaf margins (Ocfemia 1930). This threat in the fiber industry caused more than half a billion losses due to BTV infestation (Tabios 2019). This only accounts for the losses in the Bicol Region and other unaccounted areas.

The severity of losses due to viral diseases calls for proper management of the disease. As no antiviral agents are available, effective management strategies should be used to manage abaca bunchy top disease. This can be through cultural, physical, biological, and chemical control methods. The Philippine Fiber Industry and Development Authority (PhilFIDA 2022) has disease management programs such as producing and distributing clean and disease-free planting materials, detecting viral diseases in abaca through ELISA testing, and integrated abaca disease management project (ADMP).

Using resistant plants is the most practical way of managing virus disease. Conventional breeding may take 10 to 20 years, while genetic engineering may shorten the process to 3 to 5 years. However, there are hesitations in the readiness of the country to accept genetically-modified (GM) plants. Induced resistance, on the other hand, in the form of systemic acquired resistance (SAR), is the fastest and most durable mechanism of making a plant resistant to diseases. SAR makes a plant resistant in 2 to 3 days from induction through elicitor application such as salicylic acid and confers long-lasting resistance to the plant that can be retained until harvesting (Durrant & Dong 2004).

Some natural or synthetic chemicals, such as salicylic acid (SA), activate the signaling pathway, resulting in systemic acquired resistance to various plant-pathogenic viruses, bacteria, fungi, and even insects (Feng et al 2021). SA application in plants was proven to induce resistance against plant viruses such as *Soybean mosaic virus* (Zhang et al 2019) and *Bean common mosaic virus* (Mardani-Mehrabad et al 2020; Radwan et al 2008). However, there is no available data for abaca, hence this study.

The non-availability of commercial antiviral agents redirects research on enforcing plant immunity in combatting viral diseases. Effective BTD management should be implemented so that the Philippines can produce more abaca as a sustainable source of fiber. This study will induce resistance in abaca plants using SA as an elicitor in managing bunchy top disease in abaca plants through spray application.

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MATERIALS AND METHODS

Acquisition of Tissue-Cultured Abaca Seedlings

Three hundred sixty (360) one-month-old hardened tissue-cultured abaca variety *Inosa* were obtained from the Philippine Fiber Industry Development and Authority - Tissue Culture Laboratory. The seedlings were maintained and allowed to acclimatize for a week. The transplanted seedlings were watered daily to support adaption in the new soil medium.

Salicylic Acid (SA) Preparation and Exogenous Application

The initial working stock solution was 500 μ M in 500 mL of warm water. From the stock solution, 12.5, 25, 37.5, and 50 mL were added to 487.5, 475, 462.5, and 450 mL of water to obtain the concentrations 25, 50, 75, and 100 μ M, respectively, for a total SA solution of 500mL. Each test plant was sprayed with the treatment on the stem, surface, and underside of the leaf until all surface areas were covered. Six (6) treatments were used in this study, and they are as follows:

- T₁ – Negative Control (sdH₂O)
- T₂ – 25 μ M SA
- T₃ – 50 μ M SA
- T₄ – 75 μ M SA
- T₅ – 100 μ M SA
- T₆ – NARC-MH83-1 (Resistant Check)

Each treatment was replicated thrice, with ten samples per replicate for the inoculated setup. On the other hand, the uninoculated setup was unchallenged. It was sprayed only with salicylic acid, with the same number of replicated samples per treatment, making a total of 360 test plants.

Collection, Rearing and Introduction of *Pentalonia nigronervosa*

P. nigronervosa or brown aphids were collected from the NARC experimental area with BTD-infected abaca plants. The aphids were gathered with the infected plant part to ensure sufficient viral load. The aphids were reared in abaca seedlings for one week. Each abaca plant was introduced with ten adult aphids placed on its innermost or unfurled leaf.

Molecular BTV Indexing

Virus indexing using the Dellaporta extraction (Dellaporta et al 1983; Piamonte and Sta. Cruz 2018) protocol was done before treatment application to ensure the abaca plants were disease-free. The same procedures were conducted two months after treatment application for virus detection. DNA amplification through polymerase chain reaction (PCR) was carried out using the conditions optimized by Piamonte and Sta. Cruz (2018). Per reaction in each 200 μ L tube contains a total of 10 μ L product composed of the following components: 1 μ L diluted (1:10) DNA template, 1x PCR buffer, 0.35 μ L MgCl₂, 0.2mM dNTP, 0.2mM of each BBT1 (CTCGTCATGTGCAAGGTTATGTGC) and BBT2 (GAAGTTCTCCAGCTATTCATCGC) oligonucleotide primers, 0.2 μ L of commercial DNA *Taq* polymerase (Invitrogen) and 6.85 μ L of sterile distilled water.

The mixture was subjected to a thermal cycler (BOECO, TC-TE) for DNA

amplification under the following conditions: a cycle for the initial denaturation at 94°C for 1 minute and 30 seconds, 35 cycles of denaturation at 94°C for 20 seconds, annealing at 60°C for 1 minute, extension at 72°C for 1 minute, and a cycle of final extension at 72°C for 3 minutes. The amplified fragments were checked using the 1.2% agarose gel run in an electro system (ENDURO™ Gel Electrophoresis System) with 1x Tris/Borate/EDTA (TBE) buffer at 100V for 30 minutes. 1 kb DNA ladder was placed in the first lane and a mixture of 2µL loading dye and 5µL PCR product sample were placed in the succeeding wells and lanes 26-28 were loaded with blank, healthy, and positive control, respectively. The gel was placed inside a band visualizer to document the resulting bands using the gel documentation system (ENDURO™ GDS, LabNet International).

DATA GATHERED

Disease Incidence

The incidence of BTD was assessed to evaluate the effectivity of different SA concentrations in preventing infection of the said disease. The assessment was based on the symptoms exhibited by each plant. The percentage disease reduction was also noted by computing the difference between the percentage disease incidence of the control and the treatment. Upon termination, infected abaca seedlings were counted, and disease incidence for each treatment and setup was computed using the formula: Total number of infected plant samples over total number of plants assessed multiplied by 100.

Disease Severity Index

This was collected every other week to monitor the disease progress per plant. The disease severity index (DSI) was based on the disease severity score (Table 1) and computed through the formula $DS (\%) = [\text{sum (class frequency} \times \text{score of rating class)}] / [(\text{total number of plants}) \times (\text{maximal disease index})] \times 100$.

Table 1. Disease severity rating scale of BTD (Parac et al 2021) modified for *Inosa* variety

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

Incubation Period

The onset of disease infection was noted based on the appearance of early disease symptoms, such as dark green streaks on the veins and midribs of the inoculated plants. The symptoms were closely monitored to determine the exact day they appeared on the plants. This was recorded in days from inoculation up to the appearance of symptoms. The number of plants per observation period was

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also recorded.

Area Under the Disease Progress Curve (AUDPC)

To calculate AUDPC, a formula that takes into account the number of times the disease severity is evaluated, the disease severity at each evaluation time, and the length of the epidemic is used, or the area under the disease progress curve, which is used to summarize the progression of disease severity (Ngatat et al 2017). It was calculated by the formula:

$$\text{AUDPC} = \sum_{j=1}^{n-1} \left(\frac{y_j + y_{j+1}}{2} \right) (t_{j+1} - t_j)$$

Where 'y_i' is the proportion of the infected plant at the 'i'-th observation, and 't_i' is the time in days at the 'i'-th observation. Progress curves were computed using MS Excel and plotted using each treatment's midpoint rule or trapezoidal method.

Statistical Analysis

The data collected all throughout the experiment were processed in the Microsoft Excel software. The Statistical Tool for Agricultural Research (STAR) of IRRI v2.0.1 was used in analyzing all data using the one-way analysis of variance and pairwise test to further analyze significantly different variances. Graphs were processed using the Microsoft PowerPoint software for data visualization.

RESULTS AND DISCUSSION

Characteristic Symptom of BTB

Pathogenicity test was done to determine the infectivity of bunchy top virus inoculum via forced inoculation of viruliferous aphids. This is essential to check if the inoculum vectored by the aphids is pathogenic to the plant. The aphids collected from BTB-infected abaca plants were introduced to one-month-old healthy abaca seedlings. The virus was allowed to incubate and infect for a month until symptoms were visible. Typical symptoms of BTB (Fig. 1) appeared on inoculated seedlings two weeks after inoculation, which were similar to the host plant of the aphid source.

Apparent symptoms were observed on the leaves of the abaca seedlings. The plants exhibited dark patches on the leaf blade upon the onset of infection within a month from inoculation. As the disease progressed, the yellowing of leaves was observed and later became more pronounced. The emerging leaves became abnormally narrow and stiff compared to the control plants. Bunching symptom was also observed during the later part, where the leaves clustered at the end of the pseudostem without developing petioles (Ocfemia 1926; Raymundo & Bajet 2000).

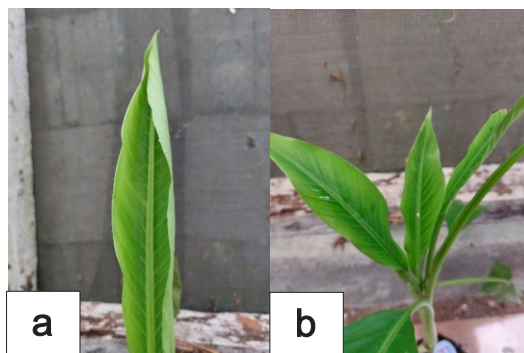


Figure 1. Bunchy top disease symptoms in abaca seedlings 28 days after inoculation: (a) dark green patches on leaf blades and (b) bunched-top appearance of leaves

Effects of SA concentration on BTB incidence

The effect of different SA concentrations on the incidence of BTB was confirmed by PCR using BBT1/2 primer pairs. The unchallenged set up revealed no BTB infection upon indexing. Among the different SA concentrations applied in the challenged set up (Table 2), differences in the disease incidence were observed. Both 25 μ M and 50 μ M have higher incidence where more than half the total samples tested were infected which is comparable with the control. SA at 75 μ M and 100 μ M have lowered the BTB incidence by more than half and is comparable with the resistant check. This implies that at 75-100 μ M concentrations, SA has most effectively induced resistance in abaca against the incidence of the BTB compared to other concentrations.

Table 2. Incidence of bunchy top disease in the challenged set up after two months from SA application and inoculation

TREATMENTS	DISEASE INCIDENCE (%) **	% DISEASE REDUCTION
Negative Control	100.00	0.00
25 μ M	66.67	33.33
50 μ M	66.67	33.33
75 μ M	33.33	66.67
100 μ M	33.33	66.67
NARC MH83 -1	0.00	100.00

Incubation Period

The number of days from inoculation to the appearance of symptoms in each treatment was recorded. Four assessment periods (14, 28, 42, and 56 days after inoculation) were conducted, with a two-week interval from each time point. Symptoms of the disease were first noted at 14 days after inoculation (DAI), among control plants only (Table 3). At the 28 DAI, it was observed that 25 μ M SA

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had the greatest number of plants that exhibited bunchy top symptoms, followed by 50 μ M and 100 μ M SA concentrations applied. Meanwhile, 75 μ M had the least number of infected plants at the time of observation.

Table 3. Number of abaca seedlings that has been infected per observation time among different treatments

TREATMENTS	NO. OF INFECTED PLANTS					
	0 DAI	14 DAI	28 DAI	42 DAI	56 DAI	NO INFECTION
Negative Control	0	9	12	8	1	0
25 μ M	0	0	5	7	5	13
50 μ M	0	0	4	7	1	18
75 μ M	0	0	2	8	1	19
100 μ M	0	0	3	8	3	16
NARC MH83 -1	0	0	0	0	0	30

However, 25 μ M and 50 μ M SA had fewer infected plants in the third observation period (42 DAI) compared to 75 μ M and 100 μ M, though the differences were not that significant. Upon the last assessment period (56DAI), 50 μ M and 75 μ M SA had the least number of infected plants compared to 25 μ M and 100 μ M SA concentrations. The number of plants where no infection was observed was also assessed, of which 75 μ M and 50 μ M had the highest count compared to 25 μ M and 100 μ M SA concentrations. During the third observation (42 DAI), the number of infected samples peaked. This implies that the treatments applied delayed the treated plants' infection compared to the negative control, where a higher number of infected samples were observed at the first two observation periods (14 & 28 DAI). Another implication is that there could be a resistance break at 42 DAI.

The average number of days from inoculation to symptom appearance was obtained by dividing the sum of all incubation periods by the total number of infected plants. This graph shows that the 100 μ M and 25 μ M SA concentrations had the highest average IP, which signifies that the plants treated with these SA concentrations had the most delayed onset of infection. Though the difference between the said concentrations with 75 μ M SA is not that significant, the latter is lower than the former but is still considerably different from the negative control. These findings do not coincide with the disease incidence and disease severity over time, as it was consistent that 75 μ M SA induces resistance to the plant more effectively than the other concentrations.

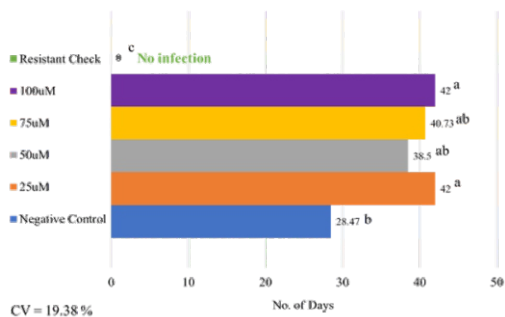


Figure 3. Average number of days of incubation period among infected plants per treatment after 56 days of observation

Quantifying the magnitude or degree of disease infection by determining disease severity, such as BTB, is common. This study used an established rating scale for the severity of BTB modified for the *Inosa* variety (Table 2). The same assessment time points as the incubation period were used to assess the severity of the bunchy top disease in abaca. The time of inoculation was included in the graph to visualize the progress of severity over time in two-week intervals (Fig. 4).

Disease Severity

Among the treatments applied, the 75µM SA concentration consistently resulted in the lowest BTB severity among treated seedlings, while the 25µM SA concentration had the highest severity comparable with the negative control throughout all observation periods (Fig. 4). Two weeks after inoculation, the rating obtained for all treatments was the same since no symptoms had yet been observed. After four weeks of observation, the 100µM concentration had lesser disease severity is less severe than the 50µM concentration. However, the 25µM, 50µM, and 100µM concentrations have the same severity level at 42 DAI. At the last assessment period, 50µM obtained lower severity than the 100µM SA concentration.

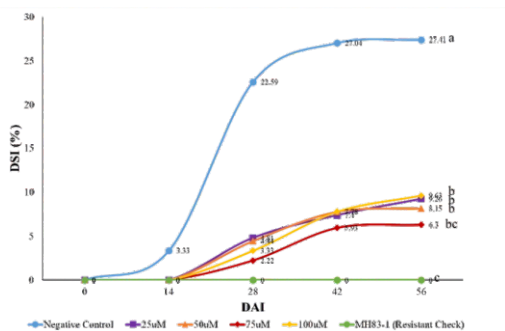


Figure 4. Severity of BTB in abaca seedlings treated with different concentrations of SA

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Overall, the result is consistent with the disease incidence and onset of disease, and the 75 μ M SA concentration reduced the disease severity among the treatments over time. This result implies that the 75 μ M SA concentration can induce resistance against BTB at a significantly lower concentration. Even though there is a presence of infection, plants with a low level of severity can still be harvested and utilized for fiber. SA was found to significantly lower both the incidence and severity of various plant diseases, consistent with the following studies. Plants sprayed with SA are found to have increased levels of endogenous SA, which elicits a cascade of signals to activate resistance response in plants against pathogen infections such as yellow mosaic virus (Radwan et al 2008), soybean mosaic virus (Zhang et al 2019), tomato leaf curl disease (Ong et al 2016) and bean common mosaic virus (Mardani-Mehrabad et al 2020).

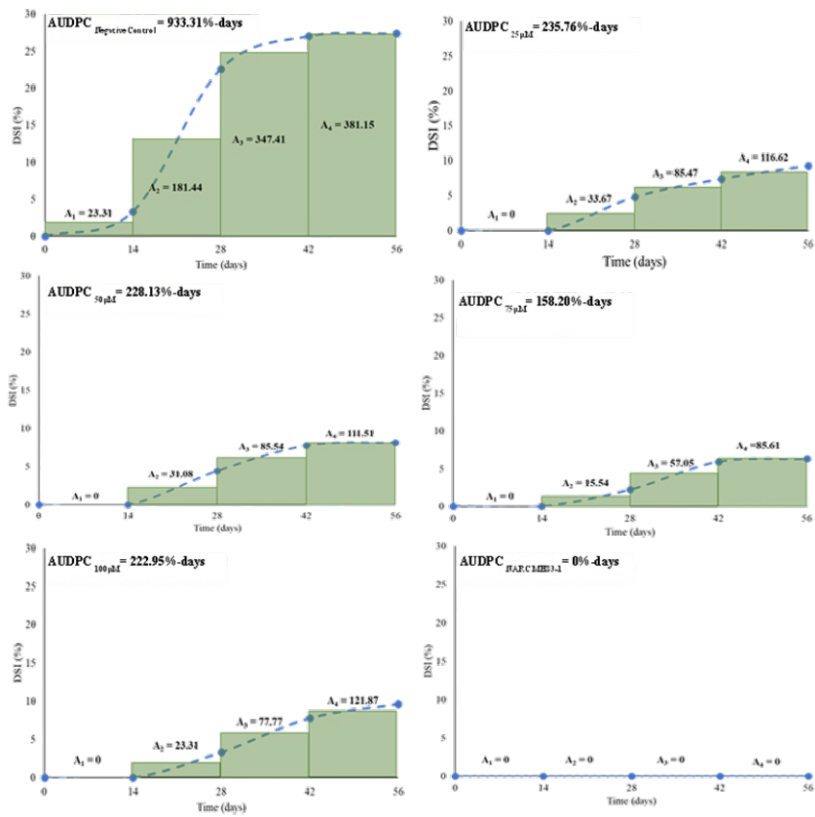


Figure 5. The cumulative area under the disease progress curve of BTB in abaca among different treatments after 56 days from inoculation

The resulting cumulative AUDPC of the different treatments exhibited similar trend in the incidence, onset of disease and severity of infection. The negative control plants model a pattern of BTB infection within 56 days of observation.

There has been exponential progress of BTB during 14 to 28 DAI, and from 28 to 56 DAI, the rate of progress has entered the lag phase. The 75 μ M SA concentration has the lowest total amount of disease over time among all treatments. However, the 100 μ M SA has a comparable mean to 75 μ M throughout the assessment periods. All the concentrations tested, including the negative control, exhibited a monomolecular curve pattern, while the NARC MH83-1 (resistant check) had no curve since the disease was absent. However, Xu (2006) also emphasizes that disease curve models can be affected by many factors, and reliability depends on properly identifying the factors being evaluated.

Table 4. Total amount of disease and % reduction of BTB infection

TREATMENTS	AMOUNT OF DISEASE (% -DAYS)	
	TOTAL AUDPC	% REDUCTION
Negative Control	933.31	0.00
25 μ M	235.76	74.74
50 μ M	228.13	75.56
75 μ M	158.20	83.05
100 μ M	222.95	76.11
NARC MH83 -1	0.00	100.00

This implies that SA can reduce disease progress compared with the negative control tested with time. However, this should not negate the need to reapply the treatment to reinforce resistance for longer periods, as the presence of a disease curve indicates the breakdown of SA-induced resistance to the bunchy top disease. It is crucial if this is applied at the field level since abaca takes approximately two years to mature before it can be harvested for its fiber. Reapplication will be necessary considering the resulting progress of the disease over time, even with the treatment.

Kamle et al (2020) describe SAR as a response to attacks by herbivores or infections; plants produce various chemicals that either lessen or prevent the enemy's onslaught. Plants react locally, in the organ that was initially damaged, and systemically, in other sections of the plant that are unaffected. The primary characteristic of SAR is the molecularly increased expression of pathogenesis-related (PR) genes in the local and systemic tissue, which involves different metabolic pathways.

Salicylic acid is thought to be a crucial signaling molecule that helps plants respond to different pathogenic threats by developing endemic and local disease resistance (Alvarez 2000). An established defense response against different pathogenic diseases is established and signaled by SA, a well-known naturally occurring signaling molecule (Malamy et al 1990; Durner et al 1997), which is also known to induce systemic acquired resistance (SAR) in plants. After a localized infection, some mediator for long-distance communication is needed to induce SAR. SA travels through the phloem of plants from diseased to non-infected organs.

Hayat et al (2010) describe the general mechanism of SA in plants. Different

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biosynthetic pathways in endogenous SA follow long-distance signaling. Upon exogenous application of SA, long-distance signaling is cascaded because of other stressors, which could be biotic or abiotic. Upon entry, methylated SA is more rapidly distributed across cell membranes and is converted to SA upon activation. This results in elevated plant activity in terms of SAR, hypersensitive response, proline, PR proteins, antioxidant activity, and N metabolism, consequently mitigating the stress in the plant system.

The study of Ohashi et al (2004) reported that SA was translocated rapidly through exogenous application at the cut end of the petiole in tobacco plants. Their experiment showed that within 10 minutes, the signal reached six neighboring upper leaves and three adjacent lower leaves, and this signal also accumulated within 50 minutes throughout the entire plant body. This indicates that the smooth and rapid transport of SA signals is enough to allow systemic distribution in the plant body in a short period, consequently providing tolerance to infections.

Singh et al (2004) found that in plants, salicylic acid triggered a series of actions that prevented viral replication and long-distance cell-to-cell transmission. Furthermore, SA alters the activity of ROS metabolism and redox homeostasis in plants. The key mechanism to this is when SA indirectly activates biomolecules by binding with enzymes such as APX, CAT, aconitase, and carbonic anhydrase (Lamb & Dixon 1997; Ruffer et al 1999; Slaymaker et al 2002), which consequently induces a hypersensitive reaction or defense response against viral agents and other pathogenic microbes.

SA does not only induce resistance in plants against pathogen infection but also aids in the recovery of plant from the disease (Kamle et al 2020). Moreover, SA meets the criteria of a good elicitor which should be effective in low concentrations, no biocidal activity, have long-lasting effects, and is cost-effective (Piamonte & Borines 2012). Mohase and van der Westhuizen (2002) reported the potential of SA in inducing resistance reaction against the infestation of Russian wheat aphid (*Diuraphis noxia* Mordvilko) in wheat. These evidences confirm the potential of SA for effective BTM management leading to increased abaca productivity.

CONCLUSION

The application of SA has proved to be effective in inducing resistance to BTM in one-month-old tissue-cultured abaca seedlings. SA at 50-75 μ M significantly reduced the incidence of disease infection as confirmed by molecular detection through polymerase chain reaction. SA at 25, 75 and 100 μ M concentrations most delayed the disease onset, but 75 μ M had the greatest number of uninfected plants among the treatments. The severity of infection was also greatly reduced by SA at 75 μ M. Furthermore, the amount of disease experienced by the sample plants over time (AUDPC) was the lowest at 75 μ M. SA has induced resistance in abaca, causing a significant decrease in the BTM incidence, severity, onset of disease, and rate of progress over time at 75 μ M concentration. Bunchy top disease in abaca can be effectively controlled through the safe, affordable, and environmentally friendly application of SA elicitor.

ACKNOWLEDGEMENTS

The author would like to thank the Department of Science and Technology – Accelerated Science and Technology Human Resource Development Program National Science Consortium (DOST-ASTHRDP-NSC) for funding this study. The author is also grateful for Dr. Robelyn T. Piamonte, Dr. Elvira L. Oclarit and Dr. Mannylen A. Merioles.

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