ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 10, pp.74440-74448, October, 2018

DOI: https://doi.org/10.24941/ijcr.32696.10.2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ESTABLISHMENT OF SYSTEMIC ACQUIRED RESISTANCE (SAR) IN PAPAYA BY EXTERNAL SALICYLIC ACID APPLICATION AS A STRATEGY TO CONTROL DIEBACK DISEASE

[2]Normahfuzah Husna Samsuddin, [1]*Norliza Abu Bakar, [2,4]Noor Azmi Shaharuddin, [2]Azzreena Mohamad Azzeme, [3]Rozeita Laboh, [1]Rafidah Badrun and [3]Azlan Azizi Muhamad Nor

[1]Biotechnology and Nanotechnology Research Centre, Malaysian Agriculture Research and Development Institute, PO Box 12301, General Post Office, 50774 Kuala Lumpur Malaysia
[2]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Malaysia
[3]Crop and Soil Sciences, Malaysian Agriculture Research and Development Institute, PO Box 12301, General Post Office, 50774 Kuala Lumpur Malaysia
[4]Institute of Plantation Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor Malaysia

ARTICLE INFO

Article History:
Received 20th July, 2018
Received in revised form
25th August, 2018
Accepted 08th September, 2018
Published online 31st October, 2018

Key Words:

Carica papaya, Papaya Dieback Disease, Systemic Acquired Resistance, Pathogenesis-related (PR) Proteins, Salicylic acid.

ABSTRACT

Erwinia mallotivora has been identified as the causal pathogen of papaya dieback disease in Malaysia, and has become one of the major threats to the papaya industry. Pathogenesis-related (PR) proteins are considered as plant defense proteins, where they are capable to prevent or reduce the effect of pathogen and insect attacks. Accumulation of PR proteins is associated with the development of systemic acquired resistance (SAR) in plants. The PR gene expressions can be induced by signaling compounds such as abscisic acid (ABA), ethylene, jasmonic acid (JA), salicylic acid (SA), mechanical wounding, osmotic stress and microbial infection. In this study, salicylic acid (SA) was used as a chemical inducer and their effectiveness in inducing SAR in papaya for enhanced disease resistance to papaya dieback was evaluated. *Carica papaya* was grown in a glasshouse with complete randomized block design (CRBD) arrangement. Salicylic acid (SA) treatment with three concentrations (1 mM, 3 mM, 5 mM) and observation of papaya seedlings physiology were carried out to evaluate the effect of SA on the plant growth. *Erwinia mallotivora* was inoculated to the papaya seedlings after 1 month of SA applications, and evaluation of disease assessment was done weekly. From statistical analysis (proc ANOVA), no significance difference on the seedlings growth was observed when different concentrations of SA applied. As for the disease assessment, highly significantly differences were recorded between the control and the treatments. Among 10 PR genes analyzed using quantitative real Time PCR, only Osmotin, CPBI_13 and CPBI_17 were down regulated for all SA-treatments; whereas increased of expression were observed for the rest of the genes.

Citation: Normahfuzah Husna Samsuddin, Norliza Abu Bakar, Noor Azmi Shaharuddin, Azzreena Mohamad Azzeme. 2018. "Establishment of Systemic Acquired Resistance (SAR) in Papaya by External Salicylic Acid Application as a Strategy to Control Dieback Disease", *International Journal of Current Research*, 10, (09), 74440-74448.

INTRODUCTION

Papaya (*Carica papaya*) is an important herbaceous plant in the family *Caricaceae*. It is cultivated widely for consumption as a fresh fruit or products like drinks and jams candies. It is also a very popular fruit tree because of its nutritious and medicinal qualities. *Carica papaya* continues to be broadly cultivated in most parts around the globe including South-East Asia region, leading to elevated global production because of popular demands from consumers (Evans & Ballen 2012;). This plant is an important crop in Malaysia with an export value of about RM100–120 million per year (Rabu *et al.,* 2005; Abu Bakar *et al.,* 2018). In 2004, Malaysia was the second most important exporter of papaya in the world with a total volume of 58,149 mt accounting for 21% of the global trade. After 2005, Malaysian papaya production declined rapidly due to two reasons which are the outbreak of the bacterial dieback (*Erwinia*) disease and fruit fly quarantine restrictions from China. These situations have affected Malaysian papaya export industry leading to decrease of production by 60% (Chan and Baharuddin, 2010). The disease was caused by *Erwinia*

Corresponding author: **Norliza Abu Bakar,**
Biotechnology and Nanotechnology Research Centre, Malaysian Agriculture Research and Development Institute, PO Box 12301, General Post Office, 50774 Kuala Lumpur Malaysia.

mallotivora (Noriha *et al.,* 2011). *Erwinia mallotivora* (EM) enters its papaya host through stomata and wound openings. Subsequently, the whole areas of the papaya plant including shoot, leaf, frond and bar as well as the fruit are going to be infected leading to early slimy, greasy, water-drenched patches in addition to signs and symptoms around the foliage and petioles. Eventually, this leads to necrosis, blemished or premature fruit drop and death of the papaya plant (Abu Bakar *et al.,* 2017).When attacked by pathogen, plants will defend themselves through activation of defense mechanism which includes oxidative burst of cells, changes in cell wall composition and *de-novo* synthesis of compounds like phytoalexin and increased expression of pathogenesis-related (PR) proteins. Production of PR genes/proteins can lead to increase resistance against pathogen attack (Adrienne and Barbara 2006; Baskaran *et al.,* 2015). The increased resistance of the whole plant is known as Systemic Acquired Resistance (SAR) which is an inducible defense response found in a large range of plant species including papaya (Qiu *et al.,* 2004). PR proteins can be triggered by exposing the plant to virulent, avirulent and nonpathogenic microbes, or artificially with low molecular weight and sometimes volatile molecules such as salicylic acid and methyl jasmonate (Delaney *et al.,* 1994; Xu *et al.,* 1994; Wu and Bradford 2003).

Strategies for combating disease include traditional technologies such as plant breeding and chemical applications. Currently, technologies such as generation of transgenic plants that express components of known defense signaling pathways, application of salicylic acid (SA) analogs for SAR responses and the adaptation of newer technologies such as RNA silencing of pathogen and plant transcripts are also adopted (Matthew *et al.,* 2002). Many of these strategies show great promise and exciting opportunities in the development of new tools for combating plant pests (Matthew *et al.,* 2002). Salicylic acid is a natural signaling molecule naturally found in plants and plays an important role in plant defense against infection by various pathogen (Kawano and Furuichi, 2007; Chaturvedi and Shah 2007). Its role in plant disease resistance has been extensively characterized in dicotyledonous plants, where it is required for basal resistance against pathogens as well as for the inducible SAR response which confers resistance against a broad-spectrum of pathogens. The activation of SAR is associated with heightened level of expression of the pathogenesis-related proteins, some of which possess antimicrobial activity. Studies in the model plant *Arabidopsis thaliana* have provided important insights into the mechanism of SA signaling in plant defense (Chaturvedi and Shah 2007). The induction of SAR using SA has been investigated in the past in plants such as tobacco against tobacco mosaic virus (TMV) (White 1979; Antoniw and White 1980; Sticher *et al.,* 1997) and *Arabidopsis thaliana* (Mauch *et al.,* 2001). Since then, application of SA and its functional analogs, for example, 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole S-methyl ester (BTH) have been found to induce expression of the *PR* genes and confer resistance against viral, bacterial, oomycete and fungal pathogens in a variety of dicotyledonous (Malamy and Klessig, 1992; Ryals *et al.,* 1996; Shah and Klessig, 1999) and monocotyledonous plants (Wasternack *et al.,* 1994; Kogel *et al.,* 1994; Gorlach *et al.,* 1996; Morris *et al.,* 1998; Pasquer *et al.,* 2005; Makandar *et al.,* 2006). In case of virus infections, SA is also shown to promote the inhibition of viral replication, cell-to-cell movement and also long-distance movement of the virus (Singh *et al.,* 2004).

Nevertheless, there is still lack of information and study to determine the effect of salicylic acid as a potential SAR inducer in controlling the papaya dieback disease. This study was conducted to investigate the effect of exogenous salicylic acid (SA) application in papaya seedling and to study their effectiveness in combating papaya dieback disease. This can be achieved through the study of the concentrations of salicylic acid that were applied to papaya and their effect in the expression of selected *PR* genes in papaya.

MATERIALS AND METHODS

Plant growth: *Carica papaya* (Eksotika I) seeds were germinated in the greenhouse in flats containing potting soil. Four-week-old papaya seedlings were divided into four groups of 12 plants each to serve as experimental replicates for disease resistance and induced activity experiments. Each replicate contained four treatment blocks of four plants each, randomized in a complete block design. Plants were grown in the greenhouse MARDI in natural condition (modified from Zhu *et al.,* 2003).

Salicylic acid treatment, pathogen inoculation and observation: The papaya seedlings were treated at three different concentrations of Salicylic Acid treatments, 1 mM, 3 mM and 5 mM through foliar application. To assure complete solubility, each solution was stirred with a magnet bar for at least 1 h and also vigorously shaken before application. Sterile distilled water was used as the non-treated control. The treatment was repeated 1 month after the first spraying. Physiological data on parameters such as stem height and gird size were taken weekly, using Mitutoya Vernier Calliper. For molecular analysis, leaves were collected on day 3, day 10 and day 31 after treatment, frozen in liquid nitrogen, and stored at -80 °C until further analysis (modified from Belhadj *et al.,* 2006). Pathogen inoculation was done one week after the second treatment. Each seedling (at the first three shoot) was inoculated with 10 ml of pathogen (*Erwinia mallativora*) (OD = 0.4 – 0.7) at the infection house.

Symptom evaluation: After inoculation of pathogen, disease assessments which included disease incidence and disease severity were carried out. For disease severity, it was evaluated using score system consisting of; Stage 0: symtompless, Stage 1: leaf vein blackening, Stage 2: leaf vein blackening and slightly wilting, Stage 3: leaf stalk wilting, Stage 4: stem blackening and Stage 5: plant died.

Statistical analysis: Data of stem height and gird size were analyzed using analysis of variance (ANOVA) followed by comparison of means using Duncan Multiple Range Test (DMRT).

Primer Design: Sequences data for PR genes that are associated with SAR especially in papaya were obtained from public databases and publicly submitted information. The gene-specific primers for PCR (**Table 1**) were designed using web-based tools primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/)

Tissue collection and total RNA extraction: Leaf tissue amounting to 30g were grounded to a fine powder using liquid nitrogen, and divided into approximately 100mg into each 2ml tubes. Total RNA was extracted using RNeasy Plant Mini Kit (Qiagen) method, following the manufacture's instruction. For qPCR analysis, small scale RNA isolation were performed for

approximately 100mg grounded leaf tissue followed by DNAse treatment to remove genomic DNA contamination. After the DNAse treatment, 2ul of RNA were viewed using 1% agarose gel. RNA concentrations were estimated after DNAse treatment using NanoDrop 1000v. All RNA was transcripts were converted into cDNA using Biorad Reverse Transcription. The total RNA were stored at -80°C, and while the cDNA were stored in -20°C.

Synthesis of complimentary DNA (cDNA) and PCR analysis: Total RNA was extracted from leaves of four month-old seedlings at day 3 and day 10 after chemical inducer treatments. About 2 ug of DNase treated total RNA were used for first strand cDNA synthesis using iScript™ Reverse Transcription Supermix for RT-qPCR (Biorad). kit. PCR products were separated on 2% agarose gels and stained with ethidium bromide to determine the size of the PCR products.

Primer testing: The performance of the designed primer sets were evaluated according to their standard curve and melting curve. Good quality standard curve was chosen based on the value of primer efficiency which is between 90 –110 and R^2 value which must be as near as possible to the value of 1.0. Good sharp peaks of a melting curve are an indicator of specific primer amplification.

Characterization of PR genes via Real Time PCR: The resulting cDNA (5 ul) were diluted with 20 ul of ultrapure H_2O (scale 1:4) and this dilution were used as template for qPCR. Real Time qPCR were performed using Fast SYBR Green qPCR with 20 mM each primer. Cycling profile was at 95°C (2 mins); 40 cycles of 95°C (5 sec); 60°C (30 s); 95°C (15 sec); 60°C (1 min) followed by denaturation 95°C (15s) for melting curve analysis. The fluorescence thresholds for determining CT were set at 0.15 for all experiments. Primers were designed to amplify fragments of 90–150 bp PCR products using primer design software from Genescript.com.

RESULTS AND DISCUSSION

Physiological parameter analysis: Salicylic acid (SA) has an essential function in regulating plant developmental processes that affect nutrient uptake and their status. This includes vascular differentiation, stem elongation, leaf development and senescence (Rubio *et al.,* 2009). Mineral nutrients are essential for growth and development of plants and microorganisms, as they are important factors in the regulation of various physiological and biochemical processes in plants. How each element affects a plant's physiological and biochemical processes, (positively or negatively), is unique to each plant. In recent years, SA has been in focus of intensive research due to its crucial role in the regulation of physiological and biochemical processes during the entire life span of the plants and its roles in regulating their growth and productivity (Arberg 1981; Rubio *et al.,* 2009). In this study, phenotypic data were taken to see if there was any effect of exogenous application of salicylic acid to papaya plants physiology. A good inducer will not affect the growth of the plant. Based on Table 2, there is no significance difference observed between the control and treated plants for the gird size parameter. Statistical analysis also showed there is no significant relationship between the control and treated plants for stem height. These results proved that foliar application of 1 mM, 3 mM and 5 mM of SA did not affect the growth of papaya. However, these results differ with the study carried out by Chinnasri (2006) where the foliar spray of SA was shown to stunt and reduced pineapple growth by $P \geq 0.05$. This however might be due to the high concentration of SA (10 mM) used during the study. Interestingly, Hussein *et al.,* (2007) reported an enhanced productivity and improvement in all growth characteristics including plant height, number and area of green leaves, stem diameter and dry weight of stem, leaves and of the wheat plant as a whole in their pot experiment where foliage of wheat plants were sprayed with SA,.

Disease Severity Assessment: Forty eight papaya seedlings were observed at a 2-day interval for the recording of the mean disease severity percentage with a method developed from Masood *et al.,* (2010). Disease severity (Table 3) showed highly significant difference between the control and the SA treatments. However there is no difference of severity between the SA treatments according to the same letter of mean value. This can be proved via Figure 1, where on day 6, the symptoms of papaya dieback disease on SA treatments were low at 20%, compared to the control seedling where the severity increased rapidly to 60%. From this result, SA exogenous application was shown to slow the infection rate of *E. mallotivora*. The defence mechanism of plant might be successfully induced by SA treatment. Figure 2 showed the morphology of papaya dieback disease according to the disease severity assessment. Initial disease symptom was brown discoloration, yellowing and necrosis along leaf edges followed by water soaked symptom on the bases of leaf stalks, crown and along leaf mid-ribs **(**Figure 2: A) (Gardan et al., 2004). The other typical external symptoms of papaya dieback disease were bunching of crown leaves, bending of the apical growing point, and chlorosis of crown leaves (Figure 2: B), followed by necrosis of young leaf and stem tissue (Figure 2: C**)** (Glennie and Chapman, 1976). Later, necrotic and water soaked areas developed on the papaya stem and spread to the internal tissues (Figure 2: D). In advanced stages bending of watersoaked leaf stalk occurred, leading to dieback and death of trees (Figure 2: E) (Gardan et al., 2004).

Disease Assessment: Disease Incidence: For the disease incidence analysis (Table 3), there was highly significant relationship between the control and the three SA concentrations used. Control plants, which were untreated, showed faster disease infection rate when compared to SA treated plants. However, no significant differences were observed for the three concentrations used. Graph 2 showed that by day 6, 100% of disease occurred in the control compared to the treated plants where the disease incidence was shown to be lower than 20%.

This evaluation showed that all the treated plants eventually succumb to the disease but there was a different in the time of the treated plants achieving the stage 5. An earlier study has reported that the expression of PR genes were greater in osmotin OsAOS2-overexpressed grain plants, contributing to higher tolerance to rice *Magnaporthe oryzae* pathogen (Mei *et al.,* 2006). This also suggested the roles of PR genes in plant defence. In this current study, gene expression profiles obtained from the qPCR analysis revealed that PR genes were regulated in *E. mallotivora*-infected papaya seedlings at different concentrations of SA as compared to control. Among the 10 PR-genes tested, only one gene showed up-regulation for all the concentrations of SA used (low-1mM, medium-3mM and high-5mM).

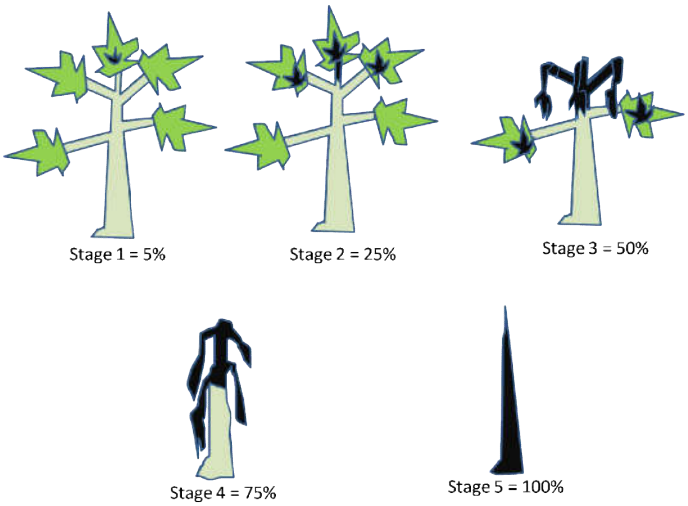


Figure 1. Disease severity (%) on papaya seedling according to scale stage 1 -5 (Asad Masood et al, 2010)

Table 1. Primer sequences of housekeeping and pathogenesis-related genes used in Real Time-PCR analysis.

Target Gene	Forward primer (5'-3')	Reverse primer (3'-5')	Product size (bp)
Pathogenesis-related genes			
Peroxidase (Qiu et al., 2004)	TGCTGCAGTTCCAATGGTAT	TCCCAACATTCCTGCATTTA	121
Osmotin (Qiu et al., 2004)	TCTACTTGCCAAGCTCCAAA	GGGCAATTGTTCCTTACTTGA	132
PR_1b Set2	CGTCCTTTTGGTTTCTGGGA	TTTTTGTTCGTTACTCACACATGA	151
PR_1d Set3	CTCGGTTCGTTTAGGGTGTG	TGGATTATTGGGAAACTTTTATTGA	155
PR1_a (Qiu et al., 2004)	CCCGGAGGATCATAGTTACAAG	ACTCAGGTTGTGTGGAGAAC	98
PR1_c (Qiu et al., 2004)	AATTGGGTGCGCGAGAGT	GGCCTTTCGCCAATGTAATTCC	93
CPBI_3	CCTATTGTTGGGATCGCAGT	GTGCCGGGAAAAGGTTTTAT	185
CPBI_4	CCGTAGGAACTGGCCAACTA	CGATCCTTATTGAGGGGTCA	198
CPBI_13	CCGAGGAAAGTGTTCCAGAG	ACTCCCCAACAGCTGCATAC	162
CPBI_17	CTTGCCAAGCTCCAAAACAT	CCTCGGTTCAACTGTCTTCC	182
Housekeeping gene			
actin	TTCCACTATGTTCCCTGGTATT	TCCTATCCAGACGCTGTATTTC	119
40sRP	TGGCAAAGCCTACAAAGACTATCA	AGGAATGGGAAGGGAGGGAGAT	78

Table 2. Parameter of gird size and stem height of seedling

SA concentration	Gird size of stem (mm)	Stem height (mm)
Control	11.1495^{b}	53.083^{b}
1 mM	11.0453^{b}	54.367^{ab}
3 mM	12.0013^{a}	55.867^{a}
5 mM	11.1925^{b}	53.775^{b}
F-test	ns	ns

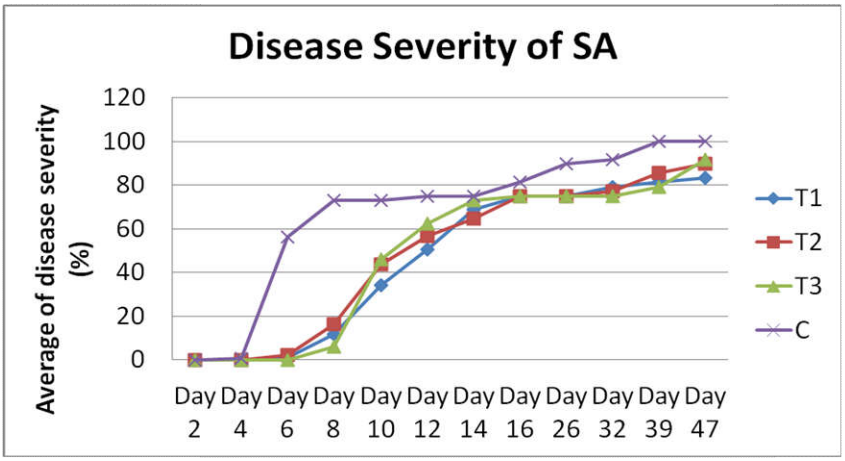
Gird size and stem height showed non-significant difference (F-test: ns) between the control and SA treatments. Means separation within columns and factors by LSD at P≤0.05. Mean values in each column with the same letter are not significantly different at P≤0.05 according to LSD.

Table 3. Observation on disease incidence and disease severity after bacterial inoculation

SA Concentration	Disease incidence (%)	Disease severity (%)
Control	0.58333a	68.264a
1 mM	0.31667b	48.715b
3 mM	0.35000b	48.611b
5 mM	0.30000b	46.606b
F-test	***	***

Disease incidence and disease severity showed highly significant (F-test:***). Mean values in each column with the same letter are not significantly different at P<0.05 according to LSD.

Three genes showed up-regulation at low and medium concentrations, two genes were up-regulated at medium concentration, one gene showed up-regulation at the highest concentration and another genes showed down-regulation for all the concentrations of SA used (Figure 3). *Osmotin*, *PBI_13* and *CPBI_17* gene expressions were downregulated for all concentrations of SA-induced compared to control. In the case where application of 1mM, 3mM and 5mM of SA did not elevate the PR genes expression after inoculation with *E. mallotivora*, there are chances of non occurrence of SAR. Qiu et al (2004) uncovered the *CPBI_17* gene during their EST (Expressed Sequence Tag) analysis which showed similarity to tomato *osmotin* precursor. Both genes were shown to function in plant defense against pathogen attack (Qiu *et al.,* 2004). Another gene, *CPBI_13* was also discovered during the analysis and was shown to be similar with chitinase that function as cell wall fungal hydrolase (Qiu *et al.,* 2004; Abiri *et al.,* 2017).



C=control, T1=1mM, T2=3Mm and T3=5mM.

Figure 1. Disease severity of SA treatment on papaya seedling

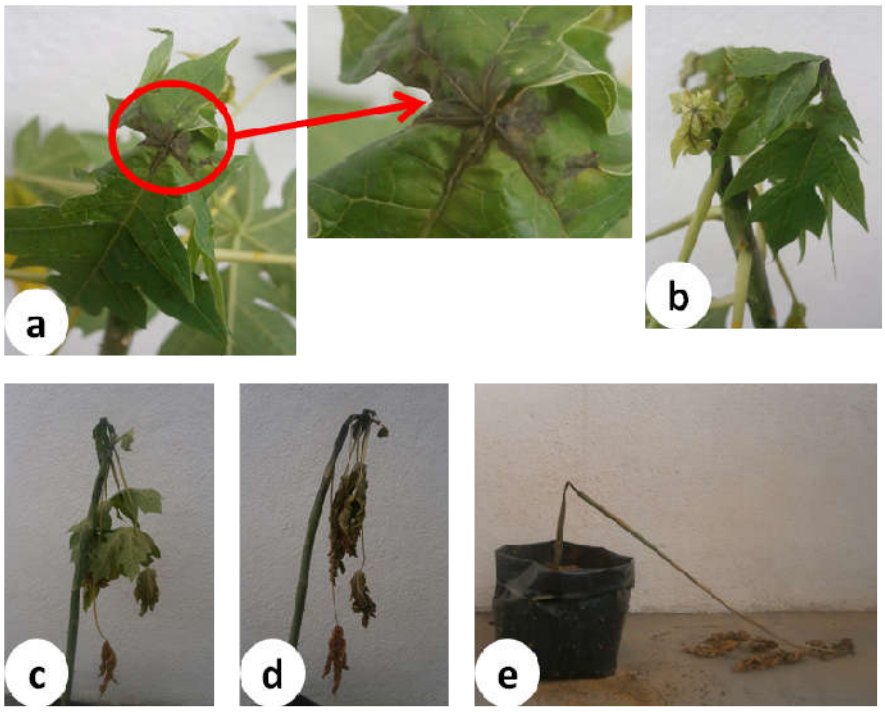
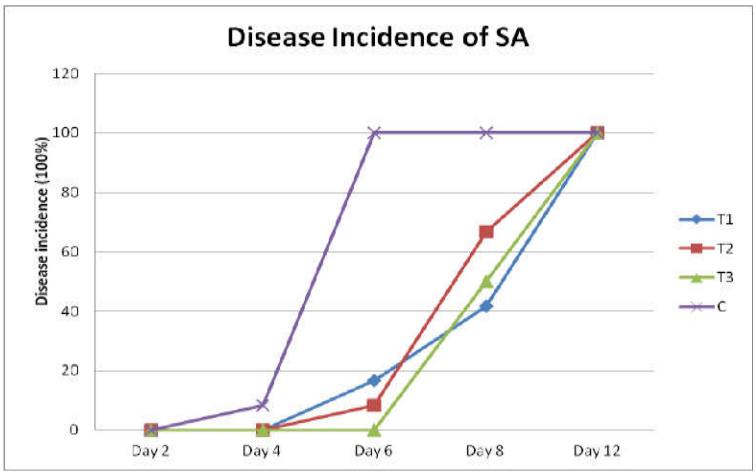


Figure 2. Disease symptom on papaya seeding after *E. mallotivora*-inoculation A) stage 1, B) stage 2, C) Stage 3, D) Stage 4 and E) stage 5



C=control, T1=1mM, T2=3Mm and T3=5mM.

Figure 2. Disease Incidence of SA treatment on papaya seedling

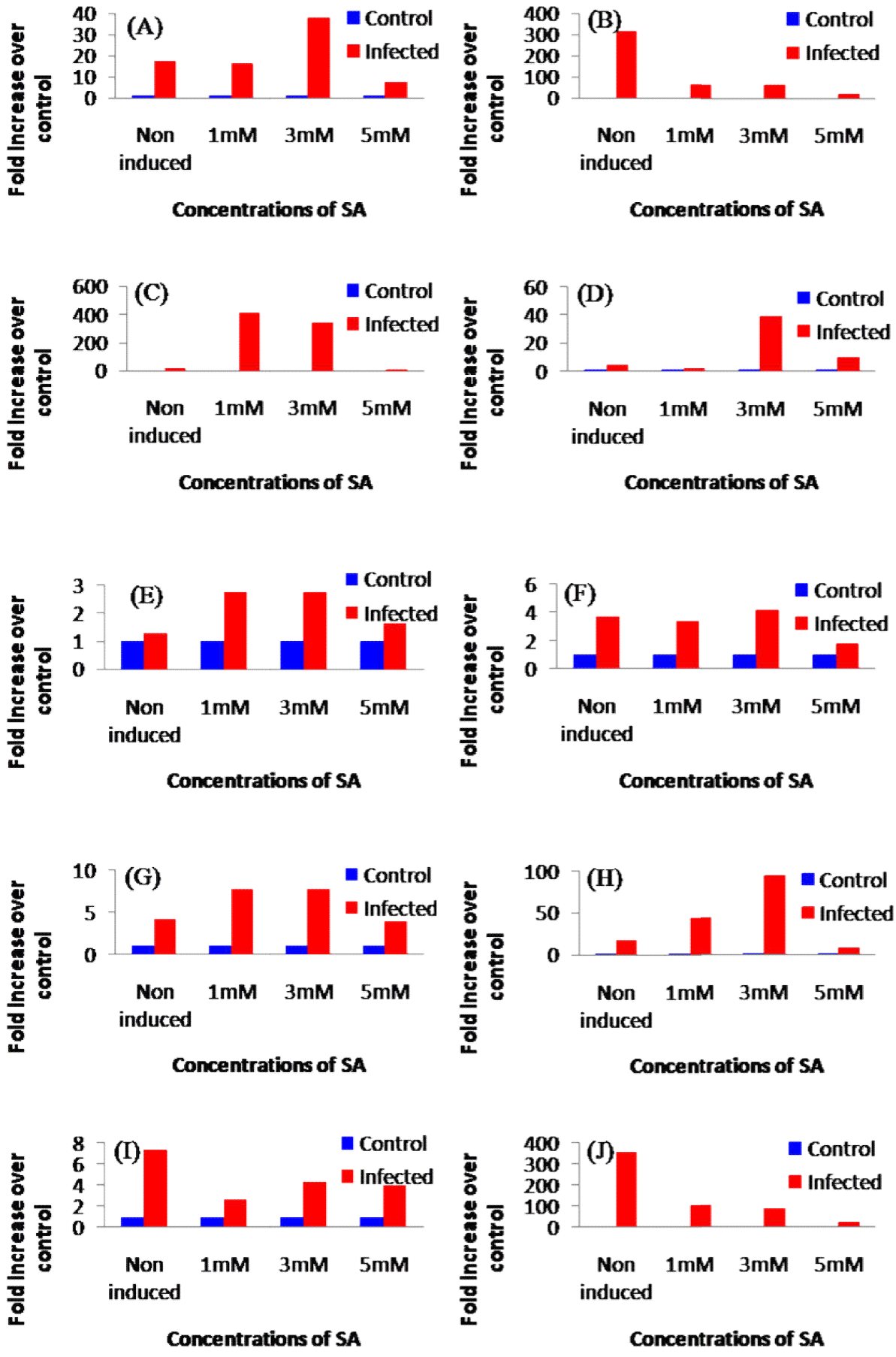


Figure 4. Expression of potential pathogenesis-related genes in *Erwinia mallotivora*-infected papaya leaf and control leaf treated with autoclaved double distilled water: (A) peroxidase, (B) osmotin, (C) *PR_1b Set2*, (D) *PR_1d Set3*, (E) *PR1_a*, (F) *PR1_c*, (G) *CPBI_3*, (H) *CPBI_4*, (I) *CPBI_13* and (J) *CPBI_17*

Peroxidase was only upregulated at medium SA concentration where the expression was 37.9658 fold compared to the control. Peroxidase gene that was used in this study was named as *CPBI_4*. The *CPBI_4* showed up regulation for both low concentration and medium concentration where their expressions were 43.7538 fold and 93.9055 fold respectively. Both Peroxidase and CPBI_4 functioned during oxidative stress response. Nie (2006) and Vidhyasekaran (2015) stated that peroxidase activity was successfully induced by SA treatment and the peroxidase activity has suppressed the replication and accumulation of *Potato virus Y* in tobacco. Peroxidase also has roles in detoxifying ROS and establishing reducing conditions in the cell during pathogen invasion.

PR1_a were found up to be regulated at all concentrations of SA. At the concentrations of 1mM and 3mM, similar level of expressions were observed with 2.7336 fold for 1mM and 2.7292 fold for 3mM. However the fold expression decreased when papaya was induced with high concentrations of SA (5mM) where only 1.626 fold of expression was observed. These results are agreeable to the statement from Kohler et al (2002) where SA at low concentrations were shown to promote faster and stronger activation of gene expression in response to pathogen or microbial elicitors. The process is known as 'priming' and contributes to induce defense mechanisms activation. In previous study, *PAL* gene and *PR_1* gene were induced by SA at the concentrations of 300µM when applied on Arabidopsis plant (Kohler *et al.,* 2002). *PR1a* has similarity with *Arabidopsis* PR-genes (Qiu et al., 2004). Study from van Verk et al., (2008) showed that *PR1* was successfully induced by SA. Apart from *PR1a*, the *PR1* genes are also divided into several groups consisting of *PR1b* to *PR1d* based on their alignment with other known *PR1* genes in other plant species (van Loon and van Strien, 1999). Nie, (2006) proved that SA induced *PR1b* expression. In this study, *PR_1b Set2* was successfully induced by low (1mM) and medium (3mM) concentrations of SA but was down regulated by 5mM of SA. The *PR1b Set2* gene was induced at 408.0386 fold by 1mM of SA and 339.7556 fold by 3mM of SA. However, for 5mM SA, the gene expression decreased to 11.369 fold. It has been shown on previous studies *PR1b* can be induced through application of other SA analog such as BTH (Zhu *et al.,* 2003; Shimono *et al.,* 2007; Jamalnasir *et al.,* 2013). This suggested that low and medium concentrations of SA were the best concentration for the development of SAR in papaya and can successfully be used to induce SAR in papaya seedling for increased protection against papaya dieback disease.

Conclusion

Application of salicylic acid as a synthetic inducer to the papaya seedlings showed no effect on the growth of the seedlings. It was concluded that salicylic acid is safe to be used as SAR chemical inducer. After the inoculation of pathogen, high significant difference was observed between the salicylic acid-treated and untreated plants. Treated papaya seedlings showed increased tolerance to the papaya dieback pathogen. However, there is no significance difference when different concentrations of SA were used. It can be concluded that for positive SAR inducement, a low concentration of salicylic acid is enough to enhance the defense system of papaya to combat papaya dieback disease. This may indicate a degree of improvement towards the tolerance to the pathogen after SA application. However, from the qPCR expression results, 3mM showed the best concentration to use as a SAR inducement, where 7 out of 10 PR genes showed high regulation compared to the control plant. They were *peroxidase*, *PR1b_Set2*, *PR1d_Set3*, *PR1_a*, *PR1_C*, *CPBI_3* and *CPBI_4*. These genes were proposed as a convenient and good candidate for SAR induction marker. *Osmotin*, *CPBI_13* and *CBBI_17* showed down regulation in all treatments. From this study, it was shown that salicylic acid is effective in inducing SAR in papaya.

Acknowledgement

This study was supported partly by MARDI Development Grant PRH405 and Science Fund Grant, MOSTI Grant 02-03-08-SF0380.

REFERENCES

Abiri, R., Shaharuddin, N.A., Mahmood, M., Balia, Z.N.Y, Atabakie, N., Sahebi, M., Valdiani, A., Kalhori, N., Azizi, P., Hanafi, M.M. 2017. Role of ethylene and the APETALA 2/ethylene response factor superfamily in rice under various abiotic and biotic stress conditions. *Environmental and Experimental Botany* 134: 33–44

Abu Bakar, N., Barun, B., Rozano, L., Ahmad, L., Mohd Raih, M.F.R., Tarmizi, A.A. 2017. Identification and validation of putative *Erwinia mallotivora* effectors via quantitative proteomics and Real Time Analysis. *J. Agric. Food. Tech*., 7(9): 10-21

Abu Bakar, N., Sohaime, M.Z., Juri, N.M., Badrun, R., Sarip J. 2018. Induction of Systemic Acquired Resistance in Papaya by Foliar Application of HrpN Recombinant Protein for Increased Resistance against Papaya Dieback Pathogen. Curr Inves Agri Curr Res 2(3)- 2018. CIACR.MS.ID.00013

Adrienne, C.S., Barbara J.H. 2006. Parallels in fungal pathogenesis on plant and animal hosts: Eukaryot. Cell. 5(12):1941–1949.

Antoniw, J.F., White, R.F. 1980. The effects of aspirin and polyacrylic acid on soluble leaf proteins and resistance to virus infection in five cultivars of tobacco. Phytopathol. 98: 331-341.

Arberg, B. 1981. Plant growth regulators. Monosubstituted benzoic acid. Swedish Agriculture Research. 11: 93–105.

Baskaran, G.A., Salvamani, S.A., Ahmad, S.A.A., Shaharuddin, N.A., Pattiram, P.D., Shukor M.Y 2015. Anti HMG-CoA reductase inhibitory activity and phytocomponent investigation of *Basella alba* leaf extract as a treatment for hypercholesterolemia. *Drug Design, Development and Therapy*. 9: 509-517

Belhadj, A., Saigne, N., Telef, S., Cluzet, J., Bouscaut, M.F., Corio-Costet, J.M., Merillon. 2006. Methyl jasmonate induces defense responses in grapevine and triggers protection against *Erysiphe necator. J. Agric., Food. Chem*., 54: 9119-9125

Chan, Y.K., Baharuddin, A.G. 2010. Rejuvenating the flagging papaya industry in Malaysia: The role of MAFC. Acta Hort. (ISHS) 851:37-40 http://www.actahort.org/ books/851/851_2.htm

Chaturvedi, R., Shah. J. 2007. Salicylic acid in plant disease resistance. In Salicylic acid-A Plant Hormone (Eds S. Hayat and A. Ahmad), 335-370. Springer.

Chinnasri, B., Sipes, S. Schmit., D.P. 2006. Effects of inducers of systemic acquired resistance on reproduction of *Meloidogne javanica* and *Rotylenchulus reniformis* in pineapple. *Journal of Nematology*. 38 (3): 319-325.

Delaney, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E., Ryals, J. 1994. A central role of salicylic acid in plant disease resistance. *Science*. 266: 1247-1250.

Edgar, C.I., McGrath, K.C., Dombrecht, B., Manners, J.M., Maclean, D.C., Schenk, P.M., Kazan, K. 2006. Salicylic acid mediates resistance to the vascular wilt pathogen *Fusarium oxysporum* in the model host *Arabidopsis thaliana. Australasian Plant Pathol*. 35:581–591

Evans, E.A., Ballen, F.H. (2012) An overview of global papaya production, trade, and consumption. Topics: Food and Resource Economics, Extension service Institute of Food and Agricultural (IFAS): 1-7.

Gorlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K.H., Oostendorop, M., Staub, T., Ward, E., Kessmann, H., and Ryals, J. 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Plant. Cell*., 8: 629–643.

Hondo, D., Hase, S., Kanayama, Y., Yoshikawa, N., Takenaka, S., Takahashi, H. 2007. The *LeATL6 -associated* ubiquitin/proteasome system may contribute to fungal elicitor-activated defense response via the jasmonic acid-dependent signaling pathway in tomato. Mol. *Plant-Microbe Interact.* 20:72–81

Hussein, M.M., Balbaa, L.K., Gaballah, M.S. 2007. Salicylic acid and salinity effects on growth of maize plants. *Research Journal Agricultural Biology Science*. 3: 321–328.

Jamalnasir, H., Wagiran, A., Shaharuddin, N.A., Samad, A.A. 2013. Isolation of high quality RNA from plant rich in flavonoids, *Melastoma decemfidum* Roxb ex. Jack. *Australian Journal of Crop Science*. 7: 911-916.

Kawano, T., Furuichi, T. 2007. Salicylic acid as a defense related plant hormone; Roles of oxidative and calcium signalling paths in salicylic acid biology. In Salicylic acid-A Plant Hormone (eds S. Hayat and A. Ahmad). 277-321. Springer.

Kim, K.C., Fan, B., Chen, Z. 2006. Pathogen-induced Arabidopsis WRKY7 is a transcriptional repressor and enhances susceptibility to *Pseudomonas syringae*. *Plant Physiol*., 142:1180–1192.

Kogel, K.H., Beckhove, U., Dreschers, J., Münch, S., Rommé, Y. 1994. Acquired resistance in barley. *Plant Physiol*., 106: 1269-1277.

Kohler A., Schwindling, S., Conrath, U. 2002. Benzothiadiazole-induced priming for potentiated responses to pathogen infection, wounding, and infiltration of water into leaves requires the NPR1/N1M1 in Arabidopsis. Plant Physiol. 128: 1046-1056.

Laurie-Berry, N., Joardar, V., Street, J.H., Kunkel, B.N. 2006. The *Arabidopsis thaliana JASMONATE INSENSITIVE 1* gene is required for suppression of salicylic acid-dependent defenses during infection by *Pseudomonas syringae*. *Mol. Plant-Microbe Interact.* 19:789–800

Makandar, R., Essig, J.S., Schapaugh, M.A., Trick, H.N., Shah, J. 2006. Genetically engineered resistance to Fusarium head blight in wheat by expression of *Arabidopsis* NPR1. Mol. Plant-Microbe Inter. 19: 123-129.

Malamy, J., Klessig, D.F. 1992. Salicylic acid and plant disease resistance. *Plant J.,* 2: 643-654.

Masood, A., Saeed, S., Iqbal, N., Malik, M.T., Kazmi, M.R. 2010. Methodology for the evaluation of symptoms severity of mango sudden death syndrome in Pakistan. *Pak. J. Bot.*, 42(2): 1289-1299

Matthew, A.C., Heather, A.F., Pamela, C.R. 2002. Engineering pathogen resistance in crop plants. *Transgenic Research*. 11: 599-613.

Mauch, F., Mauch-Mani, B., Gaille, C., Kull, B., Haas, D., Reimmann, C. 2001. Manipulation of salicylate content in *Arabidopsis thaliana* by the expression of an engineered bacterial salicylate synthase. *Plant J.,* 25: 62-77.

Morris, S.W., Vernooij, B., Titatarn, S., Starrett, M., Thomas, S., Wiltse, C.C., Frederiksen, R.A., Bhandhufalck, A., Hulbert, S., Uknes, S. 1998. Induced resistance responses in maize. *Mol. Plant-Microbe Inter.,*11: 643-658.

Navarre, D.A., Mayo, D. 2004. Differential characteristics of salicylic acid-mediated signaling in potato. Physiol. Mol. Plant Pathol. 64:179–188

Nie, X. 2006. Salicylic acid suppresses *Potato virus Y* isolate N:O-induced symptoms in tobacco plants. *Phytopathology*. 96:255–263

Niki, T., Mitsuhara, I., Seo, S., Ohtsubo, N., Ohasshi, Y. 1998. Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. *Plant. Cell. Physiol*., 39:500–507

Noriha, M.A., Hamidun, B., Rohaiza, A.R., Indu Bala, S.J. 2011. *Erwinia mallotivora* sp., a new pathogen of papaya (*Carica papaya*) in Peninsular Malaysia. *Int. J. Mol. Sci.* 12(1): 39–45.

Pasquer, F., Isidore, E., Zarn, J., Keller, B. 2005. Specific patterns of changes in wheat gene expression after treatment with three antifungal compounds. *Plant, Mol. Biol.,* 57: 693-707.

Qiu, X.H., Guan, P., Wang, M.L., Moore, P.H., Zhu, Y.J., Hud, J., Borthd, W., Albert, H.H. 2004. Identification and expression analysis of BTH induced genes in papaya. *Physiological and Molecular Plant Pathology*. 65: 21–30.

Rabu, M.R., Mat Lin, R. 2005. Prospect of papaya in the world market: Malaysia perspective. In *Proceeding of First International Symposium on Papaya*, Genting Higlands, Malaysia

Rubio, J.S., Garcia-Sanchez, F., Rubio, F., Martinez, V. 2009. Yield, blossom end rot incidence, and fruit quality in pepper plants under moderate salinity are affected by K+ and Ca2+ fertilization. *Scientia Horticulturae*. 119: 79–87.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.Y., Hunt, M.D., 1996. Systemic acquired resistance. *Plant Cell*. 8: 1809–1819.

Shah, J., Klessig, D.F. 1999. Salicylic acid: Signal perception and transduction. In: Hooykaas, P.P.J., Hall, M. A, Libbenga, K. R., eds., Biochemistry and Molecular Biology of Plant Hormones. Amsterdam, Netherlands: Elsevier. 513–541.

Singh, D.P., Moore, C.A., Gilliland, A., Carr, J.P. 2004 Activation of multiple antiviral defense mechanisms by salicylic acid. *Mol. Plant Pathol*., 5: 57-63.

Sticher, L., Mauch-Mani, B., Métraux, J.P. 1997. Systemic acquired resistance. *Annu. Rev. Phytopathol*., 35: 235–270.

Takahashi, Y., Uehara, Y., Berberich, T., Ito, A., Saitoh, H., Miyazaki, A., Terauchi, R., Kusano, T. 2004. A subset of hypersensitive response marker genes, including HSR203J, is the downstream target of a spermine signal transduction pathway in tobacco. *Plant J.,* 40:586–595

Van Loon, L.C., van Strien, E.A. 1999. The families of pathogenesis related proteins, their activities and

comparative analysis of PR-1 type proteins. Physiological and Molecular Plant Pathology. 55, 85-97.

van Verk, M.C., Pappaaioannou, D., Neeleman, L., Bol, J.F., Linthorst, H.J.M. 2008. A novel WRKY transcription factor is required for induction of *PR-1a* gene expression by salicylic acid and bacterial elicitors. *Plant Physiol*., 140:1983–1995

Vidhyasekaran 2015. Salicylic acid signaling in plant innate immunity. In book: Plant Hormone Signaling Systems in Plant Innate Immunity.27-122. Springer.

Wasternack, C., Atzorn, R., Jarosch, B., Kogel, K.H. 1994. Induction of a thionin, the jasmonate-induced 6 kDa protein of barley by 2,6,-dichloroisonicotinic acid. *J. Phytopath.* 140: 280-284.

White, R.F. 1979. Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. *Virology*. 99: 410-412.

Wu, C., Bradford, K.J. 2003. Class I chitinase and â-1,3-glucanase are differentially regulated by wounding, methyl jasmonate, ethylene and gibberellin in tomato seeds and leaves. *Plant Physiol*., 133: 263–273.

Xing, D.H., Lai, Z.B., Zheng, Z.Y., Vinod, K.M., Fan, B.F., Chen, Z.X. 2008. Stress- and pathogen-induced *Arabidopsis* WRKY48 is a transcriptional activator that represses plant basal defense. Mol. Plant 1: 459–470

Xu, Y., Chang, P.L., Liu, D., Narasimhan, M.L., Raghothama, K.G., Hasegawa, P.M., Bressan R.A. 1994. Plant defense genes are synergistically induced by ethylene and methyl jasmonate. *Plant Cell*. 6: 1077-1085

Zhu, Y.J, Qiu, X., Moore, P.H., Borth, W., Hu, J., Ferreira, S., Albert, H. 2003. Systemic acquired resistance induced by BTH in papaya. *Physiological and Molecular Plant Pathology*. 63: 237-248.

****