

Screening of PRSV-P resistance and profiling of defensive secondary metabolites in *Carica papaya* and interspecific hybrid of *Vasconcellea*

ABSTRACT

Aims: To Screen for PRSV-P resistance in *Carica papaya* and interspecific hybrid of *Vasconcellea* (IR) and to determine the secondary metabolites difference between PRSV-P resistant and susceptible papaya using LCMS-QTOF.

Study design: The experiment was carried out using completely randomized design (CRD).

Place and Duration of Study: MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia between January 2017 to June 2019.

Methodology: *C. papaya* lines L33, L90, L13 and Eksotika were germinated from seed whilst IR was imported in tissue culture from Griffith University Australia. Screening for PRSV-P resistance was carried out using completely randomized design in the glasshouse of Agrobiodiversity and Environment Research Centre, MARDI. Percentage of disease incidence and disease severity of inoculated papaya plant were evaluated weekly for eight weeks. The generated data of disease incidence and disease score were then statistically analysed using SAS 9.4 software. Secondary metabolites analysis of *Carica papaya* and interspecific hybrid of *Vasconcellea* (IR) was carried out using LCMS Q-TOF in phytochemical laboratory MARDI. The accurate mass compound in the MassHunter Qualitative analysis was confirmed by hits from existing databases [Metlin - Scripps, PCDL (MassHunter Personal Compound Database and Library - Agilent Technologies)].

Results: Screening for PRSV-P resistance amongst Malaysian local lines showed a significant difference ($\text{Alpha}=0.05$) between line L90 [disease incidence (55%); disease score (1.2)] and the PRSV-P susceptible (Eksotika) over eight weeks observation. IR that was also significantly difference to Eksotika categorized as PRSV-P resistance. In order to profile the defense-related secondary metabolites before and after PRSV-P entry in plant, *C. papaya* (Eksotika) and IR were used to represent PRSV-P susceptible and PRSV-P resistant respectively. IR was used to develop partial resistance to PRSV-P in *C. papaya* via introgression of resistance genes in other study. Analysis of secondary metabolites using LCMS-QTOF detected anthranilic acid (AA) and *p*-aminobenzoic acid (*p*ABA) in *C. papaya* (Eksotika) and IR at day five after inoculation. Interestingly, γ -aminobutyric acid (GABA) was only found in IR at *rt*, 1.881 min based on their accurate mass using LCMS Q-TOF prior to PRSV-P inoculation.

Conclusion: Findings of this study recommended the usage of L90 in future conventional breeding with other local PRSV-P susceptible varieties such as Eksotika that has better taste and market. γ -aminobutyric acid (GABA) that was only found in IR was presumed to be involved in the plant defense response to PRSV-P. It potential to be developed as a resistance chemical marker could be explored in the near future.

Keywords: Defence-related, PRSV-P resistant, PRSV-P susceptible, virus, γ -aminobutyric acid (GABA)

1. INTRODUCTION

Papaya Ringspot Virus type P (PRSV-P) is a devastating disease in papaya industries worldwide. In Malaysia, the first outbreak of PRSV-P was detected in 1991 in the district of Johor Baharu, Johor. Since then, many efforts have been made to overcome the disease and one of which focused on the development of resistant varieties. [1] reported of PRSV-P tolerance for 11 lines that had been screened at the F₅ population of Tainung No 5 x Eksotika. In other research, [2] reported *Carciflora* as a tolerant cultivar in the screening of PRSV-P resistance amongst 31 cultivars and lines of *Carica papaya*. In 2017, the effort was extended to *Vasconcellea* genus in Caricaceae family.

Vasconcellea is the largest genus containing 21 species that are often called 'highland papayas' or 'mountain papayas' [3]. *Vasconcellea* species vary in leaf shape, leaf size, fruit colour, fruit shape, flower colour and flower shape making each species uniquely different from *C. papaya* [4]. *Vasconcellea pubescens* has been consistently reported resistant to PRSV-P. Rapid amplification of cDNA ends-polymerase chain reaction (RACE-PCR) identified a sequence polymorphisms between PRSV-P susceptible (*Carica papaya*) and resistant (*Vasconcellea pubescens*) species [5]. The genetic traits of *V. pubescens* and interspecific hybrid of *V. pubescens* x *V. parviflora* (IR) have been explored by many researchers via breeding programs.

Systemic acquired resistance (SAR) is an induced defence response known to activate plant defense mechanisms against pathogen invasion by the induction of pathogen *Avr* gene. SAR leads to cell wall reinforcement (lignin), an oxidative burst, induction of pathogenesis related (PR) proteins and phytoalexins [6]. Phytoalexins is an antimicrobial chemicals that confer resistance in plants. There are numerous natural products function against predator or microbial pathogens. Different kinds of compounds as well as plant species synthesize vary secondary metabolites (SM) as a plant chemical defense depending on the pathway and sites of production. SM in plants can be divided into three main groups according to their biosynthetic origin: terpenes, nitrogen or sulphur-containing compounds (alkaloids, glucosinolates and cyanohydrins) and phenylpropanoids also known as phenolic compounds [7]. Plant phenols constitute is one of the most common defensive compounds against pests and diseases including root parasitic nematodes [8]. SM generally, but not always, occur in relatively low quantities and their production may be widespread or restricted to particular families, genera, or even species. Higher concentrations of SM might result in a more resistant plant [9] and thus, resistant plants respond more rapidly and more vigorously to pathogens than susceptible plants do.

In this study, the status of PRSV-P tolerant lines of *Carica papaya* that reported by [1] were re-determined using local PRSV-P inoculum collected under nowadays extreme climate and unpleasant

weather. Rather than that, IR that was imported from Australia has been used in this study to explore its endogenous defensive SM compared to *C. papaya* variety Eksotika (PRSV-P susceptible) before and after PRSV-P entry using mass spectrometry. Metabolomics analysis using analytical platform such as Liquid chromatography-mass spectrometry (LC-MS) could capture as many metabolites as possible in the samples. We anticipate defensive SM is available in more resistant plant. Should a defensive SM determined from the disease resistant plant, understanding of its roles could be enhanced and used as an assisted-metabolic marker for disease resistance selection in breeding program in the near future.

2. MATERIAL AND METHODS

Screening for PRSV-P resistance in *C. papaya* and IR

1. Planting of *C. papaya*

Seeds of each *C. papaya* lines (L33, L90, L13) including Eksotika (as a control positive) were germinated on germination tray containing 3:2:1 mixture of top soil: sand: organic manure. Approximately one month after germination, *C. papaya* were transferred into a pot and placed in a glasshouse for further use.

2. Multiplication of IR

Multiplication and acclimatization of imported IR in tissue culture was carried out according to [5] and [10].

3. PRSV-P incidence and severity in *C. papaya* and IR

PRSV-P has a linear stranded positive sense RNA genome. Leaves of PRSV-P infected papaya (inoculum) were extracted using Nucleospin RNA plant kit (Macherey-Nagel) followed by cDNA synthesis using PrimeScript™ RT-PCR kit (Takara) according to their manufacturer's protocol. Subsequently, PCR amplification of the PRSV-P coat protein gene was performed using Cp2 forward primer: 5' CTAACACTCGTGCCACTCAGTC 3' and Cp2 reverse primer: 5' TTCCACTGTGTGTCTCTCCGTG 3'. The derived DNA sequence of this local PRSV-P was registered in National Center for Biotechnology Information (NCBI). Upon confirmation of the PRSV-P presence and sequencing result, mechanical inoculation of PRSV-P towards those tested plants was conducted in a glasshouse. The inoculum was prepared by grinding PRSV-P infected leaves in a mortar and pestle with 0.01 M phosphate buffer, pH 7.0 in a ratio 1:3. For each lines, 3 - 9 seedlings were inoculated depending on the number of seedlings germinated. Eksotika variety was used as a control positive. The leaves were manually dusted with carborundum (600 mesh) and mechanically inoculated by hand rubbing at the 6 to 8 leaf stage. Control negative plants of each line were similarly treated with carborundum and inoculated with virus free 0.01M phosphate buffer. Excess of PRSV-P inoculum was rinse off with running tap water. Plants were kept at relative humidity near 85-90% and temperature between 28°C and 32°C in the day and 25°C and 28°C at

night. PRSV-P symptoms were determined and scored through physiological disorders shown in *C. papaya* leaves, stem and petiole. Evaluation on the degree of severity used for rating is as follows:

0=no symptoms;

1=very mild mottling/ mosaic symptoms and water-soaking streaks on stem, petiole and under leaf surface;

2=severe mottling or mosaic and water-soaking streaks on stem, petiole and under leaf surface;

3=leaf distortion and water-soaking streaks on stem, petiole and under leaf surface;

4= shoe stringing and water-soaking streaks on stem, petiole and under leaf surface.

Plants with no symptoms in 20 days after inoculation were re-inoculated. Percentage of disease incidence and disease severity of inoculated papaya plant was evaluated weekly for eight weeks and rated as following [2].

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plants}}{\text{Total number of plants inoculated}} \times 100$$

$$\text{Disease score} = \frac{\sum (\text{Number of papaya seedlings} \times \text{Degree of severity})^n}{\text{Total number of seedlings}}$$

4. Statistical analysis

The data generated were subjected to statistical analysis using one-way ANOVA without interaction (SAS 9.4). The means significance differences between the papaya lines disease incidence and disease score were then performed using Duncan's multiple range test (the minimum significance was set at $P < 0.05$).

Secondary metabolite profiling in *C. papaya* (Eksotika) and IR

In order to profile the defense-related secondary metabolites before and after PRSV-P entry in plant, *Carica papaya* (Eksotika) and IR were used in this study to represent PRSV-P susceptible and PRSV-P resistant respectively.

1. Extraction of Eksotika and IR leaves from PRSV-P inoculated plants

Inoculation of PRSV-P into Eksotika and IR were conducted similarly as described earlier. Leaves tissues of Eksotika and IR were collected at day 0 (before inoculation), day 5 and 10 (after inoculation) for SM profiling. The extraction was carried out using 0.1 gram dried sample. This samples were mixed with ratio amount of organic solvents (20:79:1) of methanol, isopropanol and acetic acid. Samples were sonicated and centrifuged with 10,000 rpm for 10 minutes. The supernatant was collected and dried. Subsequently, the extract was mixed with 85% water and 15% acetonitrile before injected to LCMS-QTOF.

2. Secondary metabolites analysis using LCMS Q-TOF

To investigate the metabolites differences between Eksotika and IR, sample analysis was performed on an Agilent 1290 Infinity HPLC system (Agilent Technologies) coupled with an Agilent 6540 Accurate-Mass Q-TOF LC/MS system. Samples were placed in cooled autosamplers (4 °C) to maintain sample stability throughout the analysis. Chromatographic separations were performed with Kinetex reverse phase C18 column (4.6 mm × 100 mm; 2.6 μm, Phenomenex). The pump was connected to a gradient quaternary solvent system: A, 0.1% formic acid in H₂O (v/v) and B, 0.1% formic acid in acetonitrile. The gradient was performed at 0.5 mL/min with an initial condition of 5% B and linearly increased to 85% B at 25min followed by a 10 min post-run. The sample injection volume was 5 μL. The instruments detection was carried out by using Dual Agilent Jet Stream ionization source, with electrospray ionization (ESI) positive mode employed for acquisition of mass spectra.

The operation parameters of the mass spectrometer were set as follows, nebulizer pressure of 45 psi, drying nitrogen gas flow of 10 L/min at 300 °C, and sheath gas flow of 12 L/min at 350 °C. Capillary voltage was set to 3500 V and nozzle voltage to 1000 V. Acquisition type for Q-TOF was Auto MS/MS (data-dependent) in High Resolution (4 GHz) mode at a rate of 2 spectra/sec in positive mode. The mass spectra were acquired in positive mode with mass to charge ratio (m/z) ranging from 50 to 1000. A differential analysis of the Q-TOF data was performed using Mass Profiler software (Agilent Technologies). Compounds were identified based on their accurate mass using LCMS-Q-TOF. The identified compound in the MassHunter Qualitative analysis was confirmed by hits from existing databases [Metlin - Scripps, PCDL (MassHunter Personal Compound Database and Library - Agilent Technologies)].

3. RESULTS AND DISCUSSION

Evaluation of PRSV-P resistance in *C. papaya* and IR

Multiplication of IR in tissue culture

IR tissue culture used in this study was imported from Australia. Single node sections from apically dominant plants of IR that cultured on multiplication medium of [10] containing 0.5 μM of each of 6-Benzylaminopurine (BAP), Naphthalene acetic acid (NAA) and 2% sucrose needs at least three months to grow in multiplication media (Figure 1A). Figure 1B shows of dissected shoots from nodal sections that were induced with 10 μM Indole-3-butyric acid (IBA) and 2% sucrose for rooting. Figure 1C shows the rooted shoots of IR plantlets that grow vigorously for approximately two months in individual containers contained of potting mix (vermiculate: sand: top soil in a ratio 1:1:1



Figure 1. Micropropagation stages of imported IR in tissue culture. Single node section from apically dominant plants in multiplication medium (A), Dissected shoots from nodal section in rooting medium (B), Plantlets in potting mix (C).

Screening of PRSV-P resistance in *C. papaya* and IR

PRSV-P-infected papaya leaves used as an inoculum for this study were tested for their purity using PCR to confirm the presence of PRSV-P DNA. The partial sequence of coat protein gene of PRSV-P DNA was then registered in NCBI under accession number MG252957. The symptoms of PRSV-P such as mild mosaic patterns and water soaked lesions on the stems were first observed as early as 10 days after inoculation (dai) in *C. papaya*. Figure 2A shows PRSV-P symptoms in the inoculated plants. Moderate mosaic symptoms developed at 15 dai on the fully developed leaf above the point of inoculation. These mosaic patterns that were normally seen as dark and yellow green then became severe together with leaf distortion and shoe stringing symptoms between 25 to 30 dai. The observed symptoms continuously developed in all the newly emerged leaves above the point of inoculation. On the other hand, all control plants from each line that were inoculated with phosphate buffer without PRSV-P inoculum were free of PRSV-P symptoms up to eight weeks after inoculation (Figure 2B). Interestingly there was no PRSV-P symptom observed in IR up to 90 days after inoculation (Figure 2C).



Figure 2. PRSV-P resistance screening at 15 dai. PRSV-P symptom in susceptible plant (A); PRSV-P free symptom in control plant (B). PRSV-P free symptom in IR plant (C).

Results of three *C. papaya* lines and IR disease incidence percentage in a glasshouse is shown in Figure 3 meanwhile their disease score and resistance level towards PRSV-P is shown in Table 1. Lines with rating 1 - 2 normally exhibited symptoms of mottling, mosaic and water soaking whilst lines with symptom rating 3 - 4 exhibited symptoms of leaf distortion and shoe stringing. In this study, line L13 had 100% disease incidence with disease score 4.0.

Disease resistance is defined as reduction of pathogen growth on or in the plant while tolerant plants exhibit disease damage and symptoms when subjected to similar levels of a pathogen. According to [2] those with disease score above 2.5 was categorized as highly susceptible of which reflected to line L13 and control positive (Eksotika) used in this study. Line L33 although showed 100% disease incidence was categorized as susceptible to PRSV-P as it had disease score 2.4 of which significantly difference to the control (Eksotika). Many researchers reported breeding for PRSV-P within the *Carica* genus has resulted in tolerant varieties only. The only tolerant PRSV-P line with least disease incidence (55%) and low disease score (1.2) was exhibited in line L90. The result showed that line L90 is potential to be used as donor parent in developing PRSV-P tolerant line despite its poor yield and low TSS as reported by [1]. Interestingly the complete resistance against PRSV-P was shown by IR with disease score value of 0. Based on the statistical analysis, both line L90 and IR were significantly difference to the control (Eksotika).

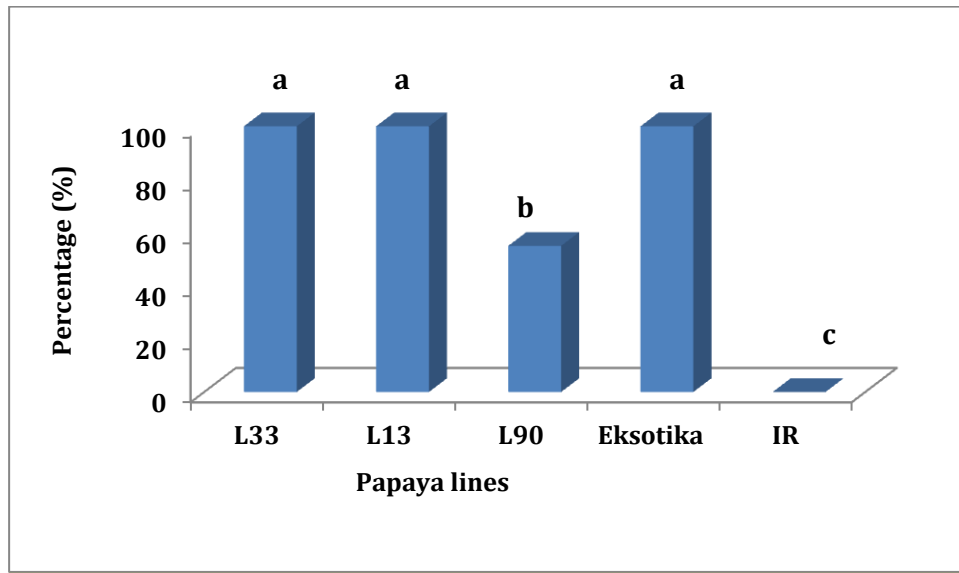


Figure 3. Mean of disease incidence percentage in different *C. papaya* lines and IR towards PRSV-P. Mean covered with the same letter are not significantly different at Alpha=0.05.

Table 1: Disease score and resistance level of different *C. papaya* lines and IR towards PRSV-P. Mean covered with the same letter are not significantly difference at Alpha=0.05.

Lines	Disease score	Resistance level
L33	2.4a	Susceptible
L13	4.0b	Highly susceptible
L90	1.2c	Tolerant
Eksotika (Pos ctrl)	3.7b	Highly susceptible
IR	0.0d	Resistant

There was none completely PRSV-P resistant determined in the screening of local *C. papaya* lines. In practical plant breeding programs, genes for plant disease resistance are frequently identified in non-commercial wild plant relatives. *V. pubescens* has been reported to be resistant against all strains of PRSV-P in all countries for more than half century and thus IR that derived by standard breeding procedures of selection and hybridization [11] was used to represent PRSV-P resistant species in this study. *C. papaya* (Eksotika) that represented PRSV-P susceptible species exhibited PRSV-P symptoms as early as ten days after inoculation differently to IR that was free of PRSV-P symptoms.

IR showed symptoms of hypersensitive response (HR) on the inoculated and first systemic leaves. Yellow spots were observed followed by rapid death cells that resulted in necrotic lesions in the center of the yellow spots (Figure 4A). This was a HR rather than more typical PRSV-P symptoms that occur as yellow spots on inoculated leaves (Figure 4B). The HR that occurred in IR was presumed as defense mechanism that inhibits the growth of pathogens within the infected tissue. HR always occurs in race-specific resistance as a response of rapid and localized cell death in the host to the invading pathogen species that colonizes primarily through the living plant tissue [12]. Cells in the vicinity of the infection synthesize a burst of toxic compounds formed by the reduction of molecular oxygen. Active oxygen species may contribute to host cell death as part of the hypersensitive response or act to kill the pathogen directly. At 45 dai, a complete HR can be seen in IR. HR were not present in the new emerge leaves in contrast to *C. papaya* that displayed PRSV-P symptoms on its newly emerged leaves.

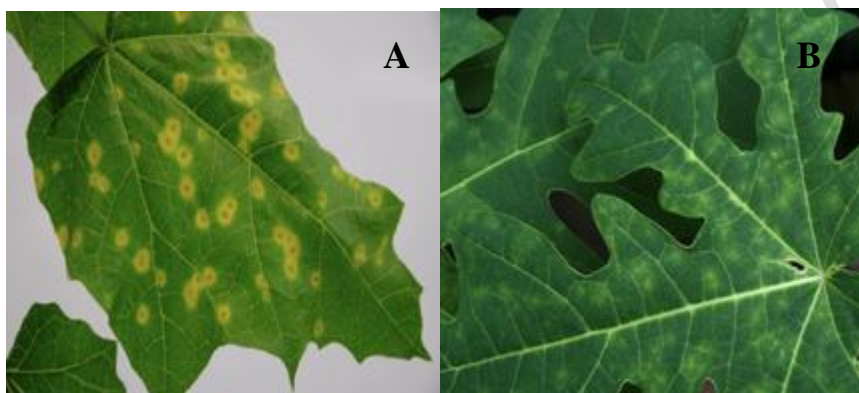


Figure 4. Hypersensitive response (HR) in IR after inoculation with PRSV-P (A). Yellow spot commonly seen as PRSV-P symptom after inoculation in *C. papaya* (B).

Analysis of secondary metabolites related to defense mechanism in *C. papaya* (Eksotika) and IR

SAR is a defence response to pathogen attack in plants. The dramatically increased concentration of Salicylic acid (SA) in plant after pathogen infection has proposed it as one signal leading to SAR [13] and [14] that can elicit the production of secondary metabolites in plants [15] and [16]. In the final step of SA synthesis, benzoic acid (BA) is converted to SA by benzoic acid 2-hydroxylase. Biosynthesis of all plants BA and their products are derived from shikimate pathway. Plant BA encompass BA and all its C6-C1 hydroxy and amino substituents [17].

The leaves tissues of *C. papaya* (Eksotika) and IR at day 0 (before inoculation), day 5 and 10 (after inoculation) were extracted and undergo chemical profiling using LCMS-QTOF. Most of the compounds belongs to the multiple classes of primary and secondary metabolites (SM). This study interest is more to discriminate the compositional differences of SM presence between *C. papaya* and IR as it is more related to the plant defense mechanism towards pathogen attack. Chemical profiling in both

plants revealed the presence of SM corresponded to the plant defense identified as anthranilic acid (AA), p-aminobenzoic acid (pABA) and γ-aminobutyric acid (GABA). Plate 1 showed the presence of those compounds in *C. papaya* and IR detected at different time points.

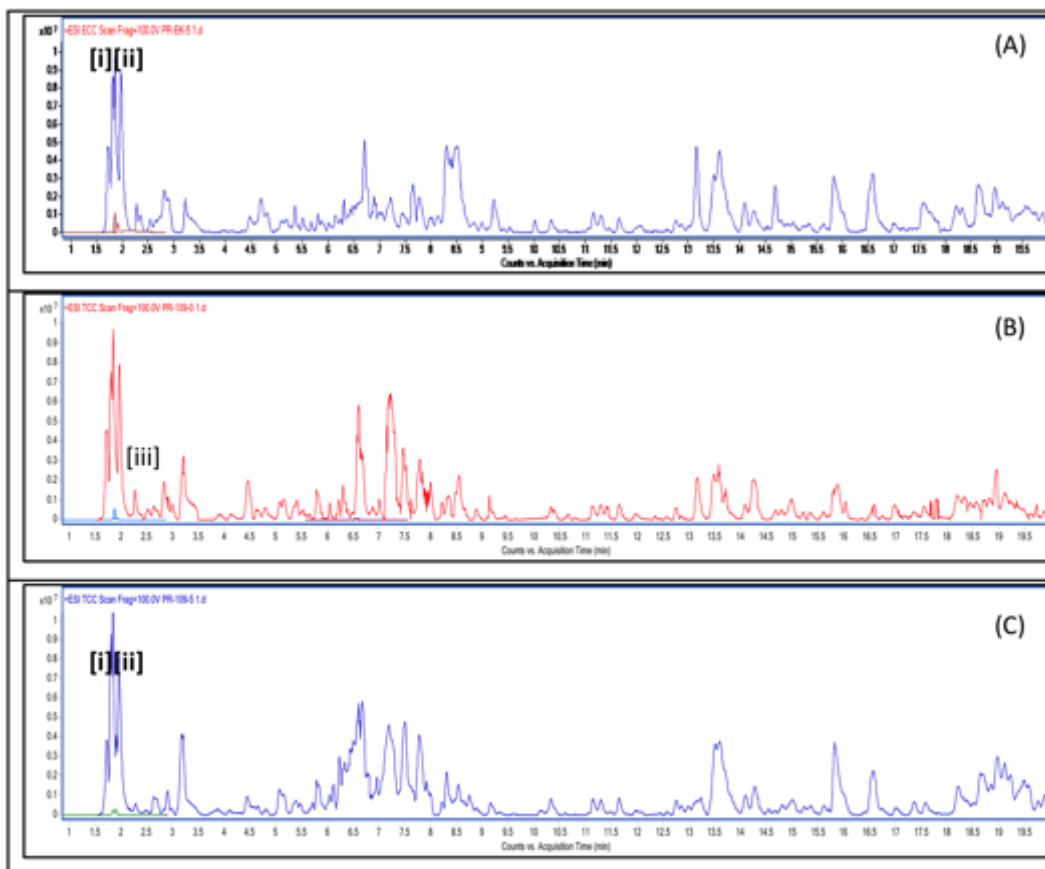


Plate 1: Chromatogram of day 5 after inoculation in *C. papaya* (A); day 0 (before inoculation) in IR (B) and day 5 after inoculation in IR (C). Anthranilic acid, AA indicated by [i], p-aminobenzoic acid (pABA) [ii] and γ-aminobutyric acid (GABA) [iii].

LCMS expansion chromatogram for these compound are shown in Figure 5-7. Chorismic acid that aminated at C-2 will yield an AA. AA was presence in *C. papaya* and IR after virus entry in plants of which represented by the samples collected at day 5 after inoculation (Plate 1A and 1C respectively). Peaks of AA were shown in the expansion chromatogram at retention time (rt), 1.851 min and 1.848 min in *C. papaya* and IR respectively (Figure 5). The presence of pABA was detected in *C. papaya* and IR at day 5 after inoculation (Plate 1A and 1C). The peak of pABA was observed at rt , 1.915 min in *C. papaya* and rt , 1.909 min in IR (Figure 6). GABA was observed only in IR at day 0 before inoculation (Plate 1B). The peak of GABA was observed at rt , 1.881 min (Figure 7).

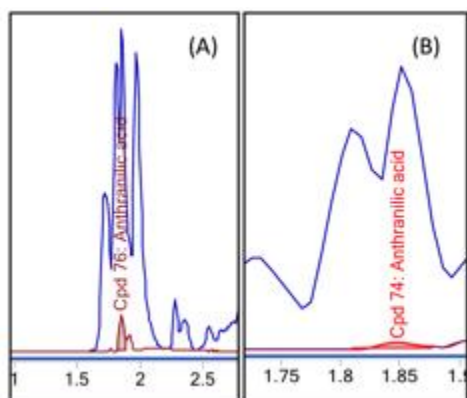


Figure 5. LCMS expansion chromatogram for AA in *C. papaya* (A) and IR(B).

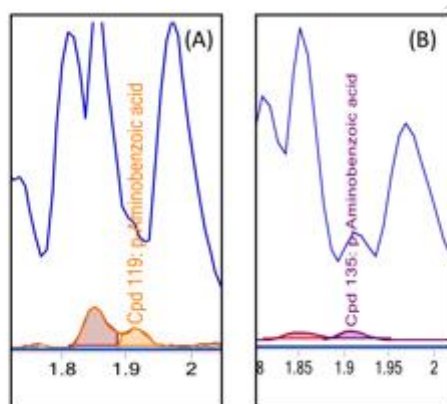


Figure 6. LCMS expansion chromatogram for *p*ABA in *C. papaya* (A) and IR (B).

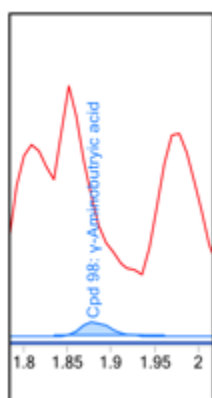


Figure 7. LCMS expansion chromatogram for GABA in IR at day 0 before inoculation.

AA is known to have a similar structure to *pABA* and is produced from the same precursor. *pABA* that is important for the synthesis of folic acid, an irreplaceable vitamin B group component has been reported to activate the synthesis of interferon, which has an important antiviral effect [18]. AA and *pABA* are the plant benzoic acid that is important as the precursor of essential compound in plant. [19] reported that *pABA* was capable to induce resistance against Cucumber mosaic virus and *Xanthomonas axonopodis*. We presumed AA and *pABA* have the capacity to induce SAR in Eksotika and IR grown under green house conditions few days after being infected by PRSV-P. This is supported by the chemical profiling of *C. papaya* (Eksotika) and IR that detected AA and *pABA* only at day five after inoculation of PRSV-P in both samples but not at day 0 (before inoculation). In other study, [20] reported the production of phytoalexin may take two or three days after microbial attack as plant needs to transcribe, translate an appropriate mRNAs and synthesizing enzymes *de novo*. Nevertheless, AA and *pABA* were not sustain up to 10 dai as they were absence in both samples. At this time point, PRSV-P symptoms were started to emerge in susceptible species (Eksotika).

PRSV-P symptoms were not emerged in IR that was detected having endogenous GABA prior to PRSV-P inoculation. GABA accumulation and/or production that commonly reported as plant responses to stress [21] was also demonstrate as an adaptive signal in plant defence resulting of pathogen restriction. GABA can act as protection agent for plant cell against oxidative damage that caused by oxidative burst of which elicit as a defence response against invading pathogens. [22] reported that GABA treatments in transgenic plants expressing SA hydrolase (NahG) partially reversed its hyper susceptibility to citrus exocortis viroid (CEVd). It had been demonstrated in other plant that exogenous treatment of GABA could play an important role in the plant defense. Moreover, exogenously applied GABA is reported capable to enhance the endogenous GABA of which providing the beneficial effects to the plant development and plant growth [23]. On basis of our result, endogenous GABA is worthy to be stated possesses a defensive role against PRSV-P where the absence of it would result in enhanced susceptibility. However additional studies are needed in future to further understand the specific roles of GABA in the defensive mechanism of broad range of plants.

4. CONCLUSION

Findings of this study recommended the usage of L90 in future conventional breeding with other local PRSV-P susceptible varieties such as Eksotika that has better taste and market in future. Secondary metabolites determined in this study were presumed to be involved in the plant defense response. This knowledge would lead to a better understanding of SM function as a new source of resistance to PRSV-P in papaya. Furthermore, the information could be used to facilitate the development of chemical markers in selection of resistant varieties in the plant breeding programs.

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

REFERENCES

1. Chan YK, Ong CA. Field performance of papaya lines selected for tolerance to ringspot virus disease. *Journal Tropical Agriculture and Food Science*. 2003; 31(2): 129–137.
2. Mohamad Roff MN. Disease Rating of Papaya Cultivars to Papaya Ringspot Virus in Malaysia. *Acta Hort*. 2007; 740: 277-282.
3. Scheldeman X, Romero J, Van Damme P. Highland papayas in Southern Ecuador: need for conservation actions. *Acta Hort*. 2001; 575:199–205.
4. Badillo VM. *Caricaceae*. 1993 ; Segundo Esquema. Revista de la Facultad de Agronomía de la Universidad Central, Alcance 43. Maracay, Venezuela.
5. Razean Haireen MR. Identification, Characterisation and Expression of PRSV-P Resistance Genes In *Carica* and *Vasconcellea*. Thesis Ph.D. Griffith University, Queensland, Australia. 2013
6. Hidalgo OB. Basic insight in plant-pathogen interaction. *Biocnologia Aplicada*. 2004; 21:1-4.
7. Croteau R, Kutcahn TM, Lewis NG. Natural products. In *Biochemistry and Molecular Biology of Plants* (Buchanan B, Gruissem W, Jones R, editors). Rockville, MD: American Society of Plant Physiologists. pp. 1250–1318. 2000
8. Wuyts N, De Waele D, Swennen R. Extraction and partial characterization of polyphenol oxidase from banana (*Musa acuminata* grand anine) roots. *Plant Physiology and Biochemistry*. 2006; 44:308-314.
9. Mazid M, Khan TA, Mohammad F. Role of secondary metabolites in defense mechanisms of plants. *Biology and Medicine* 2011; 3 (2) Special Issue: 232-249.
10. Drew RA, Smith NG. Growth of apical and lateral buds of pawpaw (*Carica papaya* L.) as affected by nutritional and hormonal factors. *Journal of Horticultural Science*. 1986; 61:535-543.
11. O' Brien CM, Drew RA. Potential for using *Vasconcellea parviflora* as a bridging species in intergeneric hybridisation between *V. pubescens* and *Carica papaya*. *Australian Journal of Botany*. 2009; 57:592-601.
12. Hammond-Kosack KE, Kanyuka K. Resistance genes (*R*) in plants. *Encyclopedia of Life Science*. 2007; John Wiley & Sons, Ltd. pg 1-21

13. Malamy J, Carr JP, Klessig DF, Raskin I. Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. *Science*. 1990; 250:1002-1004.

14. Métraux JP, Ahl-Goy P, Staub T, Speich J, Steinemann A, Ryals J, Ward E. Induced resistance in cucumber in response to 2,6-dichloroisonicotinic acid and pathogens. In Hennecke H, Verma DPS, editors. *Advances in Molecular Genetics of Plant-Microbe Interactions*, Vol 1. Kluwer Academic Publishers, Dordrecht, The Netherlands. 1991. pp 432-439.

15. Hayat R, Ali S, Amara U, Khalid R, Ahmed I. Soil beneficial bacteria and their role in plant growth promotion: A review. *Ann. Microbiol.* 2010; 60, 579–598. doi:10.1007/s13213-010-0117-1.

16. Pieterse CM, Van Loon LC. Salicylic acid-independent plant defence pathways. *Trends Plant Sci.* 1999; 4(2): 52-58.

17. Widhalm JR, Dudareva N. A familiar ring to it: biosynthesis of plant benzoic acids. *Mol Plant.* 2015; 8(1):83-97.

18. Akberova SI. New biological properties of p-aminobenzoic acid. *Biology Bulletin.* 2002; 29: 390–393.

19. Song GC, Choi HK, Ryu CM. The folate *para* aminobenzoic acid elicits induced resistance against *Cucumber mosaic virus* and *Xanthomonas axonopodis*. *Annals of Botany.* 2013;111: 925-934.

20. Grayer RJ, Harbone JB. A survey of antifungal compounds from higher plants 1982-1993. *Phytochemistry.* 1994; 37:19-42

21. Kinnersley AM, Turano FJ. Gamma Aminobutyric Acid (GABA) and Plant Responses to Stress. *Crit. Rev. Plant Science.* 2000; 19:479–509.

22. López-Gresa MP, Payá C, Rodrigo I, Bellés JM, Barceló S, Choi YH, et al. Effect of Benzothiadiazole on the Metabolome of Tomato Plants Infected by *Citrus Exocortis* Viroid. *Viruses.* 2019; 11, 437.

23. Ramos-Ruiz R, Martinez F, Knauf-Beiter G. The effects of GABA in plants. *Cogent Food & Agriculture.* 2019; 5: 1670553.