EFFICACY OF SALICYLIC ACID ELICITOR TO INDUCE SYSTEMIC RESISTANCE AGAINST BACTERIAL LEAF BLIGHT ON RICE

Wannaporn Thepbandit

A Thesis Submitted in Partial Fulfillment of the Requirements for the

Degree of Doctor of Philosophy in Crop Science

Suranaree University of Technology

Academic Year 2019

ประสิทธิภาพของกรดซาลิไซลิกอิลิซิเตอร์ในการชักนำความต้านทานต่อโรคขอบใบแห้งของข้าว

นางสาววรรณพร เทพบัณฑิต

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาพืชศาสตร์
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2562

EFFICACY OF SALICYLIC ACID ELICITOR TO INDUCE SYSTEMIC RESISTANCE AGAINST BACTERIAL LEAF BLIGHT ON RICE

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

Thesis Examining Committee

(Asst. Prof. Dr. Thitiporn Machikowa)
Chairperson

(Asst. Prof. Dr. Natthiya Buensanteai)
Member (Thesis Advisor)

(Assoc. Prof. Dr. Dusit Athinuwat)
Member

(Asst. Prof. Dr. Sodchol Wonprasaid)
Member

(Dr. Piyaporn Phansak)
Member

(Assoc. Prof. Flt. Lt. Dr. Kontorn Chamniprasart)
Vice Rector for Academic Affairs and Internationalization

(Prof. Dr. Neung Teaumroong)
Dean of Institute of Agricultural Technology

วรรณพร เทพบัณฑิต : ประสิทธิภาพของกรดซาลิไซลิกอิลิซิเตอร์ในการชักนำความต้านทานต่อโรคขอบใบแห้งของข้าว (EFFICACY OF SALICYLIC ACID ELICITOR TO INDUCE SYSTEMIC RESISTANCE AGAINST BACTERIAL LEAF BLIGHT ON RICE) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.ณัฐธิญา เบือนสันเทียะ, 185 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อทดสอบประสิทธิภาพของกรดซาลิไซลิกทางการค้า SA-Ricemate® เพื่อในการกระตุ้นความต้านทานให้กับข้าวพันธุ์ขาวดอกมะลิ 105 เพื่อควบคุมโรคขอบใบแห้งที่เกิดจากเชื้อแบคทีเรีย *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) งานวิจัยนี้แบ่งออกเป็น 3 ส่วน ได้แก่ 1) การศึกษากลไกของสิ่งกระตุ้น SA-Ricemate® ให้เกิดกลไกการชักนำความต้านทานต่อโรคขอบใบแห้งข้าวในสภาพเรือนทดลอง 2) การประเมินฤทธิ์ต้านเชื้อแบคทีเรียของสิ่งกระตุ้น SA-Ricemate® เพื่อควบคุมเชื้อสาเหตุโรคขอบใบแห้งข้าว และ3) การประเมินความรุนแรงของโรคและผลผลิตในสภาพไร่ ผลการทดลองพบว่า SA-Ricemate® สามารถลดความรุนแรงของโรคขอบใบแห้งในสภาพเรือนทดลองประมาณ 60% โดยพบว่ามีความสัมพันธ์กับการเพิ่มขึ้นของสารประกอบที่เกี่ยวข้องกับความต้านทานต่อการเข้าทำลายของเชื้อสาเหตุโรคในพืช ได้แก่ ไฮโดรเจนเปอร์ออกไซด์ มาลอนไดแอลดีไฮด์ กรดซาลิไซลิกภายในพืช และคลอโรฟิลล์ เท่ากับ 61, 65, 54 และ 24% ตามลำดับ นอกจากนี้การวิเคราะห์การเปลี่ยนแปลงของสารชีวเคมีภายในใบข้าวที่ระดับมีโซฟิลล์เซลล์ด้วยเทคนิค Synchrotron Radiation-based Fourier Transform Infrared Microspectroscopy (SR-FTIR) แสดงให้เห็นการเปลี่ยนแปลงของโครงสร้างโมเลกุลชีวภาพซึ่งสามารถสังเกตได้จากการเปลี่ยนแปลงทางชีวเคมีขององค์ประกอบของกลุ่มไขมัน เพคติน โปรตีน เอไมด์ I และเอไมด์ II สูงขึ้น ในขณะที่กลุ่มโพลีแซ็กคาไรด์ต่ำกว่าในตัวอย่างที่ได้รับการกระตุ้นความต้านทาน ผลการทดลองนี้ชี้ให้เห็นว่า SA-Ricemate® สามารถลดความรุนแรงของโรคได้โดยกระตุ้นสารตัวกลางที่ทำหน้าที่ส่งสัญญาณความต้านทานโดยกลุ่มโปรตีนและไขมันที่เป็นไปได้ นอกจากนี้การใช้ SA-Ricemate® ในระดับความเข้มข้นตั้งแต่ 200 ppm สามารถยับยั้งการเจริญเติบโตของ *Xoo* โดยให้ฤทธิ์ต้านเชื้อแบคทีเรียที่มีความเข้มข้นสูง และส่งผลต่อการสะสมไบโอฟิมล์ของเชื้อแบคทีเรีย *Xoo* ลดลง 23-100% และยับยั้งการผลิตโพลีแซคคาไรด์นอกเซลล์ 53-100% อีกทั้งพบว่า SA-Ricemate® ทำให้เซลล์สูญเสียการเคลื่อนไหวส่งผลให้การเคลื่อนย้าย บุกรุก และการยึดเกาะลดลง การวิเคราะห์การเปลี่ยนแปลงสารชีวเคมีของเซลล์ *Xoo* ชี้ให้เห็นว่า SA-Ricemate® ส่งผลให้กรดนิวคลีอิก ฟอสโฟลิปิด และ โพลีแซคคาไรด์ ซึ่งเป็นองค์ประกอบของเซลล์แบคทีเรียลดลง นอกจากนั้นการทดสอบความสามารถของ SA-Ricemate® ในการยับยั้งโรคขอบใบแห้งในสภาพไร่ พบว่าลดความรุนแรงของโรคลงอย่างมีนัยสำคัญโดยประมาณ 40-70% และผลผลิตเพิ่มขึ้น 25-60% ผลการศึกษาเหล่านี้ชี้ให้เห็นว่า SA-Ricemate® เป็นสารควบคุมโรคที่มี

ประสิทธิภาพในการควบคุมโรคขอบใบแห้งของข้าวโดยกระตุ้นกลไกความต้านทานและลดปัจจัยรุนแรงของเชื้อสาเหตุโรค

สาขาวิชาเทคโนโลยีการผลิตพืช
ปีการศึกษา 2562

ลายมือชื่อนักศึกษา_____
ลายมือชื่ออาจารย์ที่ปรึกษา_____

WANNAPORN THEPBANDIT : EFFICACY OF SALICYLIC ACID ELICITOR TO INDUCE SYSTEMIC RESISTANCE AGAINST BACTERIAL LEAF BLIGHT ON RICE. THESIS ADVISOR : ASST. PROF. NATTHIYA BUENSANTEAI, Ph.D., 185 PP.

BACTERIAL LEAF BLIGHT/ELICITOR/INDUCED RESISTANCE/SALICYLIC ACID/PLANT HEALTH

The aim of this research was to investigate the efficacy of the salicylic acid elicitor SA-Ricemate® in inducing resistance against the bacterial leaf blight disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) in rice cv. Khao Dawk Mali 105 (KDML 105). This research was divided into 3 parts: 1) study of the SA-Ricemate® mechanism to induce resistance on rice under greenhouse conditions; 2) pathogenicity assessment of *Xoo* on SA-Ricemate® treated samples in vitro; 3) evaluation of disease severity and yield production under field conditions. SA-Ricemate® can reduce disease incidence in the greenhouse at approximately 60% by increasing rice hydrogen peroxide, malondialdehyde, endogenous salicylic acid, and chlorophyll contents with 61, 65, 54, and 24%, respectively. Besides, the analysis of biochemical changes in rice mesophyll by using the Synchrotron Radiation-based Fourier Transform Infrared Microspectroscopy (SR-FTIR) technique showed variations in the bio-molecular structure which can be observed as sub-cellular biochemical changes with higher-intensity signals of lipid, pectin, protein amide I and amide II groups; whereas polysaccharides were lower in the treated samples. These findings suggest that SA-Ricemate® can minimize the severity of the disease by activating intermediated compounds as a defense signaling composed by a potential protein and

lipid community. Furthermore, the use of SA-Ricemate® treatments at concentrations of ≥ 200 ppm on rice plants can inhibit the growth of *Xoo* by supplying high concentrations of antibacterial activity. Moreover, the *Xoo* biofilm formation was substantially decreased with inhibition levels of 23-100%, and by extracellular polysaccharide inhibition levels of 53-100%. The loss of cell motility, which is needed for cell migration and adhesion, was observed in SA-Ricemate® treated samples in which *Xoo* cell components resulted in a lower-intensity of nucleic acid, phospholipids, and polysaccharides. The SA-Ricemate® treatment significantly reduced disease severity by 40-70% and produced a yield increase of 25-60% in field trials. These findings suggest that SA-Ricemate® is a viable antibacterial agent for controlling bacterial leaf blight disease of rice by mediating rice resistance mechanism in order to reduce *Xoo* virulence factor.

School of Crop Production Technology Student's Signature_____

Academic Year 2019 Advisor's Signature_____

ACKNOWLEDGMENTS

This research could not have been accomplished without the support of the Research and Researchers for Industries (RRI), The Thailand Research fund under Grant [PHD58I0071] and Bioactive Agro Industry Co., Ltd. I would like to express my sincerest appreciation and deepest gratitude to all the following individuals:

My thesis advisor, Asst. Prof. Dr. Natthiya Buensanteai, for providing me with the great opportunity to pursue my graduate research, conducting all the experiments. Her kindness support, guidance, patience, understanding, and numerous hours spent on editing this thesis and other papers are valuable treasures for me forever. My co-advisor, Assoc. Prof. Dr. Dusit Athinuwat, for providing me his profound knowledge and experiences shared, support, understanding, trust and appreciation.

I would like express thanks to Dr. Sopone Wongkaew, Dr. Toan Le Thanh, for their kindness, instructions and assistance. Dr. Kanjana Thumanu, Dr. Supatcharee Siriwong, for consultation and facilities on fourier transform infrared spectroscopy (FTIR) analysis at The Synchrotron Light Research Institute under the supervision of the Ministry of Science and Technology of the Royal Thai. Ms. Nattaya Thongprom, Ms. Nitchada Jimkuntod, Ms. Rungthip, Sangpueak, Mr. Chanon Saengchan, and all members of Plant Pathology and Biotechnology Laboratory, for their being very supportive in terms of experimental materials.

Wannaporn Thepbandit

CONTENTS

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

LIST OF TABLES

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF ABBREVIATIONS

ANOVA	=	Analysis of variance
ASM	=	Acibenzolar-S- methyl
BLB	=	Bacterial leaf blight
BTH	=	Benzo[1, 2, 3] thiadiazole-7-carbothioic acid S-methyl ester
CRD	=	Completely randomized design
DAI	=	Days after inoculation
DAPG	=	2,4-diacetylphloroglucinol
DAS	=	Days after sowing
DGDG	=	Digalactosyl diacylglycerol
DMRT	=	Duncan's Multiple Range Test
DSF	=	Diffusible signal factor
EPS	=	Extracellular polysaccharides
ET	=	Ethylene
FTIR	=	Fourier transform infrared spectroscopy
G	=	Guaiacyl
G3P	=	Glycerol-3-phosphate
H	=	Hydroxyphenyl
H	=	Hour
HAI	=	Hours after inoculation
hpa	=	hrp-associated

LIST OF ABBREVIATIONS (Continued)

HR	=	Hypersensitive response
hrc	=	hrp-conserved
hrp	=	Hypersensitive response and pathogenicity
IAA	=	Indole-3-acetic acid
INA	=	2,6-dichloroisonicotinic acid
ISR	=	Induced systemic resistance
JA	=	Jasmonic acid
KDML105	=	Khao Dawk Mail 105
LPS	=	Lipopolysaccharides
MAPK	=	Mitogen activated protein kinases
MAPKK	=	MAPK kinase
MAPKKK	=	MAPKK kinase
mg L^{-1}	=	Milligram per milliliter
µL	=	Microliter (s)
µm	=	Micrometer (s)
MGDG	=	Monogalactosyl diacylglycerol
min	=	Minute (s)
mL	=	Milliliter (s)
mM	=	Millimolar
MV	=	Midvein
NADPH	=	Nicotinamide adenine dinucleotide phosphate

LIST OF ABBREVIATIONS (Continued)

NBT	=	Nitroblue tetrazolium
NIP1	=	Necrosis-inducing protein
nm	=	Nanometer
NO	=	Nitrite oxide
NGA	=	Nutrient glucose agar
NGB	=	Nutrient broth containing 2% glucose
O2 -	=	Superoxide anion
OD	=	Optical density
pal	=	Phenylalanine ammonia lyase
PBS	=	Phosphate-buffered saline
PCA	=	Principal Component Analysis
PPBL	=	Plant Pathology and Biopesticide Laboratory
ppo	=	Polyphenol oxidase
PR proteins	=	Pathogenesis-related proteins
PSA	=	Peptone sucrose agar
QS	=	Quorum sensing
ROS	=	Reactive oxygen species
rpf	=	Regulation of pathogenicity factors
rpm	=	Round per minute
SA	=	Salicylic acid
SAG	=	Salicylic acid glucoside
SAMe	=	Methyl SA

LIST OF ABBREVIATIONS (Continued)

SAR	=	Systemic acquired resistance
SE	=	Standard error
SV	=	Secondary vein
T3SS	=	Type III secretion system
TF	=	Transcription factor
UHCA	=	Unsupervised hierarchical cluster analysis
WAKs	=	Wall-associated kinases
Xoo	=	Xanthomonas oryzae pv. oryzae

CHAPTER I

INTRODUCTION

1.1 Background of the selected topic

Rice *(Oryza sativa*) is one of the most important food crops in the world, especially in Asian countries (Nayar, 2014; Kanlayavattanakul, Lourith, and Chaikul, 2016; Le et al., 2017). In 2016/2017, the total global consumption of milled rice amounted to approximately 475 million metric tons (Thai Rice Exporters Association, 2017). Thailand is the second-largest exporter of rice in the world with 10 million metric tons which value has amounted to 106,785 million baht (3,353 million USD) per year (Thai Rice Exporters Association, 2017; Food and Agriculture Organization of the United Nations, 2015). The rice demand is continuously growing whereas cultivated areas have been decreasing due to the growth of urbanization and industry (Nation, 2014; Forssell, 2018). In addition, rice production is being affected by several factors such as poor soil quality, nutrient deficiency, pests, and diseases leading to a reduction of rice yield (Keeratipipatpong, 2010; FAO, 2015; Kawasaki and Herath, 2018; Timsina et al., 2018). In the current year, an outbreak of rice disease has occurred in Thailand including rice blast disease caused by *Pyricularia oryzae Cavara)*, bacterial leaf streak caused by *Xanthomonas oryzae* pv. *oryzicola*, brown spot disease caused by *Bipolaris oryzae* and dirty panicle disease caused by *Curvularia lunata* and *Cercospora oryzae.* Particularly, the major bacterial disease of rice is bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*)

that can cause a yield reduction of approximately 30-100% worldwide (Shimono et al., 2011; Panuwet et al., 2012; Le et al., 2017). In Thailand, jasmine rice has been the most important aromatic variety which official name is Khao Dawk Mali 105 (KDML 105). This rice variety is very susceptible to BLB disease causing between 30 and 70 percent of yield loss when attacked by the pathogen (Pinta1, Toojinda, Thummabenjapone, and Sanitchon 2013; Sombunjitt et al., 2017; Vanavichit et al., 2018). The adopted approach for rice disease management has been based on the use of several chemicals such as copper-hydroxide, copper-oxychloride, copper-sulfate, streptomycin, aureomycin, and acetylene. In the current year, the use of pesticides to control plant diseases engages approximately 1.8 billion farmers worldwide and the use of more than 5.6 billion pounds of pesticides (Singh et al., 1980; Weichenthal, Moase, and Chan, 2010; Khan et al., 2012; Panuwet et al., 2012). Pesticides are causing damage to the ecosystem balance which affects soil properties, microorganisms, and natural enemies are leading to the destruction of ecology and biodiversity (Fuller, 2005; Domene, 2016). There are many living organisms such as animals, insects, soil microorganisms, aquatic organisms, and fauna which are under the threat of harmful pesticides preventing their survival (Panuwet et al., 2012; Mahmood et al., 2015). Moreover, pesticide exposure can also cause a wide range of human health problems including metabolism impairment, neurotoxicity, carcinogenicity, reproductive and endocrine disruption, immune dysfunctions, and chronic toxic injuries by inhalation or skin absorption (Chatzi et al., 2007; Nicolopoulou-Stamati et al., 2016). Approximately 25 million agricultural workers worldwide are affected by pesticide poisonings (Aktar, Sengupta, and Chowdhury, 2009; Weichenthal et al., 2010; Alavanja et al., 2015). Pesticides have been annually

imported to Thailand at about 164,538 tons; 22,000 million Baht which have been increasing to approximately 198,317 tons; 27,922 million Baht in 2017 (Bay and Heshmati, 2016; Office of Agriculture Economic, 2017). The reported cases on agricultural pesticide toxicity during 2007-2013 show a morbidity rate of approximately 76.4 to 96.6 percent from an observed sample of 100,000 people (Tawatsin and Siriyasatien, 2015). In 2015, the Ministry of Public Health reported cases of the toxic effects of pesticides during 2007-2013 that were predominantly found in the central region of Thailand with a 31-36% of the cases followed by the Northeastern region with 27-31%, the North with 18-20%, and the south with 18-19%, respectively (Chatzi et al., 2007; Aroonvilairat et al., 2015). Therefore, the reduction of the use of pesticides is necessary to prevent health problems as well as to provide food safety in crop production. Furthermore, the search for new harmless technologies for rice plant disease control such as disease-free seeds, resistant varieties, cultural methods, biological methods, and induced resistance is considered as a priority (Buensanteai, Yuen, and Prathuangwong, 2009; Walters, Ratsep, and Havis, 2013; Kanlayavattanakul et al., 2016; Le et al., 2017; Thumanu et al., 2017).

Induced resistance (IR) is a method that contributes to plant defense against plant pathogens by activating multiple mechanisms in plant defense (Walling, 2001; Tuzun and Bent., 2006; Buensanteai et al., 2009). There are two main plant defense mechanisms: Induced Systemic Resistance (ISR) and Systemic Acquired Resistance (SAR). ISR relies on jasmonic acid (JA) and ethylene (ET) for signaling (Mandal et al., 2008; Walters et al., 2013; Walters, Newton, and Lyon, 2014;). The signaling pathway regulated by the plant hormone jasmonic acid (JA) is in charge of regulating resistance to leaf-chewing herbivores (Farmer and Ryan, 1992; Kessler and Baldwin,

2002; Howe and Jander, 2008; Erb, Meldau, and Howe, 2012) through the formation of physical barriers such as trichomes and enhanced synthesis of defensive compounds such as glucosinolates (GLS) (Howe and Jander, 2008; Erb et al., 2012). ISR responses can be mediated by rhizobacteria which has shown effectiveness against necrotrophic pathogens and herbivores insects that are sensitive to JA/ET defenses (Pieterse et al., 2014). The importance of rhizobacteria-mediated ISR has been widely reported such as *Pseudomonas* sp. and *Bacillus* sp.their ability to trigger ISR. SAR requires endogenous salicylic acid (SA) as a signal molecule and it is associated with production and accumulation of defense enzymes, and increased expression of pathogenesis-related (*PR*) genes (Malamy et al., 1990; Vidal et al., 1997; Buensanteai et al., 2009; Buensanteai et al., 2010). The stimulation of SA in the plant can be induced by elicitors or plant activators, plant stimulators, and plant inducers. They can be classified into two groups: biotic and abiotic. Elicitors are characteristically non-specific and enhance a generally effective resistance against a range of pathogens which work in a taxonomically diverse range of plants (Sticher, Mauch-Mani, and Metraux, 1997; Vallad and Goodman, 2004; Sadik Tuzun and Bent, 2006; Buensanteai et al., 2009; Le et al., 2017). Various types of elicitors have been researched previously such as salicylic acid, fatty acids, β-aminobutyric acid, ascorbic acid, potassium dihydrogen phosphate, chitosan, oligosaccharides, plant growth-promoting rhizobacteria, fungi, and other microbial elicitors (Alexandersson et al., 2016). The local and systemic accumulation of SA is critical for SAR induction. During SAR, plants produce translocated signals that activate the resistance mechanism in uninfected parts of the plant to resist further invasions (Bektas and Eulgem, 2015; Tripathi, Raikhy, and Kumar, 2019). At a molecular level, SAR is

developed under an increased expression of a large number of *PR*-gene families such as *PR1*, *PR2*, and *PR5* that serve as robust markers encoding pathogenesis-related (PR) proteins, possibly capable of hydrolyzing microbial cell wall components (Conrath, 2006; Bektas and Eulgem, 2015; Sharma and Pandey, 2017)

The fine-agrochemical SA, a type of elicitor, plays an important role in the induced resistance, plant growth, and plant development (Zhang et al., 2002; Choudhary, Prakash, and Johri, 2007; War et al., 2011; Thakur and Sohal, 2013). The SA is involved in plant signal transduction mechanisms which accumulate defense reactions to produce defense intermediate compounds and PR-proteins that protect the plant from the pathogen infection (Murphy et al., 1999; He and Wolyn, 2005; Hukkanen et al., 2007; Mandal, Mallick, and Mitra, 2009; Prakongkha et al., 2013; Hussain, Hamid, and Ghazanfar, 2015; Le et al., 2017). Besides, analogs of SA, such as Acibenzolar-S-Methyl (ASM) and 2,6-dichloroisonicotinic acid (INA), resemble SA by acting as an exogenous abiotic elicitor contributing to systemic resistance and therefore they protect plants from infections by pathogens like bacteria, fungi, or virus (Kombrink and Somssich, 1995; Dann et al., 1998; Chong et al., 1999; Ishida et al., 2008; Sood, Sohal, and Lore, 2013; Thakur and Sohal, 2013; Gharbi et al., 2017). The scientific literatures have shown disease resistance induction in several economic crops such as pepper, potato, rice, grapevine, cassava, soybean, cucumber, and tomato (Walling, 2001; Mandal et al., 2009; Walters et al., 2013; Le et al., 2017; Zehra et al., 2017 Genzel et al., 2018). Moreover, it also has been widely used in commercial products to control plant pathogens and enhance plant growth in various plants (Durrant and Dong, 2004; Hukkanen et al., 2007; Vimala and Suriachandraselvan, 2008; Buensanteai et al., 2009; Cartea et al., 2011). In 2017, Le et al. reported that SA

could reduce BLB disease caused by bacterial *Xoo* in rice cv. KDML105 in greenhouse conditions. The results showed that the treatments with 1 mM of SA significantly reduced disease severities by 38.17% and increased hypersensitive response at approximately 110% at 48 h after *Xoo* inoculation under greenhouse conditions (Le et al., 2017). However, an effectiveness assessment under field conditions and evaluates the use of exogenous SA as a commercial inducer formulation is not well studied.

Therefore, the goal of this research was to evaluate the efficacy of exogenous salicylic acid elicitor as a commercial prototype for its application in strategic management of the BLB under greenhouse and field conditions. In addition, the biology of *Xoo* during pathogenesis under the SA-Ricemate® elicitor treated conditions was also studied.

1.2 Research objectives

1.2.1 To evaluate the efficacy of abiotic SA-Ricemate® formula based on SA for inducing systemic resistance against bacterial leaf blight in rice under greenhouse and field conditions.

1.2.2 To examine the biology of *Xoo* during pathogenesis under the SA-Ricemate® elicitor treated conditions.

1.3 Research hypotheses

1.3.1 The SA-Ricemate® elicitor is able to induce resistance against *Xoo*, the BLB pathogen, under greenhouse and field conditions.

1.3.2 The pathogenesis of *Xoo* can be reduced under SA-Ricemate® elicitor treated conditions.

1.4 Designed routes of this study

In this study, the efficacy of SA-Ricemate® formula based on SA was evaluated. The study was divided into three main parts of experimental studies as the following: (1) Chapter III, the effect of SA-Ricemate® on induces resistance to reduced BLB disease severity was evaluated under the greenhouse conditions. (2) Chapter IV, *Xoo* biology during pathogenesis under the SA-Ricemate® treated condition was evaluated by observing biofilm formation, extracellular polysaccharide secretion, swimming motility, and twitching motility. (3) Chapter V, the effects of SA-Ricemate® on BLB disease severity and yield were observed under field conditions.

1.5 Scope of study

The scope of this research is to study the efficacy of the SA-Ricemate® elicitor inducing resistance in the susceptible rice; KDML105 against BLB disease. The biochemical changes associated with plant innate immunity after applying a SA-Ricemate® elicitor treatment and then inoculated with *Xoo* were monitored. Besides, a study of virulence factors in the *Xoo* was also addressed. The efficacy of SA-Ricemate® was evaluated under both greenhouse and field conditions.

1.6 Expected results

1.6.1 The SA-Ricemate® could trigger defense mechanism in rice plants against *Xoo* that causes bacterial leaf blight disease hence reducing disease severity.

1.6.2 Biofilm formation and extracellular polysaccharides (EPS) production of *Xoo* could be inhibited by the SA-Ricemate® treatment.

1.6.3 Swimming and twitching motility of *Xoo* could be inhibited by the SA-Ricemate® treatment.

1.7 References

Aktar, M. W., Sengupta, D., and Chowdhury, A. (2009). Impact of pesticides use in agriculture: Their benefits and hazards. **Interdiscip. Toxicol.** 2 (1): 1-12.

Alavanja M.C., Sandler D.P., Lynch C.F.(2015). Cancer incidence in the agricultural health study. **Scand J. Work Environ. Health.** 31 (1): 39-7.

Alexandersson, E., Mulugeta, T., Lankinen, Å., Liljeroth, E., and Andreasson, E. (2016). Plant resistance inducers against pathogens in solanaceae species-from molecular mechanisms to field application. **Int. J. Mol. Sci.** 17 (10): 1673.

Aroonvilairat, S., Kespichayawattana, W., Sornprachum, T., Chaisuriya, P., Siwadune, T., and Ratanabanangkoon, K. (2015). Effect of pesticide exposure on immunological, hematological and biochemical parameters in thai orchid farmers-a cross-sectional study. **Int. J. Environ. Res. Public Health.** 12 (6): 5846-5861.

Bay, A., and Heshmati, H. (2016). Factors associated with pesticide use behaviors among farmworkers based on health belief model. **Iran J. Public Health.** 45 (2): 276-277.

Bektas, Y., and Eulgem, T. (2015). Synthetic plant defense elicitors. **Front. Plant Sci.** 5: 804.

Buensanteai, N., Mukherjee, P. K., Horwitz, B. A., Cheng, C., Dangott, L. J., and Kenerley, C. M. (2010). Expression and purification of biologically active trichoderma virens proteinaceous elicitor sm1 in pichia pastoris. **Protein Expr. Purif.** 72 (1): 131-138.

Buensanteai, N., Yuen, G. Y., and Prathuangwong, S. (2009). Priming, signaling, and protein production associated with induced resistance by bacillus amyloliquefaciens kps46. **World J. Microbiol. Biotechnol.** 25 (7): 1275-1286

Cartea, M. E., Francisco, M., Soengas, P., and Velasco, P. (2011). Phenolic compounds in brassica vegetables. **Molecules.** 16 (1): 251.

Chatzi, L., Alegakis, A., Tzanakis, N., Siafakas, N., Kogevinas, M., and Lionis, C. (2007). Association of allergic rhinitis with pesticide use among grape farmers in crete, greece. **Ann. Occup. Environ. Med.** 64 (6): 417-421.

Chong, J., Baltz, R., Fritig, B., and Saindrenan, P. (1999). An early salicylic acid, pathogen and elicitor-inducible tobacco glucosyltransferase: Role in compartmentalization of phenolics and h_2o_2 metabolism. **FEBS Lett.** 458 (2): 204-208.

Choudhary, D. K., Prakash, A., and Johri, B. N. (2007). Induced systemic resistance (isr) in plants: Mechanism of action. **Indian J. Microbiol.** 47 (4): 289-297.

Conrath, U. (2006). Systemic acquired resistance. **Plant Signal. Behav.** 1 (4): 179-184.

Dale, R. W., Adrian C. N., and Gary D. L. (2014). **Induced resistance for plant defense**. UK: Blackwell.

Dann, E., Diers, B., Byrum, J., and Hammerschmidt, R. (1998). Effect of treating soybean with 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) on seed yields and the level of disease caused by sclerotinia sclerotiorum in field and greenhouse studies. **Eur. J. Plant Pathol.** 104 (3): 271-278.

Domene, X. (2016). A critical analysis of meso- and macrofauna effects following biochar supplementation. In T. Komang and H. Caroline (eds.). **Biochar application** (pp. 268-292). Amsterdam: Elsevier.

Durrant, W. E., and Dong, X. (2004). Systemic acquired resistance. **Annu. Rev. Phytopathol.** 42: 185-209.

Erb, M., Meldau, S., and Howe, G. A. (2012). Role of phytohormones in insect-specific plant reactions. **Trends Plant Sci.** 17 (5): 250-259.

Food and Agriculture Organization of the United Nations. (2015). **Total production for rice in thailand** [On-line]. Available: http://www.fao.org/statistics/en/.

Farmer, E. E., and Ryan, C. A. (1992). Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. **Plant Cell.** 4 (2): 129-134.

Forssell, S. (2018). Rice price policy in thailand [On-line] (Master dissertation, Lung University). **LUP Student Papers**. Abstract from: https://lup.lub.lu.se/student-papers/search/publication/1389016.

Fuller, M. E. (2005). Pollutants effects on microorganisms. In Daniel Hillel (ed.) **Encyclopedia of soils in the environment** (pp. 258-264). Oxford: Elsevier.

Genzel, F., Franken, P., Witzel, K., and Grosch, R. (2018). Systemic induction of salicylic acid-related plant defences in potato in response to *Rhizoctonia solani ag3pt*. **Plant Pathol.** 67 (2): 337-348.

Gharbi, E., Martínez, J., Ben Ahmed, H., Dailly, H., Quinet, M., and Lutts, S. (2017). The salicylic acid analog 2,6-dichloroisonicotinic acid has specific impact on the response of the halophyte plant species solanum chilense to salinity. **Plant Growth Regul.** 82, 517–525.

Gnanamanickam, S. (2009). **Biological control of rice diseases.** Dordrecht: Springer.

He, C. Y., and Wolyn, D. (2005). Potential role for salicylic acid in induced resistance of asparagus roots to *fusarium oxysporum* f. Sp. *asparagi*. **Plant Pathol.** 54: 227-232.

Howe, G. A., and Jander, G. (2008). Plant immunity to insect herbivores. **Annu. Rev. Plant Biol.** 59 (1): 41-66.

Hukkanen, A. T., Kokko, H. I., Buchala, A. J., McDougall, G. J., Stewart, D., Kärenlampi, S. O., and Karjalainen, R. O. (2007). Benzothiadiazole induces the accumulation of phenolics and improves resistance to powdery mildew in strawberries. **J. Agric. Food Chem.** 55 (5): 1862-1870.

Hussain, M., Hamid, M. I., and Ghazanfar, M. U. (2015). Salicylic acid induced resistance in fruits to combat against postharvest pathogens: A review. **Arch. Phytopath. Plant Protect.** 48 (1): 34-42.

Ishida, A. K. N., Souza, R. M., Resende, M. L. V., Cavalcanti, F. R., Oliveira, D. L., and Pozza, E. A. (2008). Rhizobacterium and acibenzolar-s-methyl (ASM) in

resistance induction against bacterial blight and expression of defense responses in cotton. **Trop. Plant Pathol.** 33: 27-34.

Kanlayavattanakul, M., Lourith, N., and Chaikul, P. (2016). Jasmine rice panicle: A safe and efficient natural ingredient for skin aging treatments. **J. Ethnopharmacol.** 193: 607-616.

Kawasaki, J., and Herath, S. (2018). Impact assessment of climate change on rice production in Khon Kaen province, Thailand. **J. ISSAAS** 17 (2): 14-28.

Keeratipipatpong, W. (2010). **Disease threat to rice crop** [On-line]. Available: https://www.bangkokpost.com/learning/learning-news/208868/disease-threat-to-rice-crop.

Kessler, A., and Baldwin, I. (2002). Plant responses to insect herbivory: The emerging molecular analysis. **Annu. Rev. Plant biol.** 53: 299-328.

Khan, J., Siddiq, R., M. I. Arshad, H., S. Anwar, H., Saleem, K., and F. Jamil, F. (2012). Chemical control of bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae*. **AGRIS. J.** 24 (2): 955-958.

Kombrink, E., and Somssich, I. E. (1995). Defense responses of plants to pathogens.. **Adv. Bot. Res**. 21: 1-34.

Le Thanh, T., Thumanu, K., Wongkaew, S., Boonkerd, N., Teaumroong, N., Phansak, P., and Buensanteai, N. (2017). Salicylic acid-induced accumulation of biochemical components associated with resistance against xanthomonas oryzae pv. oryzae in rice. **J. Plant Interact.** 12 (1): 108-120.

Mahmood, I., Imadi, S., Shazadi, K., Gul, A., and Hakeem, K. (2015). Effects of pesticides on environment. In Hakeem K., Akhtar M., Abdullah S. (eds). **Plant, Soil and Microbes** (pp. 253-269)**.** Cham: Springer.

Malamy, J., Carr, J. P., Klessig, D. F., and Raskin, I. (1990). Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. **Science.** 250 (4983): 1002-1004.

Mandal, B., Mandal, S., Csinos, A. S., Martinez, N., Culbreath, A. K., and Pappu, H. R. (2008). Biological and molecular analyses of the acibenzolar-s-methyl-induced systemic acquired resistance in flue-cured tobacco against tomato spotted wilt virus. **Phytopathology.** 98 (2): 196-204.

Mandal, S., Mallick, N., and Mitra, A. (2009). Salicylic acid-induced resistance to *fusarium oxysporum* f. Sp. *Lycopersici* in tomato. **Plant Physiol. Biochem.** 47 (7): 642-649.

Murphy, A. M., Chivasa, S., Singh, D. P., and Carr, J. P. (1999). Salicylic acid-induced resistance to viruses and other pathogens: A parting of the ways? **Trends Plant Sci.** 4 (4): 155-160.

Nation, T. (2014). **Thailand to benefit from growing world rice demand, market monitors say** [On-line]. Available: https://www.nationthailand.com/31447.

Nayar, N. M. (2014). Chapter 4 - oryza species and their interrelationships. **Origin and phylogeny of rices** (pp. 59-115). San Diego: Academic Press.

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., and Hens, L. (2016). Chemical pesticides and human health: The urgent need for a new concept in agriculture. **Front. Public Health.** 4: 148.

Office of Agricultural Economics (2017). **Agriculture pesticides imported 2011-2017** [On-line]. Available: http://www.oae.go.th/oaenew/OAE.

Panuwet, P., Siriwong, W., Prapamontol, T., Ryan, P. B., Fiedler, N., Robson, M. G., and Barr, D. B. (2012). Agricultural pesticide management in thailand: Situation and population health risk. **Environ. Sci. Policy.** 17: 72-81.

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Wees, S. C. M. V., and Bakker, P. A. H. M. (2014). Induced systemic resistance by beneficial microbes. **Annu. Rev. Phytopathol.** 52 (1): 347-375.

Prakongkha, Sompong, Wongkaew, Athinuwat, and Buensanteai. (2013). Changes in salicylic acid in grapevine treated with chitosan and BTH against sphacelomaampelinum, the causal agent of grapevine anthracnose. **Afr. J. Microbiol.** 7 (7): 557-563.

Sharma, M., and Pandey, G. (2017). **Abiotic stress signaling in plants: Functional genomic intervention** [On-line]. Available: https://atlasofscience.org/abiotic-stress-signaling-in-plants-functional-genomic-intervention/#more-22640.

Shimono, M., Koga, H., Akagi, A., Hayashi, N., Goto, S., Sawada, M., and Takatsuji, H. (2011). Rice wrky45 plays important roles in fungal and bacterial disease resistance. **Mol. Plant. Pathol.** 13(1): 83-94.

Singh, R. A., Das, B., Ahmed, K. M., and Pal, V. (1980). Chemical control of bacterial leaf blight of rice. **Trop. Pest. Manage.** 26 (1): 21-25.

Sombunjitt, S., Sriwongchai, T., Kuleung, C., and Hongtrakul, V. (2017). Searching for and analysis of bacterial blight resistance genes from thailand rice germplasm. **Agric. Nat. Resour.** 51 (5): 365-375.

Sood, N., Sohal, B., and Lore, J. (2013). Foliar application of benzothiadiazole and salicylic acid to combat sheath blight disease of rice. **Rice Science.** 20 (5): 349-355.

Sticher, L., Mauch-Mani, B., and Metraux, J. (1997). Systemic acquired resistance. **Annu. Rev. Phytopathol.** 35 (1): 235-270.

Tawatsin, A., and Siriyasatien, P. (2015). Pesticides used in thailand and toxic effects to human health. **Med. Res. Arch.** 3.

Thai Rice Exporters Association. (2017). **Rice export satatistics** [On-line]. Available: http://www.thairiceexporters.or.th/List_%20of_statistic.htm.

Thakur, M., and Sohal, B. S. (2013). Role of elicitors in inducing resistance in plants against pathogen infection: A review. **ISRN Biochemistry.** 2013: 762412.

Thumanu, K., Wongchalee, D., Sompong, M., Phansak, P., Le Thanh, T., Namanusart, W., and Buensanteai, N. (2017). Synchrotron-based ftir microspectroscopy of chili resistance induced by bacillus subtilis strain d604 against anthracnose disease. **J. Plant. Interact**. 12 (1): 255-263.

Timsina, J., Wolf, J., Guilpart, N., van Bussel, L. G. J., Grassini, P., van Wart, J., and Ittersum, M. K. (2018). Can bangladesh produce enough cereals to meet future demand? **Agric. Syst.** 163: 36-44.

Tripathi, D., Raikhy, G., and Kumar, D. (2019). Chemical elicitors of systemic acquired resistance-salicylic acid and its functional analogs. **Curr. Plant Biol.** 17: 48-59.

Tuzun, S., and Bent, E. (2006). **Multigenic and induced systemic resistance in plants.** Boston: Springer.

Vallad, G. E., and Goodman, R. M. (2004). Systemic acquired resistance and induced systemic resistance in conventional agriculture. **Crop Sci.** 44: 1920-1934.

Vanavichit, A., Kamolsukyunyong, W., Siangliw, M., Siangliw, J., Traprab, S., Ruengphayak, S., and Tragoonrung, S. (2018). Thai hom mali rice: Origin and breeding for subsistence rainfed lowland rice system. **Rice.** 11: 20.

Vidal, S., León, I. P., Denecke, J., and Palva, E. T. (1997). Salicylic acid and the plant pathogen *erwinia carotovora* induce defense genes via antagonistic pathways. **Plant J.** 11 (1): 115-123.

Vimala, R., and Suriachandraselvan, M. (2008). Influence of antagonistic agent, plant products and chemical agents on the powdery mildew disease of bhendi and its production. **J. Biopestic.** 1 (2): 130-133.

Pinta1, W., Toojinda, T., Thummabenjapone, P., and Sanitchon, J. (2013). Pyramiding of blast and bacterial leaf blight resistance genes into rice cultivar RD6 using marker assisted selection. **Afr. J. Biotechnol.** 12: 4432-4438.

Walling, L. L. (2001). Induced resistance: From the basic to the applied. **Trends Plant Sci.** 6 (10): 445-447.

Walters, D., Newton, A., and Lyon, G. (2014). **Induced resistance for plant defence: A sustainable approach to crop protection**. Oxford: Blackwell.

Walters, Ratsep, J., and Havis, N. (2013). Controlling crop diseases using induced resistance: Challenges for the future. **J. Exp. Bot.** 64 (5): 1263-1280.

War, A. R., Paulraj, M. G., War, M. Y., and Ignacimuthu, S. (2011). Role of salicylic acid in induction of plant defense system in chickpea (*cicer arietinum* L.). **Plant Signal Behav.** 6 (11): 1787-1792.

Weichenthal, S., Moase, C., and Chan, P. (2010). A review of pesticide exposure and cancer incidence in the agricultural health study cohort. **Environ. Health Perspect** 118 (8): 1117-1125.

Zehra, A., Meena, M., Dubey, M. K., Aamir, M., and Upadhyay, R. S. (2017). Synergistic effects of plant defense elicitors and trichoderma harzianum on enhanced induction of antioxidant defense system in tomato against fusarium wilt disease. **Bot. Stud.** 58 (1): 44.

Zhang, S., Moyne, A.-L., Reddy, M. S., and Kloepper, J. W. (2002). The role of salicylic acid in induced systemic resistance elicited by plant growth-promoting rhizobacteria against blue mold of tobacco. **Biol. Control.** 25 (3): 288-296.

CHAPTER II

LITERATURE REVIEW

2.1 Rice

2.1.1 Role of rice

Rice is an important staple food for a large part of the world with the third-highest worldwide production after sugarcane and maize (FAO, 2015). Human consumption accounts for 85% of total production for rice (Kearney, 2010; Muthayya et al., 2014). In the 2018/2019 crop year, about 486.62 million metric tons of rice was consumed worldwide, up from 437.18 million metric tons (Shahbandeh, 2019). Global consumption of rice is forecasted to increase, especially in China, India, and Sub-Saharan Africa as well as its market trade which is forecasted to become the third largest volume (Mosleh, Hassan, and Chowdhury, 2015; USDA, 2020). In Thailand, rice production has a very important role as a major economy crop which is important both for consumption as well as main food of Thai people and export as a major agricultural export product of Thailand (Jaroensathapornkul, 2007; Castillo, 2011; Chakhonkaen et al, 2012). Thai rice cultivation uses the fifth-largest amount of land in the world with 9.65 million hectares (FAO, 2015) making Thailand the second largest exporter of rice with 10 million metric tons, value amounted to 106,785 million baht (Thai Rice Exports Association, 2015; FAO, 2015; Statistics Portal, 2018).

2.1.2 Cultivation of rice

There are only two cultivated species of rice: *Oryza glaberrima* Steud and *Oryza sativa* Linn. *Oryza glaberrima* is confined to West Africa where it is an upland crop (Gurdev, 1997). The *Oryza sativa*, commonly known as Asian rice contains two major subspecies: short grained japonica and long-grained indica (Nayar, 2014). Japonica varieties are usually cultivated in dry fields in temperate East Asia, upland areas of Southeast Asia, and high elevations in South Asia. Whereas, indica varieties are mainly lowland rice grown mostly submerged throughout tropical Asia (Herve and Kayano, 2006).

2.1.3 Rice growth and development

Rice is an annual cereal with round, hollow, jointed culms; narrow, flat, sessile leaf blades joined to the leaf sheaths with collars; well-defined, sickle-shaped, hairy auricles; small acute to acuminate or two cleft ligules, and terminal panicles. The life cycle of rice plant is generally ranges from 105 to 145 days depending on cultivar, photoperiod, and environment (Vergara, 1991; Tang et al., 2009). The development of the rice plant, which can be divided into three main phases included vegetative stage, reproductive stage, and ripening stage, showed in Figure 2.1 (Vergara, 1991). Germination and emergence are the first stages in the vegetative phase of growth. Germination begins with the appearance of the young shoot and root through the seed coat at one end of the seed. As the shoot elongates and reaches the soil surface, emergence occurs. When rice is grown on the soil surface, germination and emergence occur almost simultaneously. Seedling growth follows emergence and is usually determined by number of leaves, this stage occurs during the first to five weeks after planting. As rice becomes 3-4 leaf stage, it begins to the tillering stage. Tillers emerge

from the axiliary buds of the nodesas as well as secondary shoots. The number of tillers is primarily determined by plant population and cultivar, and tiller formation occurs over a two or three weeks period (Pawar and Radhakrishnan, 2016). Panicle initiation is the first stage in the reproductive phase of growth. The developing panicle is microscopic in size inside the stem, and panicle initiation can usually be associated with the beginning of stem internode formation. A total of five internodes can be produced in the formation of a stem of rice. Then, panicle differentiation stage occurs. Panicle differentiation is the first stage in the reproductive phase when the newly forming panicle becomes visible. The panicle continues to grow and develop inside the stem. When the panicle develops completely, the heading stage is identified when a portion of a panicle is growing out of the rice stem. From this time forward, growth stages are based on the state of the panicle outside of the rice stem. The flowering and grain filling stages begin within one to five days after heading stage, and grain filling is complete within three weeks. The grain filling stages are including the milk, dough and physiological maturity stages. The milk stage is observed when a milky white substance begins to accumulate, usually seven to 10 days after heading, and the dough stage occurs about a week later as the milky substance begins to change and become the texture of bread dough. The physiological maturity stage is occurred when rice grains become firm, two weeks later when the moisture content approximately 20 percent, the ripen grain ready to harvest maturity (Tanaka and Yamaguchi, 1968; Moldenhauer and Slaton, 2001).

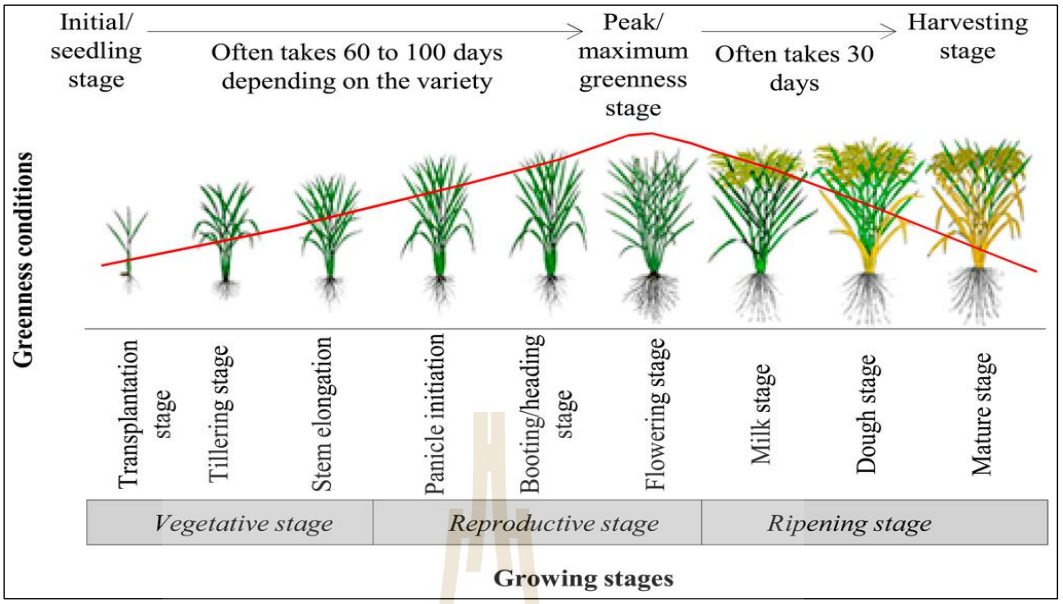


Figure 2.1 Developmental stages of the rice plant (International Rice Research Institute (IRRI), 2017).

2.1.4 Rice production

One of the challenges in Thai rice production is quality standards. Good Agricultural Practice (GAP) for rice in Thailand is significantly important for promoting and supporting quality standards for rice production encourage the quality and safety development of the rice production in order to be accepted for both domestic and international trade. The GAP applies recommendations and available knowledge to address environmental, economic, and social sustainability for farm production and post harvest processes resulting in safe and healthy food or non-food agricultural products. To participate in the Rice GAP program, farmers must have their rice plots registered and they have to follow a set of practices listed in the detailed GAP guidelines as presented in Table 2.1 (Thai Agricultural Standard, 2008).

Table 2.1 Inspections for Thailand GAP Rice.

Items	**Inspections**
1. Water sources	Inspect the surroundings. If there is any risk, verify the water quality.
2. Plantation areas	Inspect the surroundings. If there is any risk, verify the soil quality.
3. Application of pesticides	Check the record of pesticide application. - Inspect the storage of the pesticides. - If evidence or situation is in doubt of misapplication of pesticide,the produce shall be analyzed for pesticide residues.
4. Quality management in pre-harvest production	- Review the certified document or the record of seed source. - Review the record of soil preparation and off type plant elimination. - Random sampling for off type rice plant in rice field. in case of any doubts, analyze the paddy for admixing grain. - Review the record for plant damages by pest survey and control. - Review the record of pesticide application. - Visual examination of for weedy rice plant in rice field. - Visual examination of produce for defected grain by disease and insect.
5. Harvesting and post-harvest practices	- Review data record for harvesting and threshing practices. - If necessary, inspect the practices during harvesting and threshing or visual examination of the harvested produce. - If any doubt occurring, take a random sampling of the paddy to test for milling quality. - Review record for harvesting and threshing practices. - Review record of drying. - If any doubt, take a random sampling of paddy to test for moisture and/or milling quality.
6. Transportation, storage and produce collection.	- Review record of packing, transportation and storage. - Inspect equipments, containers, storage and rice collecting room. - Inspect practices for grain storage and collecting handling. - Inspect labeling in storage.
7. Recording and record keeping	- Review the records and code or sign or mark of produce source.

2.2 Bacterial leaf blight of rice

2.2.1 Symptom and damage

Xanthomonas oryzae pv. *oryzae* (*Xoo*) causes wilting of seedlings, yellowing, and drying of leaves (Afolabi et al., 2014). Rice plants become infected with *Xoo* through rice seed, stem, and roots. *Xoo* lives on the surface and in the xylem of plants spreading plant-to-plant through irrigation and storm floods. Upon contact with the host plant, the bacterium infiltrates invade through natural openings or leaf and root wounds. *Xoo* grows in the plant and infects the plant's leaf veins as well as the xylem causing blockage and plant wilting (Yadeta and Thomma, 2013; Hull, 2014). Bacteria oozes (Figure 2.2a) from the infected leaf lesions are signs of this disease as an inoculum that can be spread by rain or wind. The bacteria oozes can be observed by dipping the damaged leaves in water, which become turbid because of bacterial ooze. During rice plants vegetative stage, the BLB symptoms (Figure 2.2b) are seen as translucent lesions appearing near the leaf edge. The lesions enlarge both in length and width with a wavy margin turning straw yellow within few days covering the entire leaf. As the disease advances, the lesions cover the entire lamina which turns white or straw colored. Opaque dew drops containing bacterial masses are formed on young lesions in the early morning. Discolored spots are observed in affected grains (Pinta et al., 2013; Le et al., 2017).

Figure 2.2 Signs and symptoms of BLB a) Bacterial ooze of *Xoo*, and b) Lesionscaused by bacterial blight (IRRI, 2017)

Xoo is a rod-shaped, round-ended, gram-negative bacterium. The individual cell length varies between approximately 0.7 μm to 2.0 μm and its width between 0.4 μm to 0.7 μm. Cells are motile by means of a single polar flagellum. Colonies on solid media containing glucose are round, convex, mucoid, and yellow in color due to the production of the pigment xanthomonadin characteristic of the genus (Figure 2.3) (Yuan et al., 2010; Le et al., 2017; Sombunjitt et al., 2017).

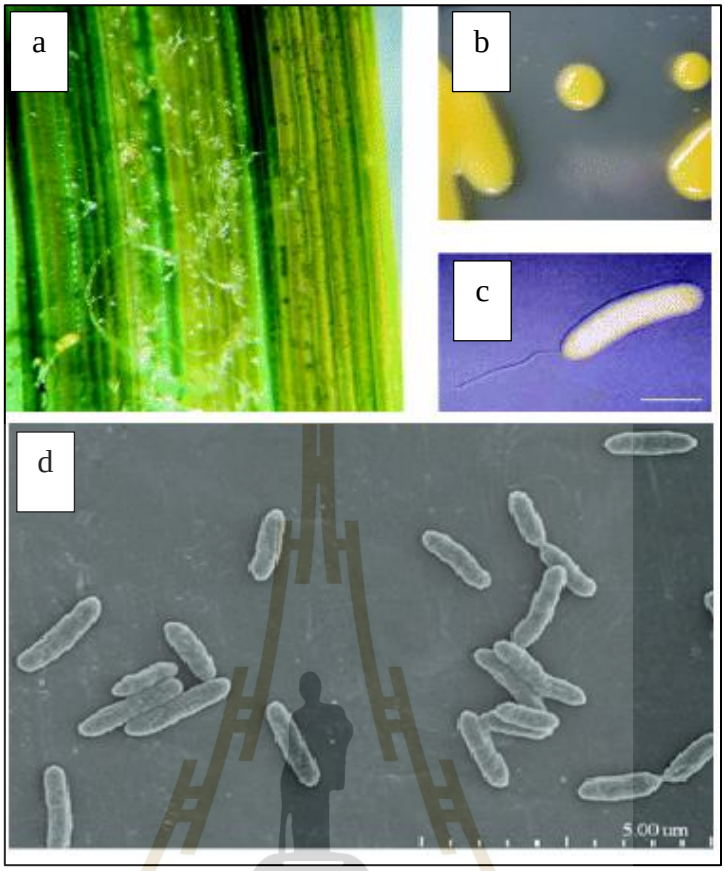


Figure 2.3 Morphology of *Xanthomonas oryzae* pv. *oryzae* a) Strands and condensed droplets of ooze consisting of *Xoo* cells coated in extracellular polysaccharide exuded on to the surface of an infected rice leaf. b) Colonies of *Xoo* on glucose yeast extract agar. c) Scanning electron micrograph of a single *Xoo* cell (bar, 1.0 µm). and d) *Xoo* cell by SEM. (Niño-Liu, Ronald, and Bogdanove, 2006; Huang et al., 2018)

2.2.2 Identification of *Xoo* virulence factors

Virulence is one of a character that possibility contributes to host-microbe interaction. To establish themselves successfully invade the host plants, the bacteria

should be able to adhere to the plant surface before invading the intercellular space of the host tissue to acquire nutrients (Pizarro-Cerdá and Cossart, 2006).

Bacterial surface structures are important virulence factors of *Xanthomonas* spp. (Schmidt and Hensel, 2004; Buttner and Bonas, 2010; Yuan et al., 2010). *Xoo* produces a characteristic extracellular polysaccharide (EPS), xanthan, which leads to the mucoid appearance of the bacterial colonies (Mhedbi-Hajri et al., 2011; Sylvestre, Karlovsky, and Wydra, 2014). Xanthan is a polymer of repeating pentasaccharide units (Jansson, Kenne, and Lindberg, 1975; Becker et al., 1998). The production of xanthan is directed by several genetic loci including the gum gene cluster which consists of 12 genes (*gumB* to *gumM*) highly conserved among *Xanthomonas* spp. (Katzen et al., 1998; Vojnov et al., 1998). Due to its highly hydrated and anionic consistency, xanthan protects bacteria from environmental stresses such as dehydration and toxic compounds. Furthermore, in vascular pathogens xanthan causes wilting of host plants by blocking the water flow in xylem vessels (Denny, 1995; Chan and Goodwin, 1999). *Gum genes* of several *Xanthomonas* spp. including *Xanthomonas campestris* pv. *campestris*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas axonopodis* pv. *citri* and *Xanthomonas axonopodis* pv. *manihotis* were observed to contribute to epiphytic survival in plant growth and disease symptom formation (Chou et al., 1997; Katzen et al., 1998; Dharmapuri and Sonti, 1999; Kemp et al., 2004; Dunger et al., 2007; Rigano et al., 2007). Interestingly, gum genes of *X. axonopodis* pv. *citri* are dispensable for bacterial growth and disease symptom formation on *Citrus sinensis*, however they contribute to bacterial virulence in *Citrus limon* suggesting that the contribution of xanthan to virulence may depend on the host plant and on the environmental conditions (Dunger et al., 2007; Rigano et al., 2007).

A biofilm is a bacterial population in which bacteria attach to each other or to biotic or abiotic surfaces. Biofilm is embedded in an extracellular polymeric matrix formed by extracellular polysaccharide, proteins, and lipids (Sutherland, 1990; Guerra et al., 2018). The formation of a biofilm presumably provides protection against antibiotics and host defense responses while contributes to bacterial epiphytic survival before colonization of the plant intercellular space and xylem vessels (Hall-Stoodley, Costerton, and Stoodley, 2004; Balcázar, Subirats, and Borrego, 2015). The initiation of biofilm formation can be divided into five stages (Khatoon et al., 2018). First, initial reversible attachment is when planktonic cells adhere to a surface in a plant host as exposed plant lesions. They exhibit behaviors that have been reversible as a typically mediated by thin proteinaceous structures such as fimbriae, pili, and flagella patterns (Hoffman et al., 2015). The initial stages of biofilm development require a certain density of bacterial cells attachment and mobility. This uses flagella functions for the initial attachment and type IV pili that allow them to form elaborate structures (Klausen et al., 2003; Conrad et al., 2011). As for the result of the second step; irreversible attachment, the third step starts from a secretion of the adhesion typically composed of polysaccharides and proteins which tethers the bacterium to the surface (Petrova and Sauer, 2012). Then, it starts producing biofilm matrix components forming small aggregates of bacteria called microcolonies. Eventually they develop into large cellular aggregates encased by a matrix. After that, in the maturation step the intercellular communication called quorum sensing is processed (Li and Tian, 2012b). The bacteria in a community convey their presence by producing, detecting, and responding to small diffusible signal molecules called auto-inducers (Li and Tian, 2012a). Bacterial QS system is a type of signaling system detected and responded by proper sensing apparatus

and regulatory control (Kievit and Iglewski, 2000). In the final step called dispersion, planktonic cells detach from biofilm matrix and start this process again (Figure 2.4).

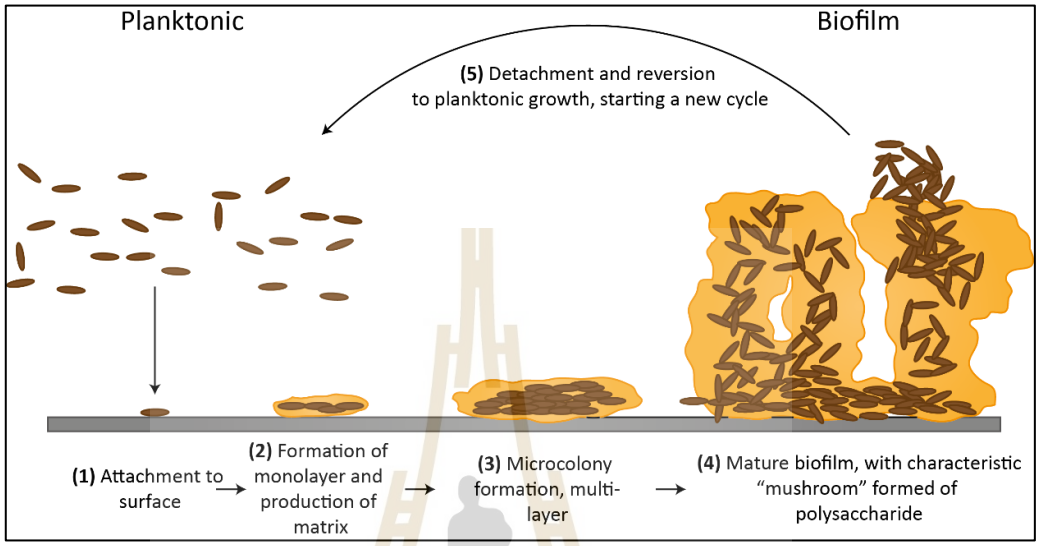


Figure 2.4 Schematic representation of a biofilm formation (Trafny, 2008).

Extracellular polysaccharides are a complex mixture of biopolymers primarily consisting of polysaccharides, proteins, lipids, and humic substances (Vu et al., 2009). They can be present in many forms including cell-bound capsular polysaccharides, unbound "slime", and as O-antigen component of lipopolysaccharide with an equally wide range of biological functions (Bazaka et al., 2011). The key functions of the EPS comprise the mediation of the initial attachment of cells to different substrata and protection against environmental stress, dehydration, and resistance to desiccation protecting them against non-specific and specific host immunity (Ding et al., 2015). For instance, the gram-negative *Pseudomonas aeruginosa* can cause disease in plants and animals including humans (Klaus, 1994). The most virulence pathogens produce growth of EPS in structured bacterial communities that can coat surfaces as

embedded bacteria and protect them from the host immune response (Maurice, Bedi, and Sadikot, 2018). The formation of EPS depends on the intercellular space of microbial aggregates structure and the architecture of the biofilm matrix (Vu et al., 2009). These components are required for motility and cellular agglutination (Li et al., 2003)

The successful infection of host plants often depends on bacterial protein secretion systems that introduces proteins into the extracellular milieu as well as transports proteins and DNA (Figure 2.5) directly into the host cell in order to counteract plant defense responses (Buttner and Bonas, 2010). Several pathogens use a combination of different protein secretion systems to ensure efficient bacterial multiplication and disease progression (Preston, Studholme, and Caldelari, 2005) from *Xanthomonas* spp. Six types of protein secretion systems are encoded.

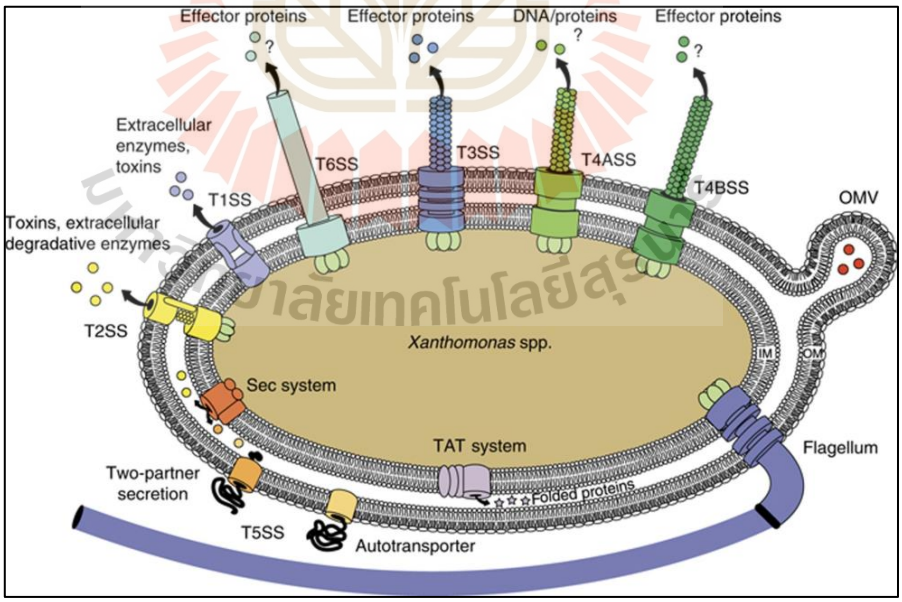


Figure 2.5 Schematic representation of protein secretion systems.

T2S and T5S systems depend on the Sec or the TAT system for protein transport across the inner membrane. T3S, T4S and T6S systems are associated with extracellular pilus structures and presumably translocate proteins into the host cell. So far, protein translocation was experimentally proven for T3S systems. Only in a few cases does protein secretion depend on the formation of outer membrane vesicles (OMV). IM, inner membrane; OM, outer membrane; TAT, twin-arginine translocation (Buttner and Bonas, 2010).

2.3 Control of bacterial leaf blight disease in rice

2.3.1 Chemical control

The use of chemicals is generally toxic which are used as disinfectants or fumigants that target specific kinds of pathogens such as bactericides or antibiotics by blocking metabolic pathway or inhibiting of pathogen.

Khan et al. (2012) investigated the antibacterial activity of six different broad-spectrum antibiotics named Benzylpenicillin, Ampicillin, Kanamycin, Streptomycin, Chloramphenicol, and Sinobionic in four different concentrations against *Xoo*. Benzylpenicillin was found to effectively control the BLB. In the other studies on efficiency of bactericide to control bacterial leaf blight, the authors found that copper oxychloride, streptomycin sulfate, oxytetracycline, and thiram inhibited growth of *Xoo* in laboratory tests (Jeffries, 2002; Agrios, 2005a; Gnanamanickam, 2009b).

2.3.2 Cultural control

To combat BLB disease in field infestation or infection of crop with *Xoo*, healthy rice and disease free rice seeds; treated with hot water at 50ºC for 20 minutes were used (Agrios, 2005a; Le et al., 2017). The disease will become severe in

susceptible varieties especially in hybrid rice and where nitrogen fertilizer has been overused (Mukherjee et al., 2005). Therefore, proper use of fertilizer, especially nitrogen, can help the plants not to be extremely succulent during the period of infection and to remove weed hosts, rice straws, and unwanted seedlings keeping the water level low during the severe flooding period (IRRI, 2017).

2.3.3 Disease resistant cultivars

Rice breeding programs at the International Rice Research Institute (IRRI) and national rice improvement programs in the Philippines, Indonesia, and India, with resistance genes including *Xa4*, *Xa5*, *Xa7*, and *Xa21* were targeted to be transferred to commercially important rice varieties (Nelson, 1996; Le et al., 2017).

2.3.4 Biological control

A variety of biological controls is available for application. Effective adoption will require a greater understanding of the complex interactions among plants as it is applied to the suppression of plant diseases. Diverse microorganisms secrete and excrete other metabolites that can interfere with pathogen growth or activities. Many microorganisms including *Bacillus spp.*; *B. lentus*; *B. cereus*; *B. circulans*, and *Pseudomonas fluorescens* (Velusamy and Gnanamanickam, 2003; Gnanamanickam, 2009a) produce and release lytic enzymes that can hydrolyze a wide variety of polymeric compounds including chitin, proteins, cellulose, hemicellulose, and DNA. Expression and secretion of these enzymes by different microbes can sometimes result in the direct suppression of plant pathogen activities (Pal, 2011).

Bacterial bio-control agents were studied on management of bacterial leaf blight disease in rice with endophytic bacteria *Bacillus subtilis* var. *amyloliquefaciens* (FZB 24), EPB 9, EPB10, EPCO 29 and EPCO 78 which recorded a significantly higher

inhibition levels of *Xoo* over control in vitro. Rice plants applied with FZB 24, recorded the lowest severity of bacterial leaf blight (31.36%) with a percent reduction of 40 over control under greenhouse conditions. In addition, the *B. subtilis* (FZB 24) treated rice plants experienced higher induction of defense related enzymes such as viz., peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase resulting in higher accumulation of total phenols compared to untreated control plants (Krishnan et al., 2014). Moreover, a commercial product of *Trichoderma* spp. was evaluated in effectiveness against bacterial leaf blight of rice under field conditions. All the bioagent formulations were significantly effective in reducing disease severity over check during Kharif season 2006 and 2007. The *T. harzianum* was found to be most effective and resulted to a 48.26 % and a 59.22 % of reduction in disease (Gangwar, 2013).

2.3.5 Induced resistance

Plant innate immunity is based on a surprisingly complex response that is highly flexible in its capability on recognizing and counteracting different invaders. To effectively combat invasion by microbial pathogens and herbivorous insects, plants can mount a systemic response establishing an enhanced defensive capacity in parts distant from the location of the primary attack (Hadrami et al., 2015). This systemically induced response protects the plant against subsequent invaders. Several biologically induced systemic defense responses have been characterized in detail such as systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Figure 2.6) (Durrant and Dong, 2004).

The onset of SAR is associated with increased levels of SA both locally at the infection location and systemically in distant tissues (Sticher, Mauch, and Métraux, 1997) which is associated with the activation of specific set of genes encoding

pathogenesis related PR proteins (Van, Bakker, and Pieterse, 1998; Heil and Bostock, 2002).

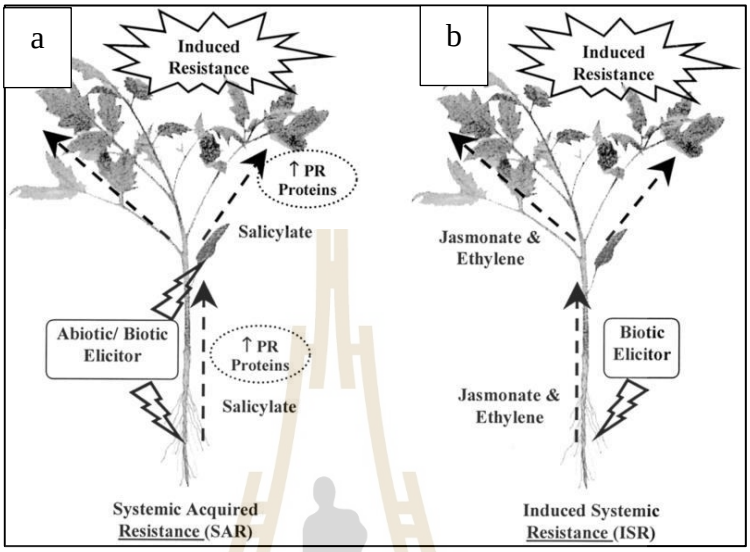


Figure 2.6 Types of induced resistance against plant disease a) Systemic Acquired Resistance and b) Induced Systemic Resistance Source: (Vallad and Goodman, 2004)

2.4 Characteristics of elicitor

2.4.1 Role of elicitors

Elicitors are generally defined as molecules that can stimulate the defense responses of plants (Thakur and Sohal, 2013). Elicitor molecules can attach to special receptor proteins located on plant cell membranes. These receptors are able to recognize the molecular pattern of elicitors and trigger intracellular defense signaling via the Octadecanoid pathway (Holopainen et al., 2009; Mejía-Teniente et al., 2013; Bektas and Eulgem, 2015). Babu et al. (2005) studied the induction of bacterial blight resistance in rice by treatment with acibenzolar-S-methyl. The results show that all the

tested concentrations of ASM (1, 10 and 100 μg mL^{-1}) were effective in inducing resistance to BLB by observing reduction in lesion length.

The synthetic elicitors including SA, benzoic acid (BA), ascorbic acid (AA), oxalic acid (OA), potassium dihydrogen phosphate (PDP), sodium saccharin dihydrate or BIT, 2,6-dichloroisonicotinic acid, riboflavin, vitamin B1, chitosan, kinetin, benzo (1,2,3)-thiadiazole-7-carbothionic acid, and S-methyl ester or acibenzolar-S- methyl (ASM) have been widely evaluated against several plant diseases (Perazzolli et al., 2008; Buensanteai, Yuen, and Prathuangwong, 2009; El-Yazied, 2011; Prakongkha et al., 2013).

Many biotic elicitors have been identified from a variety of living materials. They have been classified according to their biochemical constitution such as yeast cell wall, mycelia cell wall, and fungal spores activities which have been correlated with pectin fragment released from plant cell walls through the action of pathogen (Eschen-Lippold, Altmann, and Rosahl, 2010; Graham and Myers, 2011; Walters, Ratsep, and Havis, 2013; Lyon, 2014).

Many physical and chemical traits of abiotic elicitors are effective. Examples include the salts of heavy metals, UV irradiation, partial freezing, DNA-intercalating compounds and free radicals. All of these elicitors cause membrane perturbation and gene depression (Richard., 1993).

2.4.2 Salicylic acid (SA)

Salicylic acid ($C_7H_6O_3$); synonym 2-hydroxybenzoic acid, SA is a type of phenolic acid and a beta hydroxyl acid. This colorless crystalline organic acid is widely used in organic synthesis and functions as a plant hormone (Hayat, Khan, and Alyemeni, 2013; Zhang et al., 2017). The salicylic acid applications are related to

plant's growth and development by enhancement of chlorophyll and carotenoid pigments, flower induction, activity modification of some important enzymes, photosynthetic rate, and ion uptake in plant (Hayat et al., 2013). Salicylic acid plays a key role on plant physiological processes. This application can also be manifested in higher photosynthetic capacity via SA signaling and jasmonic acid signaling of plant with or without suffering by abiotic or biotic stress (Figure 2.7) (Pancheva, Popova, and Uzunova, 1996; Janda et al., 2014). Carotenoids and chlorophyll are well known for photosynthesis. Several researches reported that SA had a positive effect on the chlorophyll and carotenoids content (Moharekar et al., 2003). metabolites and biochemical compounds belonging to diverse biochemical structural, including phenolics, terpenoids, and alkaloids (Turkyilmaz, Aktas, and Guven, 2005). The level of secondary metabolites that are involved with defense response in plants was influenced by some factors like biotic and abiotic stresses (Gorni and Pacheco, 2016). The researches involving with SA in induced resistance in plant have been reported and introduced. Anwar et al. (2013) studied the effectiveness of seed pre-conditioning with salicylic and ascorbic acids in increasing vigor of rice seeding and founded that seed conditioning with SA (20 mg L^{-1}, 20 h) gave an increase in seed vigor of 10%. Similarly, Anaya et al. (2015) studied the influence of salicylic acid on seed germination of *Vicia faba* under salt stress concluded that 0.25 mM of SA concentration could improve germination percentage and enhance the establishment of seedlings. Ganesan et al. (2001) studied the accumulation of hydrogen peroxide in rice leaves in response to salicylic acid (SA) treatment and found that a SA treatment induced protective mechanisms that operate during some types of oxidative stress and hypersensitive response (HR).

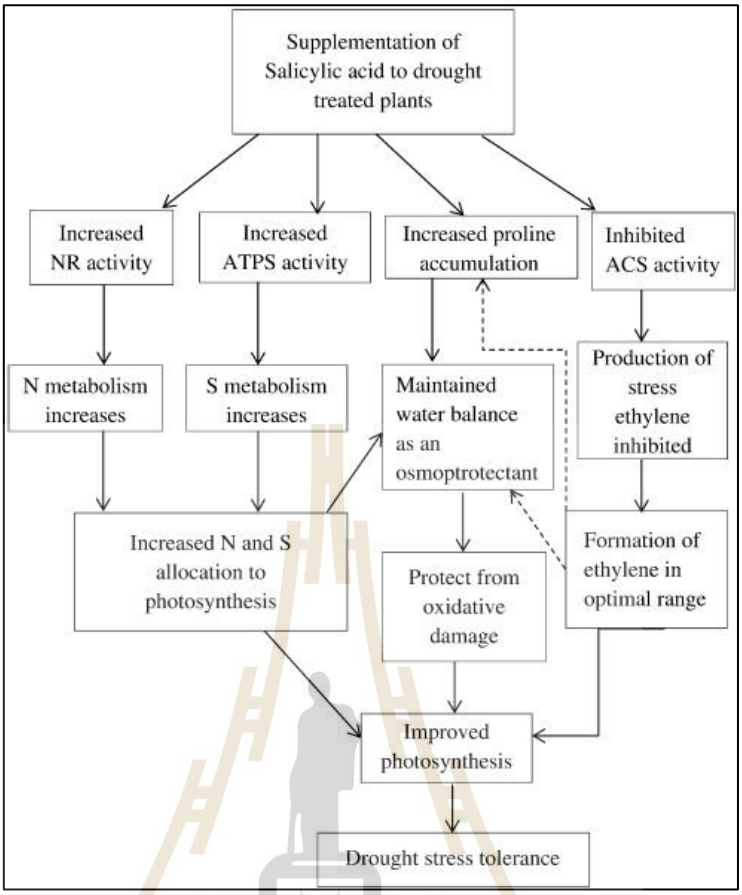


Figure 2.7 Schematic representation showing the role of SA application results in protected photosynthesis under drought stress in mustard involving proline accumulation, N metabolism, S metabolism, ethylene formation and photosynthesis. ACS, 1-aminocyclopropane carboxylic acid synthase; ATPS, ATP sulfurylase; NR, Nitrate reductase; SA, salicylic acid (dotted lines represent areas need to be investigated) Source: (Nazar et al., 2015)

Besides, salicylic acid influences the accumulation of secondary

Expression of rice pathogenesis-related protein 3, 8, 11 (*PR3, PR8, PR11*), one of the *PR* genes associated with systemic acquired resistance, was induced by SA. Moreover, the research of Makandar et al. (2012) on SA regulating basal resistance to

fusarium head blight in wheat shows that the increased accumulation of SA in fungus-infected spikes is correlated with elevated expression of the SA-inducible pathogenesis-related 1 (*PR1*) gene and FHB resistance. In addition, FHB severity and mycotoxin accumulation were curtailed in wheat plants treated with SA and in AtNPR1 wheat which is hyper-responsive to SA. Other authors observed that the exogenous SA application at different concentrations (0.5, 1.0, 2.0 mM) reduced the abiotic stress in rice plants (Li et al., 2012; Wang et al., 2012; Le et al., 2017).

2.5 Mechanism of defense resistance against pathogens

Plant defense comprise preexisting barriers as well as the defense induced upon perception of pathogen associated molecular patterns (PAMPs), microbe associated molecular patterns (MAMPs), and molecules produced from damage as a result of infection damage associated molecular patterns (DAMPs) (Garcion, 2014). There are two ways to artificially create disease resistance in plants. First, R genes of a virulent isolate of a plant pathogen are identified on natural sources of organism; then isolated and transferred to target cultivated plants by using biotechnological methods. The cultivated plants containing R genes show high level of disease resistance against one or several isolates of plant pathogen (Loebenstein, 2006). Second, the susceptible plants can be induced using inducers leading to an activation of an array of defense-related genes which process is called induced resistance (Walters et al., 2013; Le et al., 2017). In case of rice bacterial infection, there are three steps in this process including pre-entry, entry, and colonization (Agrios, 2005a; Gnanamanickam, 2009b). To gain access to the nutrients or replication machinery available within the host cell, pathogens must first breach the natural barriers presented by healthy plants. The protection from a

pathogen invasion is achieved via preformed structural and compound as physical and chemical (Figure 2.8). The physical barriers includes cuticle, stomata, and cell wall (Agrios, 2005a). The importance of the cuticle as a barrier to against penetration has been demonstrated by the dependence of many pathogens on the adhesion and the subsequent release of cutin-degrading enzymes at the time of penetration. Although cutin-degrading enzymes are also secreted by many fungi and bacteria, their primary activity is to allow access to cellulose in plant cell walls as a nutritional substrate. Different forms of cutin-degrading enzymes are used by pathogens to puncture the cell wall. The chemical barriers are based on antimicrobial chemicals as well as glucosides, saponins, terpenoids, stilbenes, and tannins. Exudates on the surfaces of plants or compounds in plant cells may stimulate or inhibit the development of pathogens. When the pathogen overcomes the preformed structural or initial barriers, inducible post-infection plant defenses are activated for protection. Firstly, OsRac GTPase is required for pathogen recognition. The Rac GTPase family belongs to the Rac superfamily of small GTPases. Members of this superfamily process GTPase activity which is used for activation of protein kinases (MAPK cascades). In plants, Rac GTPases serve diverse functions in many important cellular activities including polar growth, cell differentiation, and stress responses. In rice, seven genes encoding Rac GTPases have been characterized (Chen and Ronald, 2011; Le Thanh et al., 2017). MAPK cascades play important roles in transmission of extracellular signals to downstream components through protein phosphorylation including proliferation, differentiation, apoptosis, and stress response (Plotnikov et al., 2011). A MAPK cascade consists of three kinases such as a MAPK, a MAPK kinase (MAPKK), and a MAPKK kinase (MAPKKK). Seventeen MAPKs have been identified in rice that are associated

with pathogen infection and host defense response (Reyna and Yang, 2006). Following pathogen recognition and signal transduction, defense responses are activated that protect plants from infection leading to rapid active defenses express at the membrane including fluxes of ions K^+ , H^+ and Ca^{2+} (Atkinson et al., 1990; Tuteja and Mahajan, 2007). The oxidative burst triggered signals; at the cell wall including reinforcement of the cell wall. In addition, hosts employ hypersensitive cell death to prevent the spread of pathogens. Moreover, the antibiotic compounds such as phytoalexins create a toxic micro-environment in the infected cell which may prevent disease establishment (Jabs et al., 1997). Delayed active defenses include the containment of the pathogen, wound repair, the acquisition of induced resistance, and the expression of pathogenesis-related (PR) proteins (Sels et al., 2008). These pathways of induced resistance can enhance their defense effectiveness related situations include the application of inducers that mimic the effect of pathogen attack that give rise to proteins. (Agrios, 2005b; Ratsep, Havis, and Walters, 2013). Finally, enzymes like phenylalanine ammonialyase which are constitutively present also increase during most infections and an expression of PR proteins. PR proteins are lytic enzymes that can destroy the integrity of the pathogen cell wall and inhibit growth (Toruño, Stergiopoulos, and Coaker, 2016). Based on amino acid sequences, serological relationship, and enzymatic activities; PR proteins are classified into 17 groups: PR1 to PR17. In rice, only few groups of PR genes (PR1, PR8, and PR10) have been reported to be induced following bacterial or fungal infections (Chen and Ronald, 2011). The PR protein is described in detail in Table 2.2. Receptors perceive pathogen presence and activate inducible plant defenses including cell wall reinforcement as callose, lignin, suberin, and cell wall proteins (Guest and Brown, 1997; Dadakova et al., 2015).

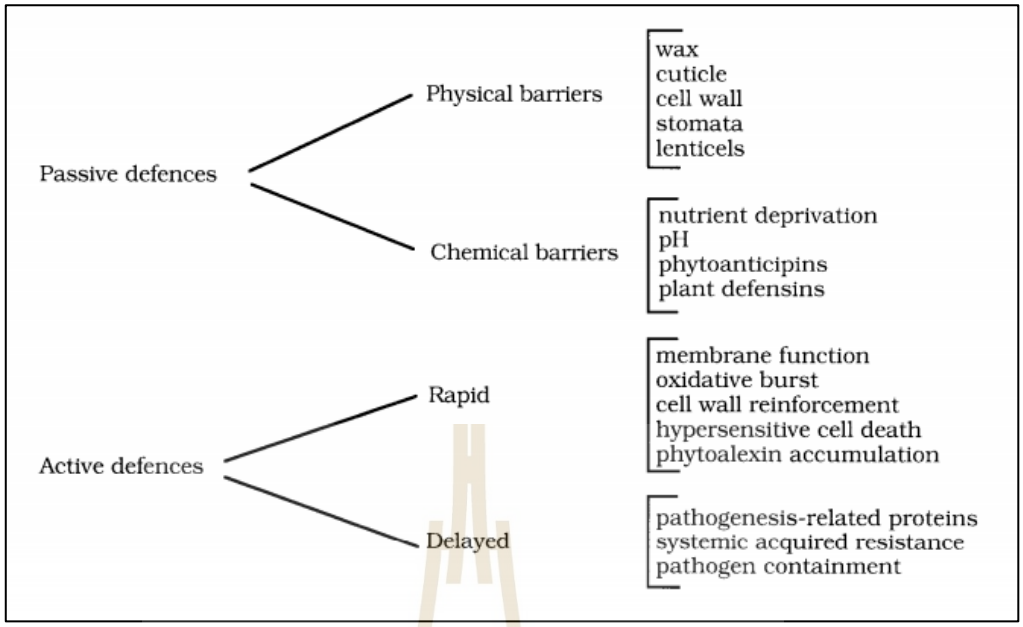


Figure 2.8 Type of the defense mechanisms in plants

Table 2.2 Main properties of classified families of PR proteins

Family	Type member	Typical size (kDa)	Properties	Proposed microbial target
PR-1	Tobacco PR-1a	15	Antifungal	Unknown
PR-2	Tobacco PR-2	30	Beta1,3-Glucanase	Beta1,3-Glucan

PR-3	Tobacco P.Q	25-30	Chitinase (class I, II, IV, V, VI)	Chitin
PR-4	Tobacco 'R'	15-20	Chitinase class I, II	Chitin
PR-5	Tobacco S	25	Thaumatin-like	Membrane
PR-6	Tomato Inhibitor 1	8	Proteinase-inhibitor	-
PR-7	Tomato P69	75	Endoproteinase	-
PR-8	Cucumber chitinase	28	Chitinase class III	Chitin
PR-9	Tabacco 'lignin-forming peroxidase'	35	Peroxidase	-
PR-10	Parsley 'PR1'	17	Ribonuclease-like	-
PR-11	Tabacco 'class V' chitinase	40	Chitinase class I	Chitin
PR-12	Radish Rs-AFP3	5	Defensin	Membrane
PR-13	*Arabidopsis* THI2.1	5	Thionin	Membrane
PR-14	Barley LTP4	9	Lipid-transfer protein	Membrane
PR-15	Barley OxOa (germin)	20	Oxalate oxidase	-
PR-16	Barley OxOLP	20	Oxalate oxidase-like	-
PR-17	Tobacco PRp27	27	Unknown	-

2.6 References

Abou El-Yazied, A. (2011). Effect of foliar application of salicylic acid and chelated zinc on growth and productivity of sweet pepper (*Capsicum annuum* L.) under autumn planting. **Res. J. Agric Biol. Sci.** 7(6): 423-433.

Afolabi, T., Milan, B., Amoussa, R., Koebnik, R., Poulin, L., Boris, S., and Silue, D. (2014). First report of *Xanthomonas oryzae* pv. *oryzicola* causing bacterial leaf streak of rice in Burundi. **Plant Dis.** 98: 1426.

Agrios, G. N. (2005a). Plant desease. **Plant pathology** (5th ed., pp. 615-703). San Diego: Academic Press.

Agrios, G. N. (2005b). Preface. **Plant pathology** (5th ed., pp. 826-874). San Diego: Academic Press.

Atkinson, M. M., Keppler, L. D., Orlandi, E. W., Baker, C. J., and Mischke, C. F. (1990). Involvement of plasma membrane calcium influx in bacterial induction of the the $K^+/H^+$and hypersensitive responses in tobacco. **Plant Physiol.** 92 (1): 215-221.

Balcázar, J. L., Subirats, J., and Borrego, C. M. (2015). The role of biofilms as environmental reservoirs of antibiotic resistance. **Front. Microbiol.** 6: 1216.

Bazaka, K., Crawford, R., Nazarenko, E., and Ivanova, E. (2011). Bacterial extracellular polysaccharides. **Adv. Exp. Med. Biol.** 715: 213-226.

Becker, A., Katzen, F., Pühler, A., and Ielpi, L. (1998). Xanthan gum biosynthesis and application: A biochemical/genetic perspective. **Appl. Microbiol. Biotechnol.** 50 (2): 145-152.

Bektas, Y., and Eulgem, T. (2015). Synthetic plant defense elicitors. **Front. Plant Sci.** 5: 804.

Buensanteai, N., Yuen, G. Y., and Prathuangwong, S. (2009). Priming, signaling, and protein production associated with induced resistance by *Bacillus amyloliquefaciens* KPS46. **World J. Microb. Biot.** 25 (7): 1275-1286

Buttner, D., and Bonas, U. (2010). Regulation and secretion of *Xanthomonas* virulence factors. **FEMS. Microbiol. Rev.** 34 (2): 107-133.

Castillo, C. (2011). Rice in thailand: The archaeobotanical contribution. **Rice.** 4 (3): 114-120.

Chakhonkaen, S., Pitnjam, K., Saisuk, W., Ukoskit, K., and Muangprom, A. (2012). Genetic structure of Thai rice and rice accessions obtained from the International Rice Research Institute. **Rice.** 5 (1): 19.

Chan, J. W. Y. F., and Goodwin, P. H. (1999). The molecular genetics of virulence of *Xanthomonas campestris*. **Biotechnol. Adv.** 17 (6): 489-508.

Chen, X., and Ronald, P. C. (2011). Innate immunity in rice. **Trends Plant Sci.** 16 (8): 451-459.

Chou, F.-L., Chou, H.-C., Lin, Y.-S., Yang, B.-Y., Lin, N.-T., Weng, S.-F., and Tseng, Y.-H. (1997). The *Xanthomonas campestris gum gene* required for synthesis of xanthan gum is involved in normal pigmentation and virulence in causing black rot. **Biochem. Biophys. Res. Commun.** 233 (1): 265-269.

Conrad, J. C., Gibiansky, M. L., Jin, F., Gordon, V. D., Motto, D. A., Mathewson, M. A., and Wong, G. C. L. (2011). Flagella and pili-mediated near-surface single-cell motility mechanisms in *Aeruginosa*. **Biophys. J.** 100 (7): 1608-1616.

Dadakova, K., Havelkova, M., Kurkova, B., Tlolkova, I., Kasparovsky, T., Zdrahal, Z., and Lochman, J. (2015). Proteome and transcript analysis of *Vitis vinifera* cell cultures subjected to *Botrytis cinerea* infection. **J. Proteomics.** 119: 143-153.

De Kievit, T. R., and Iglewski, B. H. (2000). Bacterial quorum sensing in pathogenic relationships. **Infect. Immun.** 68 (9): 4839-4849.

Denny, T. P. (1995). Involvement of bacterial polysaccharides in plant pathogenesis. **Annu. Rev. Phytopathol.** 33 (1): 173-197.

Dharmapuri, S., and Sonti, R. V. (1999). A transposon insertion in the gum g homologue of *Xanthomonas oryzae* pv. *oryzae* causes loss of extracellular polysaccharide production and virulence. **FEMS Microbiol. Lett.** 179 (1): 53–59.

Ding, Z., Bourven, I., Guibaud, G., van Hullebusch, E., Panico, A., Pirozzi, F., and Esposito, G. (2015). Role of extracellular polymeric substances (EPS) production in bioaggregation: Application to wastewater treatment. **Appl. Microbiol.** 99: 9883–9905.

Dunger, G., Relling, V. M., Tondo, M. L., Barreras, M., Ielpi, L., Orellano, E. G., and Ottado, J. (2007). Xanthan is not essential for pathogenicity in citrus canker but contributes to *Xanthomonas* epiphytic survival. **Arch. Microbiol.** 188 (2): 127-135.

Durrant, W. E., and Dong, X. (2004). Systemic acquired resistance. **Annu. Rev. Phytopathol.** 42: 185-209.

El Hadrami, A., Islam, M. R., Adam, L. R., and Daayf, F. (2015). A cupin domain-containing protein with a quercetinase activity (VDQASE) regulates *verticillium dahliae*'s pathogenicity and contributes to counteracting host defenses. **Front. Plant Sci.** 6: 440.

Eschen-Lippold, L., Altmann, S., and Rosahl, S. (2010). Dl-β-aminobutyric acid–induced resistance of potato against phytophthora infestans requires salicylic acid but not oxylipins. **Mol. Plant Microbe Interact.** 23 (5): 585-592.

Food and Agriculture Organization of the United Nations. (2015). **Total production for rice in thailand** [On-line]. Available: http://www.fao.org/statistics/en/.

Gangwar, G. (2013). Field efficacy of formulation of fungal bioagents against bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Uyeda and Ishiyama). **J. Nat. Appl. Sci.** (2): 423-426.

Garcion, C., Lamotte, O. , Cacas, J. and Métraux, J. (2014). Mechanisms of defence to pathogens: Biochemistry and physiology. In D.Walters, A.C.Newton and G.D. Lyon (eds.). **Induced resistance for plant defense** (pp. 106-125). Oxford: Blackwell.

Gnanamanickam, S. (2009). **Biological control of rice diseases** (pp. 67-78). Dordrecht: Springer.

Gnanamanickam, S. S. (2009b). Rice and its importance to human life. **Biological control of rice diseases** (pp. 1-11). Dordrecht: Springer.

Gorni, P., and Pacheco, A. (2016). Growth promotion and elicitor activity of salicylic acid in achillea millefolium l. **Afr. J. Biotechnol.** 15: 657-665.

Graham, J. H., and Myers, M. E. (2011). Soil application of sar inducers imidacloprid, thiamethoxam, and acibenzolar-s-methyl for citrus canker control in young grapefruit trees. **Plant Dis.** 95 (6): 725-728.

Guerra, M., B. Malafaia, C., Macedo, A., V. Silva, M., Mariano, R., and Souza, E. (2018). Biofilm formation by *Xanthomonas campestris* pv. *Viticola* affected by abiotic surfaces and culture media. **Trop. Plant Pathol.** 43: 146–151.

Guest, D., and Brown, J. (1997). Plant defences against pathogens. In J. F. Brown and H. J. Ogle (Eds.). **Plant pathogens and plant diseases** (pp. 263-286). Armidale: Rockvale Publications.

Gurdev S. K. (1997). Origin, dispersal, cultivation and variation of rice. **Plant Mol. Biol.** 35: 25–34.

Hall-Stoodley, L., Costerton, J. W., and Stoodley, P. (2004). Bacterial biofilms: From the natural environment to infectious diseases. **Nat. Rev. Microbiol.** 2: 95-108.

Hayat, S., Khan, N. A., and Alyemeni, M. (2013). **Salicylic acid: Plant growth and development**. Dordrecht: Springer.

Heil, M., and Bostock, R. M. (2002). Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. **Ann. Bot.** 89 (5): 503-512.

Herve, P., and Kayano, T. (2006). Japonica rice varieties (*Oryza sativa* , Nipponbare, and others). In K. Wang (eds). **Methods in molecular biology** (pp 123-222). Humana: Springer.

Hoffman, M. D., Zucker, L. I., Brown, P. J. B., Kysela, D. T., Brun, Y. V., and Jacobson, S. C. (2015). Timescales and frequencies of reversible and irreversible adhesion events of single bacterial cells. **Anal. Chem.** 87 (24): 12032-12039.

Holopainen, J., Heijari, J., Nerg, A. M., Vuorinen, M., and Kainulainen, P. (2009). Potential for the use of exogenous chemical elicitors in disease and insect pest management of conifer seedling production. **Open J. For. Sci. J.** 2: 17-24.

Huang, D., Shao, Z. Z., Yu, Y., Cai, M. M., Zheng, L. Y., Li, G. Y., and Hao, F. H. (2018). Identification, characteristics and mechanism of 1-deoxy-n-acetylglucosamine from deep-sea *virgibacillus dokdonensis* MCCC 1A00493. **Mar. Drugs.** 16 (2): 52.

Hull, R. (2014). Plant to plant movement. **Plant virology** (5th ed., pp. 669-751). Boston: Academic Press.

International Rice Research Institute. (2017). **Learn about best practices in rice farming** [On-line]. Available http://irri.org/our-work/research/rice-and-the-environment.

Jabs, T., Tschöpe, M., Colling, C., Hahlbrock, K., and Scheel, D. (1997). Elicitor-stimulated ion fluxes and $O_2$from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley. **Proc. Natl. Acad.Sci.** 94 (9): 4800-4805.

Janda, T., Gondor, O. K., Yordanova, R., Szalai, G., and Pál, M. (2014). Salicylic acid and photosynthesis: Signalling and effects. **Acta. Physiol. Plant.** 36 (10): 2537-2546.

Jansson, P.-e., Kenne, L., and Lindberg, B. (1975). Structure of the extracellular polysaccharide from *Xanthomonas campestris*. **Carbohydr. Res.** 45 (1): 275-282.

Jaroensathapornkul, J. (2007). The economic impact of public rice research in thailand. **Cu. J. Econ.** 19(2): 111-134.

Jeffries, P. (2002). Biotic interactions in plant-pathogen associations (Vol. 16).

Katzen, F., Ferreiro, D. U., Oddo, C. G., Ielmini, M. V., Becker, A., Pühler, A., and Ielpi, L. (1998). *Xanthomonas campestris* pv. *Campestris* gum mutants: Effects on xanthan biosynthesis and plant virulence. **J. Bacteriol. Res.** 180 (7): 1607-1617.

Kearney, J. (2010). Food consumption trends and drivers. **Philosophical transactions of the Royal Society of London. Series B, Biological sciences.** 365 (1554): 2793-2807.

Kemp, B. P., Horne, J., Bryant, A., and Cooper, R. M. (2004). *Xanthomonas axonopodis* pv. *Manihotis gum D* gene is essential for EPS production and pathogenicity and enhances epiphytic survival on cassava (*manihot esculenta*). **Physiol. Mol. Plant Pathol.** 64 (4): 209-218.

Khatoon, Z., McTiernan, C. D., Suuronen, E. J., Mah, T.-F., and Alarcon, E. I. (2018). Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. **Heliyon.** 4 (12): e01067-e01067.

Klaus W. E. Rudolph, M. G., Firous Ebrahim-Nesbat, Matthias Nöllenburg, Alim Zomorodian, Kerstin Wydra, Michael Neugebauer, Ursula Hettwer, Wagih El-Shouny, Bernd Sonnenberg, and Zoltan Klement. (1994). The role of extracellular polysaccharides as virulence factors for phytopathogenic *Pseudomonads* and *Xanthomonads*. In C. Kado and J.H. (eds) **Molecular mechanisms of bacterial virulence.** Dordrecht: Springer.

Klausen, M., Heydorn, A., Ragas, P., Lambertsen, L., Aaes-Jørgensen, A., Molin, S., and Tolker-Nielsen, T. (2003). Biofilm formation by *Pseudomonas aeruginosa* wild type, flagella and type IV pili mutants. **Mol. Microbiol.** 48 (6): 1511-1524.

Krishnan, N., Gandhi, K., Peeran, M. F., Muthurajan, R., Kuppusamy, P., and Thiruvengadam, R. (2014). Management of bacterial leaf blight disease in rice with endophytic bacteria. World Appl. Sci. J. 28 (12): 2229-2241.

Sticher, L., Mauch, B., and Métraux, J. (1997). Systemic acquired resistance. **Annu. Rev. Phytopathol.** 35 (1): 235-270.

Van Loon, L.C., Bakker, M., and Pieterse, C. M. J. (1998). Systemic resistance induced by rhizosphere bacteria. **Annu. Rev. Phytopathol.** 36 (1): 453-483.

Le Thanh, T., Thumanu, K., Wongkaew, S., Boonkerd, N., Teaumroong, N., Phansak, P., and Buensanteai, N. (2017). Salicylic acid-induced accumulation of biochemical components associated with resistance against xanthomonas oryzae pv. Oryzae in rice. **J. Plant Interact.** 12 (1): 108-120.

Li, W., Xu, Y., Zhang, Z., Cao, W., Li, F., Zhou, X., and Cai, X. (2012). Identification of genes required for nonhost resistance to *Xanthomonas oryzae* pv. *oryzae* reveals novel signaling components. **PLoS One.** 7 (8): e42796.

Li, Y.-H., and Tian, X. (2012a). Quorum sensing and bacterial social interactions in biofilms. **Sensors.** 12: 2519-2538.

Li, Y.-H., and Tian, X. (2012b). Quorum sensing and bacterial social interactions in biofilms. **Sensors.** 12 (3): 2519-2538.

Li, Y., Sun, H., Ma, X., Lu, A., Lux, R., Zusman, D., and Shi, W. (2003). Extracellular polysaccharides mediate pilus retraction during social motility of *Myxococcus xanthus* **Proc. Natl. Acad. Sci.** 100 (9): 5443-5448.

Loebenstein, G., and Carr, J. P. . (2006). **Natural resistance mechanisms of plants to viruses**. Dordrecht: Springer.

Lyon, D. R. W. C. N. D. (2014). **Induced resistance for plant defense**. Oxford: Blackwell.

Maurice, N. M., Bedi, B., and Sadikot, R. T. (2018). *Pseudomonas aeruginosa* biofilms: Host response and clinical implications in lung infections. **Am. J. Resp. Cell Mol.** 58 (4): 428-439.

Mejía-Teniente, L., de Dalia Durán-Flores, F., Chapa-Oliver, A. M., Torres-Pacheco, I., Cruz-Hernández, A., González-Chavira, M. M., and Guevara-González, R. G. (2013). Oxidative and molecular responses in *Capsicum annuum* l. after

hydrogen peroxide, salicylic acid and chitosan foliar applications. **Int. J. Mol. Sci..** 14 (5): 10178-10196.

Mhedbi-Hajri, N., Darrasse, A., Pigné, S., Durand, K., Fouteau, S., Barbe, V., Jacques, M.-A. (2011). Sensing and adhesion are adaptive functions in the plant pathogenic *Xanthomonads*. **BMC. Evol. Biol.** 11: 67-67.

Moharekar, S. T., Lokhande, S. D., Hara, T., Tanaka, R., Tanaka, A., and Chavan, P. D. (2003). Effect of salicylic acid on chlorophyll and carotenoid contents of wheat and moong seedlings. **Photosynthetica.** 41 (2): 315.

Moldenhauer, K., and Slaton, N. (2001). Rice growth and development. **Rice Production Handbook**: 7-14. Louisiana.

Mosleh, M., Hassan, Q., and Chowdhury, E. (2015). Application of remote sensors in mapping rice area and forecasting its production: A review. **Sensors.** 15: 769-791.

Mukherjee, A., K. Mohapatra, N., V. Suriya Rao, A., and Nayak, P. (2005). Effect of nitrogen fertilization on the expression of slow-blasting resistance in rice. **J. Agric. Sci.** 143 (5): 385-393.

Muthayya, S., Sugimoto, J., Montgomery, S., and Maberly, G. (2014). An overview of global rice production, supply, trade, and consumption. **Ann. N. Y. Acad. Sci.** 1324.

Nayar, N. M. (2014). The origin of asian rice. In N. M. Nayar (Ed.), **Origin and phylogeny of rices** (pp. 169-253). San Diego: Academic Press.

Nazar, R., Umar, S., Khan, N. A., and Sareer, O. (2015). Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline

accumulation and ethylene formation under drought stress. **S. Afr. J. Bot.** 98: 84-94.

Nelson, R. J., Ardales, E., Baraoidan, M., Yap, I., George, M. L. C., and Chen, D. H. (1996). Rice genetics III. Manila: World Scientific.

Niño-Liu, D. O., Ronald, P. C., and Bogdanove, A. J. (2006). *Xanthomonas oryzae* pathovars: Model pathogens of a model crop. **Mol. Plant Pathol.** 7 (5): 303-324.

Pal, T. (2011). Induction of systemic resistance in rice by leaf extract of cymbopogan citrus and ocimum sanctum against sheath blight disease. **Arch. Appl. Sci. Res.** 2011. 3 (1): 392-400.

Pancheva, T. V., Popova, L. P., and Uzunova, A. N. (1996). Effects of salicylic acid on growth and photosynthesis in barley plants. **J. Plant Physiol.** 149 (1): 57-63.

Pawar, S. and Radhakrishnan, V. (2016). The importance of optimum tillering in rice-an overview. **South Indian J. Biol. Sci.** 2: 125.

Perazzolli, M., Dagostin, S., Ferrari, A., Elad, Y., and Pertot, I. (2008). Induction of systemic resistance against *Plasmopara viticola* in grapevine by *Trichoderma harzianum* T39 and benzothiadiazole. **Biol. Control.** 47 (2): 228-234.

Petrova, O. E., and Sauer, K. (2012). Sticky situations: Key components that control bacterial surface attachment. **J. Bacteriol. Res.** 194 (10): 2413-2425.

Pinta1, W., Toojinda, T., Thummabenjapone, P., and Sanitchon, J. (2013). Pyramiding of blast and bacterial leaf blight resistance genes into rice cultivar RD6 using marker assisted selection. **Afr. J. Biotechnol.** 12: 4432-4438.

Pizarro-Cerdá, J., and Cossart, P. (2006). Bacterial adhesion and entry into host cells. **Cell.** 124 (4): 715-727.

Plotnikov, A., Zehorai, E., Procaccia, S., and Seger, R. (2011). The mapk cascades: Signaling components, nuclear roles and mechanisms of nuclear translocation. **Biochim. Biophys. Acta.** 1813 (9): 1619-1633.

Prakongkha, Sompong, Wongkaew, Athinuwat, and Buensanteai. (2013). Changes in salicylic acid in grapevine treated with chitosan and bth against *Sphacelomaampelinum*, the causal agent of grapevine anthracnose . **Afr. J. Microbiol.** 7 (7): 557-563.

Preston, G. M., Studholme, D. J., and Caldelari, I. (2005). Profiling the secretomes of plant pathogenic proteobacteria. **FEMS Microbiol. Rev.** 29 (2): 331-360.

Ratsep, J., Havis, N. D., and Walters, D. R. (2013). Controlling crop diseases using induced resistance: Challenges for the future. **J. Exp. Bot.** 64 (5): 1263-1280.

Reyna, N., and Yang, Y. (2006). Molecular analysis of the rice map kinase gene family in relation to magnaporthe grisea infection. **Mol. Plant Microbe. Interact.**19 (5): 530-40.

Richard., S. N. (1993). **Plant disease control: Towards environmentally acceptable methods**. London: Springer.

Rigano, L. A., Siciliano, F., Enrique, R., Sendín, L., Filippone, P., Torres, P. S., Marano, M. R. (2007). Biofilm formation, epiphytic fitness, and canker development in *Xanthomonas axonopodis* pv. *Citri*. **Mol. Plant Microb. Interact.** 20 (10): 1222-1230.

Schmidt, H., and Hensel, M. (2004). Pathogenicity islands in bacterial pathogenesis. **Clin. Microbiol. Rev.** 17 (1): 14-56.

Sels, J., Mathys, J., De Coninck, B. M. A., Cammue, B. P. A., and De Bolle, M. F. C. (2008). Plant pathogenesis-related (PR) proteins: A focus on pr peptides. **Plant Physiol. Biochem.** 46 (11): 941-950.

Shahbandeh, M. (2019). **Total rice consumption worldwide from 2008/2009 to 2019/2020** [On-line]. Available: https://www.statista.com/statistics/255977.

Sombunjitt, S., Sriwongchai, T., Kuleung, C., and Hongtrakul, V. (2017). Searching for and analysis of bacterial blight resistance genes from Thailand rice germplasm. **Agric. Nat. Resour.** 51 (5): 365-375.

Sutherland, I. W. (1990). Biotechnology of microbial exopolysaccharides. Cambridge: Cambridge University Press.

Sylvestre, C., Karlovsky, P., and Wydra, K. (2014). Biochemical approach for virulence factors' identification in *Xanthomonas oryzae* pv. *oryzae*. J. Plant Pathol. Microb. 5 (2).

Tanaka, A., and Yamaguchi, J. (1968). The growth efficiency in relation to the growth of the rice plant. **Soil Sci. Plant Nutr.** 14 (3): 110-116.

Tang, L., Zhu, Y., Hannaway, D., Meng, Y., Liu, L., Chen, L., and Cao, W. (2009). Ricegrow: A rice growth and productivity model. **NJAS-Wagen J. Life Sc.** 57 (1): 83-92.

Thai Agricultural Standard. (2008). **Good agricultural practices for rice national bureau of agricultural commodity and food standards**: Ministry of agriculture and cooperatives.

Thakur, M., and Sohal, B. S. (2013). Role of elicitors in inducing resistance in plants against pathogen infection: A review. **ISRN Biochem.** 2013: 762412-762412.

Toruño, T. Y., Stergiopoulos, I., and Coaker, G. (2016). Plant-pathogen effectors: Cellular probes interfering with plant defenses in spatial and temporal manners. **Ann. Rev. Phyto.** 54: 419-441.

Trafny, E. A. (2008). Biofilms and their role in infection pathogenesis. **Postepy Mikrobiologii.** 47: 353-357.

Turkyilmaz Unal, B., Aktas, L., and Guven, A. (2005). Salicylic acid induced some biochemical and physiological changes in phaseolus vulgaris l. **Sci. Eng. J. Firat Univ.** 17: 319-326.

Tuteja, N., and Mahajan, S. (2007). Calcium signaling network in plants: An overview. **Plant Signal Behav.** 2 (2): 79-85.

Vallad, G., and M. Goodman, R. (2004). Systemic acquired resistance and induced systemic resistance in conventional agriculture. **Crop Sci.** 4: 66.

Velusamy, P., and Gnanamanickam, S. S. (2003). Identification of 2,4-diacetylphloroglucinol production by plant-associated bacteria and its role in suppression of rice bacterial blight in india. **Curr. Sci.** 85(9):1270-1273

Vergara, B. S. (1991). Rice plant growth and development. In B. S. Luh (Ed.), **Rice: Production and Utilization** (pp. 13-22). Boston: Springer.

Vojnov, A. A., Zorreguieta, A., Dow, J. M., Daniels, M. J., and Dankert, M. A. (1998). Evidence for a role for the *gumB* and *gumC* gene products in the formation of xanthan from its pentasaccharide repeating unit by *Xanthomonas campestris*. **Microbiol.** 144 (6): 1487-1493.

Vu, B., Chen, M., Crawford, R. J., and Ivanova, E. P. (2009). Bacterial extracellular polysaccharides involved in biofilm formation. **Molecules.** 14 (7): 2535-2554.

Walters, Ratsep, J., and Havis, N. (2013). Controlling crop diseases using induced

resistance: Challenges for the future. **J. Exp. Bot.** 64 (5): 1263-1280.

Wang, J., Zhu, J., Huang, R., and Yang, Y. (2012). Investigation of cell wall composition related to stem lodging resistance in wheat (*Triticum aestivum* L.) by ftir spectroscopy. **Plant Signal. Behav.** 7 (7): 856-863.

Yadeta, K. A., and J. Thomma, B. P. H. (2013). The xylem as battleground for plant hosts and vascular wilt pathogens. **Front. Plant Sci.** 4: 97.

Yuan, M., Chu, Z., Li, X., Xu, C., and Wang, S. (2010). The bacterial pathogen xanthomonas oryzae overcomes rice defenses by regulating host copper redistribution. **Plant Cell.** 22 (9): 3164-3176.

Zhang, Y., Zhao, L., Zhao, J., Li, Y., Wang, J., Guo, R., Zhang, K. (2017). S5H/DMR6 encodes a salicylic acid 5-hydroxylase that fine-tunes salicylic acid homeostasis. **Plant Physio.** 175 (3): 1082-1093.

CHAPTER III

APPLICATION OF SALICYLIC ACID ELICITOR FOR CONTROLLING BACTERIAL LEAF BLIGHT DISEASE ON RICE AND EXAMINING OF PLANT DEFENSE MECHANISM USING SR-FTIR MICROSPECTROSCOPY

ABSTRACT

These research aimed to examine the application of salicylic acid as SA-Ricemate® elicitor for controlling bacterial leaf blight disease and investigate rice resistance mechanism against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) by using a Synchrotron Radiation-based Fourier-Transform Infra-Red (SR-FTIR) microspectroscopy. Rice plants cv. KDML 105 were treated with SA-Ricemate® elicitor or mock-water. After that, leaves were inoculated with *Xoo* and disease severity was evaluated. Leaves supernatants were used to detect changed of endogenous salicylic acid. Furthermore, treated leaves and control were cut and subjected to SR-FTIR. The result showed SA-Ricemate® reduced disease severity approximately 60% at three weeks post inoculation by increase of endogenous salicylic acid approximately 50%. The SR-FTIR variation of bio-molecular structure and intensity changes at mesophyll result in sub-cellular biochemical changes which

higher intensity groups of lipid, pectin and protein amide I and amide II whereas polysaccharides lower in treated sample. These results indicated that the SA-Ricemate® can reduce disease severity by activate compounds such as endogenous SA and possibility group of protein and lipid. The SR-FTIR can be a practical instrument to examine the sub-cellular level that related to plant defense mechanism. SA-Ricemate® also can be controlling bacterial leaf blight disease on rice with the mechanism of induced resistance.

Keywords: abiotic elicitor, bacterial leaf blight disease, biochemical composition, induced resistance, SR-Fourier transform infrared microspectroscopy

3.1 Introduction

Bacterial leaf blight (BLB) disease as a result of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is wide in South-East Asia and Japan with averaged losses of 20-50% on rice production (Walters, Newton, and Lyon, 2008; Shaheen et al., 2019). It is considered one of the most disastrous rice diseases in Thailand due to the suitable climate for developing of *Xoo*. Chemical control as bactericides, especially copper hydroxide has been recommended and popular method but its application is often ineffective because of rainfall and can be harmful to environment. In recent years, the use of resistance abiotic elicitors has been extensively used to reduce plant diseases with mode of induced resistance (IR) in different crops such as rice, chili, grapevine, chickpea, tomato, and maize (Khan et al., 2012; Deenamo et al., 2018; Ahmed et al., 2018). IR is a type of plant defense that contributes to increasing plant resistance against plant pathogens involving multiple mechanisms that can be induced by elicitors (Liyanage et al., 2017). In 2017, Le et al., (2017) reported that the resistance

in rice plant can be activated by spraying with salicylic acid (SA), leading to reduced BLB disease severity more than 38%. Similarly to Shao et al. (2018), this author used *streptomyces* JD211 as a new potential resistance induces against *Magnaporthe oryzae* on rice plant as an induction of defense (Le et al., 2017; Shao et al., 2018). The important characteristic of IR is the priming on plants to increase expressions of defense metabolites upon bacterial pathogen infection (Thakur and Sohal, 2013b). The plants generally expressed activities of defense responses by secondary metabolites interactions between plants and pathogens including major groups as a phenolic compound, terpenes, sulphur containing secondary metabolites and nitrogen-containing secondary metabolites (Shao et al., 2018; Zaynab et al., 2018). Exogenous SA acid has been used as a resistance elicitor against BLB disease and acts as a signal molecule in plants by activating expression of plant pathogenesis-related (*PR*) genes from the cinnamate pathway that provides precursors for various phenylpropanoid compounds (Shao et al., 2018). Synchrotron Fourier-Transform Infra-Red (SR-FTIR) microspectroscopy has been advanced as a novel bio-analytical technique by directly examining non-destructive samples (Yu, 2004; Theophilou et al., 2018). This novel technique, using of synchrotron light which characteristics small and brightness that can identify the molecular chemistry in a biological tissues including, structural and non-structural lignin, proteins, lipids, carbohydrates and their ratios (Yu, 2004; Thumanu et al., 2015). For example, a vibration peak of 1700–1600 refer to Amide I, 1600–1500 refer to Amide II, 1300–1200 refer to stretching hemicelluloses and lignins (Sivakumar et al., 2014). Therefore, SR-FTIR has ability to help tracking biochemical changes in plant tissues.

This study has been performed in order to characterize the resistance

mechanism induced by abiotic elicitor, including salicylic acid combining with inert-ingredients (SA-Ricemate®) against BLB disease in rice by examining the endogenous SA and using SR-FTIR microspectroscopy technique by monitoring biochemical changes involved with mechanisms of plant defense.

3.2 Materials and methods

3.2.1 Rice cultivar

Thai jasmine rice variety Khao Dawk Mali 105 (*Oryza sativa* L.) was used in this research as a susceptible variety.

3.2.2 *Xoo* strains and culture conditions

The selected virulence *Xoo* strain SUT1-121 was obtained from the stock culture of Plant Pathology and Biopesticide Laboratory (PPB Lab), Suranaree University of Technology, Thailand. It was originally provided by Prae Rice Research Institute. The *Xoo* was transferred to a nutrient glucose agar (NGA) medium then incubated at 28 ± 1°C for 48 h subsequently, the *Xoo* was multiplied in nutrient glucose broth (NGB) at 28 ± 1°C incubated with constant shaking at 180 rpm for 48 h. Concentration of the *Xoo* suspension was measured by specific adjusted to OD 0.2 with sterile distilled water to have approximately $1x10^8$ cfu mL^{-1} (Buensanteai, Yuen, and Prathuangwong, 2008; Krishnan et al., 2014; Le et al., 2017)

3.2.3 Preparation of a commercial abiotic elicitor product (SA-Ricemate®)

The exogenous salicylic acid elicitor (SA-Ricemate®) prototype is a product of the Bioactive Agro Industry Co .Ltd., developed at the PPB Laboratory, Suranaree University of Technology, Thailand.

3.2.4 Efficacy of the SA-Ricemate® elicitor in inducing resistance against BLB

Six concentrations of SA-Ricemate® varying 50, 100, 150, 200, 250, and 300 ppm of SA-Ricemate® were tested. The treatments were included positive control including commercial elicitor; chitooligosaccharide and copper hydroxide 77% WP and negative control; Water. The experiment was done in CRD with five replications, and two pots per one replication. Rice seeds cv. KDML 105 was soaked with sterile distilled water for 24 h before planting. Subsequently, one germinating seed was transferred into each of 30 cm diameter pots that contained 5 kg of Suranaree University of Technology farm soil. The pots were kept under a greenhouse condition with 12 hours photoperiod, 28 ± 4°C and 60-75% humidity. After sowing at 15, 30 and 45 days, the rice plants were sprayed with SA-Ricemate® elicitor at the stated concentrations in each treatment. The rice plants were inoculated at 50 days post sowing with a suspension at $1x10^8$ cfu mL^{-1} on top-leaves by cutting leaf 3 cm from the leaf tip then, covered with transparent plastic bags and incubated at 24 h 28°C (Chithrashree et al., 2011; Mizobuchi et al., 2013; Xu et al., 2013; Ke, Hui, and Yuan, 2017; Le et al., 2017).

The BLB disease severity was recorded three times every 7 days post-inoculation (DPI) using the disease score chart of the International Rice Research Institute (IRR) for assessing BLB symptom. Then, the percentage of disease severity was calculated by using the following formulas (1)

$$\text{Disease severity (\%)} = \left(\frac{\sum_{i=1}^{n} r_i}{n \times m} \right) \times 100 \quad (1)$$

Where ‘r’ is the set of numerical ratings, ‘n’ is the total of evaluations per sample, and ‘m’ is the maximum value used for the evaluations (Ferreira et al., 2017; Le et al., 2017). The reduction of disease severity was calculated using the formulated equation (2).

$$\text{Reduction on disease severity (\%)} = \frac{DSn - Dst}{DSn} \times 100 \quad (2)$$

Where ‘DSn’ is the calculated disease severity from untreated samples and ‘DSt’ is the calculated disease severity from elicitor-treated samples.

3.2.5 Determination of endogenous SA

0.5 g of rice leaf samples from each treatment were soaked in liquid nitrogen and homogenized with 1 mL of extraction buffer (methanol: glacial acetic acid: water; 90:9:1 by volume) then, centrifuged at 14000 x g for 10 min, under 4°C. After that 0.5 mL of the supernatant was mixed with 0.02 M ferric ammonium sulfate solution at equal volume and incubated for 5 min at 30°C. The absorbance at 530 nm was read by the microplate reader (Bio-Tek ,USA) and, compared with that of the standard

reference to calculate the endogenous SA in the sample (Clayton and Thiers, 1966; Rozhon et al., 2005)

3.2.6 Biochemical change analyses using SR-FTIR microspectroscopy

Leaf samples were selected from the best concentration treatment of the previous experiment. The leaves were fixed with the Optimal Cutting Temperature Compound (O.C.T.) (Tissue-Trek®, USA), then rapid cooled in liquid nitrogen, and transversely cut with a cryostat microtome (Leica 3050 S, Germany) at 7 microns subsequently, the cut samples were put on 13x2 mm infrared transparent barium fluoride (BaF_2) optical windows and subjected to FTIR microspectroscopy (Le et al., 2017; Thumanu et al., 2017).

3.2.7 Data analysis of SR-FTIR microspectroscopy

The spectral data were collected and imaged at the beamline 4.1 IR Spectroscopy, Synchrotron Light Research Institute (SLRI). The determinations were carry out by using mode of mapping with an aperture size as 10×10 μm, 4 cm^{-1} of spectral resolution and 64 scans for background (Wang et al., 2015). Spectral derivative and equipment were performed by OPUS 7.2 software (Bruker Ltd., Germany) then, data analyzed by cytospecTM software and unscramblerX 10.0 software (Clayton and Thiers, 1966; Le et al., 2017; Thumanu et al., 2017; Durak and Depciuch, 2020).

3.3 Results

3.3.1 The effectiveness of the SA-Ricemate® elicitor on inducing resistance against BLB

This experiment confirmed the effectiveness of SA-Ricemate® in controlling BLB when it was sprayed 3 times before the inoculation of *Xoo*. The results showed that at 100 ppm or higher concentrations. SA-Ricemate® elicitor can 41-67% reduced the disease severity which was significantly when compared with the untreated treatment but was not significant when compared with that of the positive control (57-58%). Whereas, 50 ppm SA-Ricemate® elicitor reduced 12-26% controlling of BLB that was not significantly with untreated sample. Therefore, ≥100 ppm SA-Ricemate® elicitor showed high efficiency to BLB control on rice leaf (Table 3.1).

Table 3.1 Effectiveness of SA-Ricemate® elicitor on the disease severity and reduced disease severity of BLB in rice cv. KDML 105 inoculated with *Xanthomonas oryzae* pv. *oryzae*. strain SUT1-121

Treatment [1/]	Disease severity (%)[2/]			Reduced disease severity (%)		
	7 DPI	14 DPI	21 DPI	7 DPI	14 DPI	21 DPI
SA-Ricemate® 50 ppm	26.04 ± 2.75 [c]	35.42 ± 4.54 [c]	39.58 ± 2.75 [c]	13.80 ± 7.00 [a]	12.88 ± 1.96 [a]	26.77 ± 1.83 [a]
SA-Ricemate® 100 ppm	17.71 ± 2.75 [b]	18.75 ± 1.04 [b]	21.88 ± 1.04 [b]	41.39 ± 4.81 [b]	53.88 ± 1.18 [b]	59.53 ± 3.91 [b]
SA-Ricemate® 150 ppm	15.63 ± 3.12 [b]	16.67 ± 1.04 [b]	22.92 ± 1.52 [b]	48.28 ± 8.51 [b]	59.00 ± 3.12 [b]	57.60 ± 5.19 [b]
SA-Ricemate® 200 ppm	16.67 ± 2.77 [b]	19.79 ± 2.11 [b]	21.88 ± 1.00 [b]	44.83 ± 7.39 [b]	51.31 ± 3.25 [b]	59.53 ± 2.99 [b]
SA-Ricemate® 250 ppm	13.54 ± 2.71 [b]	17.71 ± 2.08 [b]	20.83 ± 2.18 [b]	55.18 ± 7.73 [b]	56.44 ± 3.49 [b]	61.46 ± 3.99 [b]
SA-Ricemate® 300 ppm	12.50 ± 2.85 [b]	16.67 ± 1.41 [b]	17.71 ± 1.44 [ab]	58.63 ± 6.58 [b]	59.00 ± 5.86 [b]	67.24 ± 3.95 [cb]
Chitooligosaccharides-commercial	12.50 ± 2.14 [b]	17.50 ± 1.61 [b]	22.50 ± 1.12 [b]	57.13 ± 5.98 [b]	58.00 ± 3.60 [b]	58.38 ± 3.12 [b]
Copper hydroxide 77% WP	8.33 ± 1.04 [a]	10.42 ± 1.24 [a]	12.50 ± 1.20 [a]	72.42 ± 4.13 [c]	74.38 ± 2.88 [c]	76.88 ± 1.71 [c]
Control (water)	30.21 ± 4.10 [c]	40.63 ± 2.98 [d]	54.17 ± 3.75 [d]	0	0	0
F-Test	**	**	**	**	**	*
CV (%)	27.4	15.51	11.44	30	18.5	20.09

[1/] Rice plants were treated by foliar sprays at 15, 30, and 45 DPS, with SA-Ricemate® elicitor at different concentrations and water used as the control. Rice leaves were inoculated with *Xoo* SUT1-121strain at 50 DPS.[2/] Disease severity was evaluated at 7, 14, 21 days post-inoculation (DPI). Each value represents a mean of five replicate. The mean in the column followed by the same letter is non-significant difference according to Duncan's multiple range test at P = 0.05.

3.3.2 The accumulation of endogenous SA content

The endogenous SA content in rice plants was increased at 24 h post-inoculation by all treatments as shown in Table 3.2.

Table 3.2 Effectiveness of SA-Ricemate® elicitor on the accumulation of endogenous salicylic acid in rice leaves cv. KDML 105

Treatment [1/]	Endogenous salicylic acid (µg g^{-1} of fresh weight) [2/]		Increase of SA Activity (%)
	Pre inoculation	Post Inoculation 24 h	
SA-Ricemate® 50 ppm	12.12 ± 0.17	17.60 ± 0.03 [b]	30.78 ± 0.30 [b]
SA-Ricemate® 100 ppm	12.20 ± 0.18	18.46 ± 0.10 [b]	51.27 ± 1.00 [b]
SA-Ricemate® 150 ppm	12.39 ± 0.14	18.34 ± 0.08 [b]	48.00 ± 0.80 [b]
SA-Ricemate® 200 ppm	11.78 ± 0.26	18.14 ± 0.21 [b]	54.05 ± 2.10 [b]
SA-Ricemate® 250 ppm	12.28 ± 0.22	18.28 ± 0.26 [b]	48.84 ± 2.60 [b]
SA-Ricemate® 300 ppm	12.19 ± 0.21	18.46 ± 0.25 [b]	51.44 ± 2.50 [b]
Chitooligosaccharides commercial	11.99 ± 0.38	16.61 ± 0.37 [b]	38.33 ± 2.50[b]
Copper hydroxide 77% WP	11.69 ± 0.08	13.03 ± 0.32 [a]	5.19 ± 0.32[a]
Control (water)	13.21 ± 0.10	13.89 ± 0.07 [a]	11.4 ± 0.70[a]
F-Test	ns	**	**
CV (%)	6.5	3.8	18.46

[1/] Rice plants were treated by foliar sprays at 15, 30, and 45 DPS, with SA-Ricemate® elicitor at different concentrations and water used as the control. Rice leaves were challenged with *Xoo* SUT1-121 strain at 50 DPS. [2/] Endogenous salicylic acid was evaluated pre-inoculation and 24 h post-inoculation. Each value represents a mean of five replicates. The mean in the column followed by the same letter is non-significance difference according to Duncan's multiple range test at P = 0.05.

The endogenous SA contents in rice plants treated with SA-Ricemate® elicitor at 50, 100, 150, 200, 250, 300 ppm and with oligosaccharide-commercial elicitor were significantly increased by 30.78, 51.27, 48.00, 54.05, 48.84, 51.44, and 38.33% respectively. This is considered an important difference when comparing with the treatment based on copper hydroxide which had an endogenous SA increase of 5.19% against the 11.40% from the untreated samples.

3.3.3 SR-FTIR microspectroscopy

The SR-FTIR spectra were used for investigating changes on rice biochemical and cellular compositions after being treated with SA-Ricemate®. The results showed a clear discrepancy between the untreated (water-mock) and treated clusters of 100 ppm of SA-Ricemate® with showed the PCA score plot to separately the treated and untreated group, which was explained by 51% PC1 and 26% PC2 (Figure 1a). The high positive loading from PC1 at 2935, 1660, and 1552 cm^{-1} corresponded with the positive score plot from the treated sample. Whereas, the high negative loading from the PC1 at 1162 and 1039 cm^{-1} corresponded with the negative score plot from the untreated sample (Figure 1b). SA-Ricemate® treated samples were different in the SR-FTIR spectra changes in the mesophyll revealed three distinguishable regions (Figure. 1c). The first region (3000-2800 cm^{-1}) according to the CH2, CH3 from lipid groups with a clearly peak at 2964, 2929 and 2854 $cm^{-1}$of the treated sample higher than the untreated sample. The second region (1700-1500 cm^{-1}) composed of proteins and peptides with amide group showed a peak at 1654 and 1546 cm^{-1} from the treated sample higher than the untreated sample. The third region (1300-900 cm^{-1}) involving polysaccharides and carbohydrates showed peaks at 1160, 1106, 1037, and 989 cm^{-1} from the untreated sample higher than the treated sample.

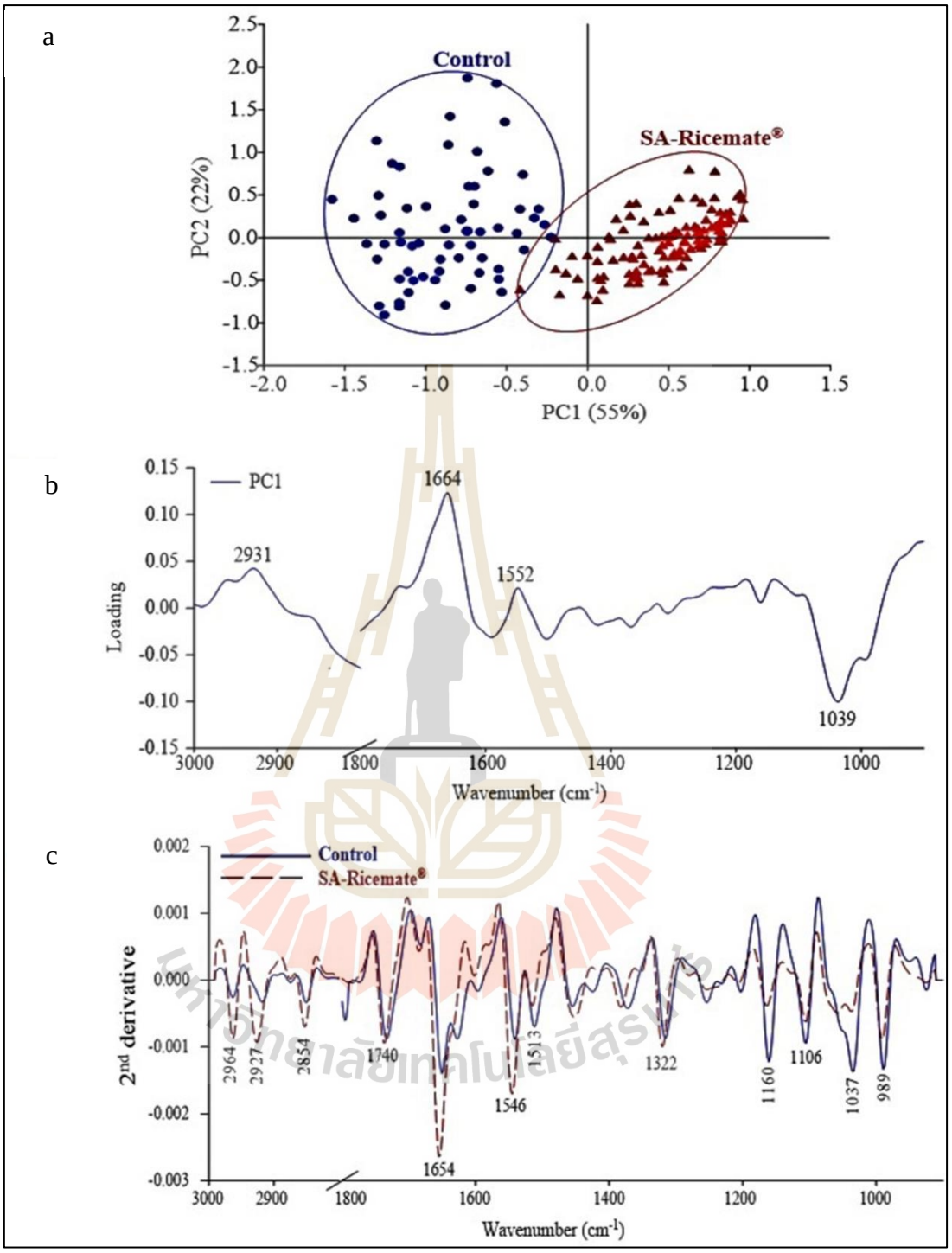


Figure 3.1 Principal Component Analysis (PCA) of SR-FTIR spectra obtained from mesophyll of rice leaf tissue a) Comparison of SA-Ricemate® treated and untreated, the scoreplot based on PC1 and PC2 from PCA shows that the

group of treated and untreated mesophyll cell spectral signatures can be differentiated. b) loading plots from PCA analysis of mesophyll treated and untreated group and c) Overlay of the average 2nd derivative spectrum of mesophyll of rice leaf tissue between treated with SA-Ricemate® compared to the untreated and then challenge inoculation with *Xoo*

3.4 Discussion

In this study, the abiotic elicitor SA-Ricemate® based on the plant defense-elicitor of salicylic acid has been described as a possible new bio-stimulant act as resistance inducer. The concentrations of SA-Ricemate at 100 ppm to 300 ppm is an optimal concentration range for controlling the BLB disease. The use of lower concentrations than 100 ppm is considered not effective against rice BLB. The obtained results are in accordance with War et al. (2011) who reported that chickpea (*Cicer arietinum*) responded to a SA treatment at 1.5 mM with higher induction of plant defense enzymes as a POD, PPO, H_2O_2, and defense proteins activities more than the use of SA at 1 and 2 mM. These results indicate that SA at 1.5 mM can be appropriate for activating plant immune (War et al., 2011). The evidence of the salicylic acid in plant defense response and involved in endogenous signal-mediated local and systemic plant defense were previously appreciated in several research works. According to Wani et al. (2017) who described the application of salicylic acid on plant improved initiation of pathogenesis-related gene expression and synthesis of defensive compounds involved in local resistance and systemic acquired resistance (Wani etal., 2017). The same report was also obtained by Yang et al. (2019)

that the effect of pretreatment with salicylic acid, with resulted in lower of rice blast disease (*Magnaporthe oryzae*) and higher expression levels of the rice defense-related genes *PR1*, *PAL*, *HSP90*, and *PR5* on rice leaves (Yang et al., 2019).

The mechanism of induce resistance (IR) provides protection in distant parts of rice plants via a signaling transduction pathway. The salicylic acid (SA) pathway is a major signaling pathway during rice BLB resistance by crosstalk between signaling that can provides a potential for efficient energy and accumulation of potential enzyme to fight against pathogen. (Lopez-Gresa et al., 2016; Adam et al., 2018). According to Sticher et al. (1997), SA signaling pathway can be triggered by exogenous SA which increases disease resistance because this pathway is related to systemic acquired resistance (SAR) that can occur by the accumulation of endogenous SA to be activated after plant pathogen infection.

Endogenous SA is involved in secondary metabolites of plant defense such as terpenes, phenolic compounds, and alkaloids that play a role on plant defense (Gharbi et al., 2017; Genzel et al., 2018). The concentration of endogenous SA can determine the selective activation of defense responses during pathogen infection and invasion that can change physiological, biochemical through molecular levels of plant (War et al., 2011; Le et al., 2017). SA activates variaty of enzymes such as peroxidase (POD), peroxidase (POX), superoxide dismutase (SOD), polyphenol oxidase (PPO) and phenylalanine ammonialyase (PAL) these enzymes play a role in protecting cell from toxic effects and active role in metabolism (Sahebani and Hadavi, 2009; War et al., 2011). Our results showed that the accumulations of endogenouse SA content in treated rice plant after infection with *Xoo* who higher than the non-treated by approximately 50%. An elevated concentration of endogenous SA was similarly

observed in tomato and citrus plants after a treatment with the elicitor ASM 1 mM for Tomato Virus and Citrus Viroid (Lopez-Gresa et al., 2016). Babu et al. (2003) also found in their study that in the susceptible rice variety IR50 significant reduction of BLB development and BLB lesion length around 20 % due to a pre-treatment with SA at 1000 μmol L^{-1} by significantly increase 44.35 % of endogenous SA accumulation. Thus, the concentration of endogenous SA can be an important component of this resistant mechanism in rice and can determine the selective activation of immune during pathogen infection and invasion (Metraux et al., 1990; Aranega et al., 2014).

The association of the biomolecular and its intensity from the average spectra suggest higher accumulations of lipids and proteins. Lipids are the major part of a cell membrane, play a roles in several cellular system like energy storage, protection communication, structural support and also hydrocarbon as a monomer that prevent water loss, protect cells and nutrients of plant and coat plant leaves surface to fight against pathogen attack. Zhang et al. (2015) reported that phospholipids phosphatidic acid (PA) belonging to the membrane lipid bilayer act as a signalling immunity and link to ROS activity and SA accumulation to Gao et al. (2017) also reported that lipids and lipid metabolites who important in rice plant to against rice bacteria blight and rice blast by plant-microbe interactions (Zhang and Xiao, 2015; Gao et al., 2017). Moreover, Enzymes including beta-glucanases and chitinases are some part of pathogenesis-related (*PR*) proteins that have important role in the plant cells to protect them from pathogen infection (Buensanteai et al., 2012; Thakur and Sohal, 2013a; Thumanu et al., 2015; Thumanu et al., 2017). When plants recognized the attack by insect, fungal, bacterial, or virus-viroid then, Beta-1,3-glucanase or chitinase, activate the plant defenses to against fungal infection. Anita et al. (2014) reported systemic

induce resistance in rice against rice root knot can occur by increasing chitinase enzyme activity (Leubner-Metzger and Meins, 2000; Wu et al., 2001; Anita and Samiyappan, 2012). Amide I and amide II associated with secondary proteins that have important amino acid which implicated in disease resistance such as L-phenylalanine is a precursor contributing plant defense metabolites by phenylpropanoid and lignin pathway similar Macoy et al. (2015) reported that several plant amide groups such as hydroxycinnamic acid amides have shown important interaction between plant and pathogen. (Buchanan et al., 2012; Macoy et al., 2015; Lahlali et al., 2017). Furthermore, polysaccharide as carbohydrates or sugars groups are necessary to supply the energy source to the defenses and can be used as regulation signals for defense genes that can be helpful to controlling plant diseases (Bolton, 2009; Buensateai et al., 2012; Trouvelot et al., 2014). During infection, the plants will modify or change their sugar source and activate their defense responses as increase PR proteins and some sugars use as source of activate agents to combat pathogens (Tauzin and Giardina, 2014; Zhao et al., 2018).

3.5 References

Adam, A. L., Nagy, Z. Á., Kátay, G., Mergenthaler, E., and Viczián, O. (2018). Signals of systemic immunity in plants: Progress and open questions. **Int. J. Mol. Sci.** 19 (4): 1146.

Anita, B., and Samiyappan, R. (2012). Induction of systemic resistance in rice by *Pseudomonas fluorescens* against rice root knot nematode meloidogyne graminicola. **J. Biopestic.** 5: 53-59.

Aranega-Bou, P., de la O Leyva, M., Finiti, I., García-Agustín, P., and González-Bosch, C. (2014). Priming of plant resistance by natural compounds. Hexanoic acid as a model. **Front. Plant Sci.** 5: 488-488.

Bolton, M. D. (2009). Primary metabolism and plant defense-fuel for the fire. **Mol. Plant Microbe Interact.** 22 (5): 487-497.

Buchanan, E. G., III, W. H. J., Choi, S. H., Guo, L., Gellman, S. H., Müller, C. W., and Zwier, T. S. (2012). Single-conformation infrared spectra of model peptides in the amide i and amide ii regions: Experiment-based determination of local mode frequencies and inter-mode coupling. **J. Chem. Phys..** 137 (9): 094301.

Buensanteai, N., Thumanu, K., Sompong, M., Athinuwat, D., and Prathuangwong, S. (2012). The FTIR spectroscopy investigation of the cellular components of cassava after sensitization with plant growth promoting rhizobacteria, *Bacillus subtilis* CaSut007. **Afr. J. Microbiol. Res.** 6(3):603-610.

Buensanteai, N., Yuen, G., and Prathuangwong, S. (2008). The biocontrol bacterium bacillus amyloliquefaciens kps46 produces auxin, surfactin and extracellular proteins for enhanced growth of soybean plant. **Thai J. Agric. Sci.** 41(3-4): 101-116.

Chithrashree, Udayashankar, A. C., Chandra Nayaka, S., Reddy, M. S., and Srinivas, C. (2011). Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. **Biol. Control.** 59 (2): 114-122.

Clayton, A. W., and Thiers, R. E. (1966). Direct spectrophotometric determination of salicylic acid, acetylsalicylic acid, salicylamide, caffeine, and phenacetin in tablets or powders. **J. Pharm. Sci.** 55 (4): 404-407.

Durak, T., and Depciuch, J. (2020). Effect of plant sample preparation and measuring methods on ART-FTIR spectra results. **Environ. Exp. Bot.** 169: 103915.

Ferreira, D., Falahati, F., Linden, C., Buckley, R. F., Ellis, K. A., Savage, G., Westman, E. (2017). A 'disease severity index' to identify individuals with subjective memory decline who will progress to mild cognitive impairment or dementia. **Sci. Rep.** 7: 44368.

Gao, M., Yin, X., Yang, W., Lam, S. M., Tong, X., Liu, J., He, Z., et al. (2017). GDSL lipases modulate immunity through lipid homeostasis in rice. **PLoS Pathog.** 13 (11): e1006724-e1006724.

Genzel, F., Franken, P., Witzel, K., and Grosch, R. (2018). Systemic induction of salicylic acid-related plant defences in potato in response to *Rhizoctonia solani* Ag3pt. **Plant Pathol.** 67 (2): 337-348.

Gharbi, E., Martínez, J., Ben Ahmed, H., Dailly, H., Quinet, M., and Lutts, S. (2017). The salicylic acid analog 2,6-dichloroisonicotinic acid has specific impact on the response of the halophyte plant species *Solanum chilense* to salinity. Plant Growth Regul. 82: 517-525.

Xanthomonas oryzae pv. *oryzae* inoculation and growth rate on rice by leaf clipping method. **Bio. Protocol.** 7 (19): e2568.

Krishnan, N., Gandhi, K., Peeran, M. F., Muthurajan, R., Kuppusamy, P., and Thiruvengadam, R. (2014). Management of bacterial leaf blight disease in rice with endophytic bacteria. **World Appl. Sci. J.** 28 (12): 2229-2241.

Lahlali, R., Song, T., Chu, M., Yu, F., Kumar, S., Karunakaran, C., and Peng, G. (2017). Evaluating changes in cell-wall components associated with clubroot resistance using fourier transform infrared spectroscopy and RT-PCR. **Int. J. Mol. Sci..** 18: 2058.

Le Thanh, T., Thumanu, K., Wongkaew, S., Boonkerd, N., Teaumroong, N., Phansak, P., and Buensanteai, N. (2017). Salicylic acid-induced accumulation of biochemical components associated with resistance against *Xanthomonas oryzae* pv. *oryzae* in rice. **J. Plant Interact.** 12 (1): 108-120.

Leubner-Metzger, G., and Meins Jr, F. (2000). Sense transformation reveals a novel role for class I β-1,3-glucanase in tobacco seed germination. **The Plant J.** 23 (2): 215-221.

Liyanage, S., Dassanayake, R. S., Bouyanfif, A., Rajakaruna, E., Ramalingam, L., Moustaid-Moussa, N., and Abidi, N. (2017). Optimization and validation of cryostat temperature conditions for trans-reflectance mode FTIR microspectroscopic imaging of biological tissues. **MethodsX.** 4: 118-127.

Lopez-Gresa, M. P., Lisón, P., Yenush, L., Conejero, V., Rodrigo, I., and Bellés, J. M. (2016). Salicylic acid is involved in the basal resistance of tomato plants to citrus exocortis viroid and tomato spotted wilt virus. **PLoS One.** 11 (11): e0166938-e0166938.

Macoy, D. M., Kim, W.-Y., Lee, S. Y., and Kim, M. G. (2015). Biotic stress related functions of hydroxycinnamic acid amide in plants. **J. Plant Biol.** 58 (3): 156-163.

Metraux, J. P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., Inverardi, B., et al. (1990). Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. **Science.** 250 (4983): 1004-1006.

Mizobuchi, R., Sato, H., Fukuoka, S., Tsushima, S., Imbe, T., and Yano, M. (2013). Identification of QRBSL, a QTL involved in resistance to bacterial seedling rot in rice. **Theor. Appl. Genet.** 126 (9): 2417-2425.

Rozhon, W., Petutschnig, E., Wrzaczek, M., and Jonak, C. (2005). Quantification of free and total salicylic acid in plants by solid-phase extraction and isocratic high-performance anion-exchange chromatography. **Anal. Bioanal. Chem.** 382 (7): 1620-1627.

Sahebani, N., and Hadavi, N. (2009). Induction of H_2O_2 and related enzymes in tomato roots infected with root knot nematode (*M. Javanica*) by several chemical and microbial elicitors. **Biocontrol Sci. Technol.** 19 (3): 301-313.

Shaheen, R., Zahid Sharif, M., Amrao, L., Zheng, A., Manzoor, M., Majeed, D., Ali, A., et al. (2019). Investigation of bacterial leaf blight of rice through various detection tools and its impact on crop yield in punjab, pakistan. **Pak. J. Bot.** 51 (4).

Shao, Z., Li, Z., Fu, Y., Wen, Y., and Wei, S. (2018). Induction of defense responses against *Magnaporthe oryzae* in rice seedling by a new potential biocontrol agent *streptomyces* JD211. **J. Basic Microb.** 58 (8): 686-697.

Sivakumar, S., Khatiwada, C. P., and Sivasubramanian, J. (2014). Studies the alterations of biochemical and mineral contents in bone tissue of mus musculus due to aluminum toxicity and the protective action of

desferrioxamine and deferiprone by FTIR, ICP-OES, sem and xrd techniques. **Spectrochim. Acta A Mol.** 126: 59-67.

Tauzin, A. S., and Giardina, T. (2014). Sucrose and invertases, a part of the plant defense response to the biotic stresses. **Front. Plant Sci.** 5: 293-293.

Thakur, M., and Sohal, B. S. (2013a). Role of elicitors in inducing resistance in plants against pathogen infection: A review. **ISRN Biochem.** 2013: 762412.

Thakur, M., and Sohal, B. S. (2013b). Role of elicitors in inducing resistance in plants against pathogen infection: A review. **ISRN Biochem.** 2013: 10.

Theophilou, G., Morais, C. L. M., Halliwell, D. E., Lima, K. M. G., Drury, J., Martin-Hirsch, P. L., Martin, F. L. (2018). Synchrotron- and focal plane array-based fourier-transform infrared spectroscopy differentiates the basalis and functionalis epithelial endometrial regions and identifies putative stem cell regions of human endometrial glands. **Anal. Bioanal. Chem.** 410 (18): 4541-4554.

Thumanu, K., Sompong, M., Phansak, P., Nontapot, K., and Buensanteai, N. (2015). Use of infrared microspectroscopy to determine leaf biochemical composition of cassava in response to *Bacillus Subtilis* CaSut007. **J. Plant Interact.** 10 (1): 270-279.

Thumanu, K., Wongchalee, D., Sompong, M., Phansak, P., Le Thanh, T., Namanusart, W., Buensanteai, N. (2017). Synchrotron-based ftir microspectroscopy of chili resistance induced by bacillus subtilis strain d604 against anthracnose disease. **J. Plant Interact.** 12 (1): 255-263.

Trouvelot, S., Héloir, M.-C., Poinssot, B., Gauthier, A., Paris, F., Guillier, C., . . . Adrian, M. (2014). Carbohydrates in plant immunity and plant protection: Roles and potential application as foliar sprays. **Front. Plant Sci.** 5: 592-592.

Walters, D., Newton, A., and Lyon, G. (2014). **Induced resistance for plant defence: A sustainable approach to crop protection**. Oxford: Blackwell.

Wang, M., Lu, X., Yin, X., Tong, Y., Peng, W., Wu, L., . . . Zhang, J. (2015). Synchrotron radiation-based fourier-transform infrared spectromicroscopy for characterization of the protein, peptide distribution in single microspheres. **Acta Pharmaceutica Sinica B.** 5 (3): 270-276.

Wani, A. B., Chadar, H., Wani, A. H., Singh, S., and Upadhyay, N. (2017). Salicylic acid to decrease plant stress. **Environ. Chem. Lett.** 15 (1): 101-123.

War, A. R., Paulraj, M. G., War, M. Y., and Ignacimuthu, S. (2011). Role of salicylic acid in induction of plant defense system in chickpea (*Cicer arietinum*). **Plant Signal Behav.** 6 (11): 1787-1792.

Wu, C. T., Leubner-Metzger, G., Meins, F., Jr., and Bradford, K. J. (2001). Class i beta-1,3-glucanase and chitinase are expressed in the micropylar endosperm of tomato seeds prior to radicle emergence. **Plant physiol.** 126 (3): 1299-1313.

Xu, J., Audenaert, K., Hofte, M., and De Vleesschauwer, D. (2013). Abscisic acid promotes susceptibility to the rice leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae* by suppressing salicylic acid-mediated defenses. **PLoS One.** 8 (6): e67413.

Yang, J., Wang, Y., Liu, L., Liu, L., Wang, C., Wang, C., and Li, C. (2019). Effects of exogenous salicylic acid and ph on pathogenicity of biotrophy-associated

secreted protein 1 (Bas1) overexpressing strain, *Magnaporthe oryzae*. **Environ. Sci. Pollut. Res.** 26 (14): 13725-13737.

Yu, P. (2004). Application of advanced synchrotron radiation-based fourier transform infrared (SR-FTIR) microspectroscopy to animal nutrition and feed science: A novel approach. **Br. J. Nutr.** 92 (6): 869-885.

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M. H., and Bahadar, K. (2018). Role of secondary metabolites in plant defense against pathogens. **M icrob. Pathog.** 124: 198-202.

Zhang, Q., and Xiao, S. (2015). Lipids in salicylic acid-mediated defense in plants: Focusing on the roles of phosphatidic acid and phosphatidylinositol 4-phosphate. **Front. Plant Sci.** 6: 387-387.

Zhao, D., You, Y., Fan, H., Zhu, X., Wang, Y., Duan, Y., . . . Chen, L. (2018). The role of sugar transporter genes during early infection by root-knot nematodes. **Int. J. Mol. Sci.** 19 (1): 302.

CHAPTER IV

SALICYLIC ACID ELICITOR INHIBITING *XANTHOMONAS ORYZAE* GROWTH, MOTILITY, BIOFILM, POLYSACCHARIDES PRODUCTION, AND BIOCHEMICAL COMPONENTS DURING PATHOGENESIS ON RICE

ABSTRACT

Application of salicylic acid elicitor (SA-Ricemate®) to rice plant can control bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). It exhibited antibacterial activity at high concentrations of ≥250 ppm and can significantly reduce growth of *Xoo* and disease severity. Salicylic acid affected the bacterial population, swimming motility, twitching motility, biofilm formation, polysaccharides production, and cell component of the *Xoo*. By using Synchrotron Radiation-based Fourier Transform Infra-Red (SR-FTIR) microspectroscopy changes on biochemical components of *Xoo* were detected changes in the cell membrane was identified as fatty acid (2825 cm^{-1}), and in the cell wall as nucleic acid, and phospholipid groups (1162 and 1040 cm^{-1}) when comparing with the untreated *Xoo*. The results indicated that SA-Ricemate® can be used as an antibacterial agent to control bacterial leaf blight of rice by changing biochemical components and reducing the virulence factors of this pathogen.

4.1 Introduction

Xanthomonas oryzae pv. *oryzae* (*Xoo*) causing bacterial leaf blight (BLB) is considered one of the important bacterial pathogens in rice plant production system which that can cause grain weight reduction and yield average losses by 30% and up to 100% under extreme conditions (Walters, Newton, and Lyon, 2008; Shaheen et al., 2019). *Xoo* grows within rice plant by infecting them through wounds and natural openings. The pathogen can invade leaf veins as well as the xylem blocking and inhibiting some agent in xylem. This contributes to plant wilt showing symptoms on leaves as pale green-yellow and brown-gray areas (Yadeta and Thomma, 2013; Hull, 2014). The main factor that provides the pathogen of resistance to extreme environment are the biofilm formation and the extracellular polysaccharides (EPS) allowing them to survive and to invade the rice plant (Schmidt and Hensel, 2004; Buttner and Bonas, 2010; Yuan et al., 2010). EPS composed of polysaccharides, proteins, nucleic acids, and lipids that protect bacteria from dehydration, toxic compounds, and mechanical instability as well as acting like molecular glue allowing adhere of cells to one on other and xylem plugging leading to BLB lesion development in rice plant (Flemming and Wingender, 2010; Batoni, Maisetta, and Esin, 2016). Formations of bacterial biofilms are likely to occur on plant surfaces and inside the plant such as in xylem. The form of biofilm depends on bacterial motility which contribute to the bacteria motion to stick on a surface (Minamino, Imada, and Namba, 2008; Yaron and Römling, 2014). The bacterial motion by swimming and twitching motilities have an important role in the pathogenicity as responses by chemotactic that requires flagella and type IV pili, respectively (Weller-Stuart et al., 2017; Bae et al., 2018). This factor fosters competition to establish symbiotic and

pathogenic relations between plants and pathogens (Kerchove and Elimelech, 2008; Vicario, Dardanelli, and Giordano, 2014). The method to control this pathogen reduces its ability to spread and induces plant immune. Although chemicals such as copper hydroxide, copper oxychloride, etc. are commonly used to control BLB but they are considered to be harmful to humans and the environment. Moreover, the chemicals cannot limit the spread of BLB because they non systemic. Recently, several researches on induced resistance by using inducers such as salicylic acid and benzoic acid to activate plant immunity are stating that such inducers can importantly reduce pathogenicity and virulence of the pathogens.

Salicylic acid is a phytohormone that regulates physiological responses and reduces its damage under biotic or abiotic stress. Mechanism of defending pathogen stress of salicylic acid can be divided into two systems. On the one hand, salicylic acid has ability to enhance plant immunity as a priming agent leading to induced defense mechanism. The activation of salicylic acid dependent pathway can contribute to production antimicrobial molecules such as pathogenesis-related proteins (PR-protein) and phytoalexins leading to hypersensitive response (HR) of systemic acquired resistance in plant (Le et al., 2017; Trunk, Khalil, and Leo, 2018). These may result in the reduction of pathogenic infections and disease symptom. Salicylic acid has been reported as an elicitor inducing systemic resistance in rice plant against *Xoo* infection via accumulation of superoxide anion, hypersensitive response induction, and alteration of monomer composition of lignin and pectin in the rice cell wall (Le et al., 2017). On the other hand, direct antimicrobial activity of salicylic acid targeted at the bacterial cell as well as the degradation of proteins, nucleic acid, lipopolysaccharide, and carbohydrates that are main component of bacterial cell (Auer and Weibel, 2017).

Such effects were perhaps mediated with reduction of the cell density contributing to reduction in quorum sensing signaling molecules (Bosund, 1963; Bandara et al., 2006; Koilpillai, 2014). Salicylic acid has been proposed as an alternative biocide-free agent capable of significantly reduce virulence factor from both bacterial and fungal adhesion (Faize and Faize, 2018). Furthermore, it importantly contributes on reducing biofilm thickness while increasing the performance of antimicrobial agents (Lattab et al., 2017; Cattò et al., 2018). In our previous study, salicylic acid had been demonstrated as an inducer to combat BLB on rice by increasing the defense enzymes in plant tissue; however, its direct mode of action to *Xoo* inhibition had not studied. The aim of this research was to investigate the effect of a salicylic acid elicitor on the function of *Xoo* virulent factor during pathogenesis including biofilm formation, EPS production, swimming motility, twitching motility, and cell component. In addition, bacterial growth inhibition and disease control efficiency on rice plant were also investigated.

4.2 Materials and methods

4.2.1 Antibacterial activity

Six concentrations of SA-Ricemate® elicitor were tested in order to assess the antibacterial efficacy against virulent strain of *Xoo* by paper disc diffusion agar method (Sandle, 2016). The *Xoo* suspension ($1x10^8$ cfu mL^{-1}) was mixed with nutrient glucose agar (NGA) then, poured into a Petri dish. Salicylic acid concentrations at 0, 50, 100, 150, 200, 250, and 300 ppm and copper hydroxide, the control treatment were loaded onto 0.5 cm in diameter sterile filter paper discs and air dried in a laminar flow hood. The discs were then placed on NGA mixed with *Xoo* as mentioned. The inhibition

zone was recorded after incubation at 28 ± 1°C for 24 h. In the orther experiment, 5 mL of *Xoo* suspension was mixed with 95 mL of nutrient glucose broth (NGB) containing 0, 50, 100, 150, 200, 250, and 300 ppm each final concentration of salicylic acid and copper hydroxide 1000 ppm then, incubated at 28 ± 1°C with constant shaking 180 rpm for 24 h. The minimum inhibitory concentration (MIC) of SA-Ricemate® was determined as the lowest concentration that inhibited visible growth (turbidity) of the *Xoo* measured by using a microplate reader (Bio-Tek, USA) at 600 nm. The minimum bactericidal concentration (MBC) is estimated as the lowest concentration that can kill the bacterium by colony growth observation on NGA (Seligy and Rancourt, 1999; Andrews, 2001).

4.2.2 Efficacy of the SA-Ricemate® to control BLB

Xoo was inoculated on Khow Dawk Mali 105 (KDML 105) rice plants by spot inoculation technique. After sowing at 45 days, the rice leaf was cut at 10 cm from the leaf top and *Xoo* suspension $1x10^8$ cfu mL^{-1} was spotted on the surface by sterile toothpick then, kept incubated on 1% agar plate for 24 h (Jia, Valent, and Lee, 2003; Chithrashree et al., 2011; Mizobuchi et al., 2013; Xu et al., 2013; Ke, Hui, and Yuan, 2017; Le et al., 2017). At 24 h post inoculation, the leaves were sprayed with 5 mL of SA-Ricemate® at different concentrations of 50, 100, 150, 200, 250, and 300 ppm and mock-water was used as a negative control and copper hydroxide 1000 ppm as a positive control; then incubated at 28ºC for 24 h. The BLB disease index and disease severity were estimated at 7 days post inoculation. The percentage of disease index and disease severity were calculated by using the following formulas (1) and (2) (Ferreira et al., 2017; Le et al., 2017)

$$\text{Disease index (\%)} = \left(\frac{\sum_{i=1}^{p} d_i}{p}\right) \times 100 \qquad (1)$$

$$\text{Disease severity (\%)} = \left(\frac{\sum_{i=1}^{n} r_i}{n \times m}\right) \times 100 \quad (2)$$

Where ‘d’ is a binary value determining plant infection, ‘p’ is the number of assessed plants, ‘r’ is the set of numerical ratings, ‘n’ is the total of evaluations per sample, and ‘m’ is the maximum value used for the evaluations.

4.2.3 Assessment of biofilm formation

The *Xoo* culture was grown in NGB at 28 ± 1°C and incubated with constant shaking at 180 rpm for 24 h. The culture suspension ($1x10^8$ cfu mL^{-1}, 2 mL) was incubated in individual glass tubes containing SA-Ricemate® solutions with concentrations of 0, 50, 100, and 150 ppm under constant shaking at 180 rpm and 28 ± 1°C for 72 h (Shi et al., 2015b). After pouring the culture out, the glass tubes were washed twice with phosphate buffer and air-dried. The crystal violet solution (0.1%) was added to each glass tube, incubated at 28 ± 1°C for 15 min. The crystal violet was poured out then, washed twice and air dried. The crystal violet bound cells were dissolved with acetic acid and the biofilm was measured as the absorbance reading at 570 nm using a microplate spectrophotometer (BioTek, USA) (O'Toole and Kolter., 1998; Niba et al.,2007; Shukla et al., 2017). The inhibition rate was calculated using the formulated equation (3) (Boling et al., 2011; Rann et al.,2016).

$$\text{Inhibition rate (\%)} = \frac{C - T}{C} \times 100 \quad (3)$$

where ‘C’ was the values of *Xoo* growth on blank control and ‘T’ was the *Xoo* growth on salicylic acid treated

4.2.4 Swimming motility assay

The swimming motility was evaluated on soft swimming medium including 0.03% peptone, 0.03% yeast extract, and 0.3% agar. *Xoo* pre culture was grown in NGB for 24 h and adjusted to a final concentration of 1×10^8 cfu mL^{-1}, then, 3-µL of *Xoo* suspensions were dropped on the center of swimming medium containing 50, 100, and 150 ppm of salicylic acid. The petri dishes were incubated at 28 ± 1°C and swimming motility observed after 24 h by measuring the diameter of the swimming area (Xu et al., 2015).

4.2.5 Quantification and mapping of *Xoo* twitching motility

The twitching motility was evaluated on twitching plates composing of 0.03% peptone, 0.03% yeast extract, and 1.5% agar. *Xoo* were picked from NGA by sterile toothpick then spotted on agar surface containing 50, 100, and 150 ppm of salicylic acid. The plates were then incubated at 28 ± 1°C for 24 h and the edge morphology of the colonies were recorded. The concentration of SA-Ricemate® that showed bacteria edge morphology different and incapable killed bacteria was chosen to quantify (Lim et al., 2008; Chow et al., 2011; Athinuwat et al., 2018).

4.2.5.1 Preparation of samples

The twitching medium that contains 100 ppm salicylic acid and untreated without SA-Ricemate® were poured onto separate glass slides and spread to around 2 mm thick by gravity, then left dry for 15 min. A circle was painted on the backside as a mark to indicate where to test. *Xoo* was transferred by using the tip of a sterile toothpick and gently applied on the medium at the center of the marked circle without having contact with the medium. A coverslip was placed over the point of inoculation and everything was introduced into a petri dish containing wet paper to

keep the moisture. The samples were then incubated for 4 h at 28 ± 1°C (Smith et al., 2018).

4.2.5.2 Image processing and analysis

After the incubation, image frames of the bacteria samples were captured every 5 min interval for 30 min starting from the inoculation time by using a compound microscope with a 60×/1.4NA oil-immersion lens. The collected images were processed in order to empirically detect and quantify spatial variation of twitching motility in each sample for a specific range of time. The image was processed and analyzed by using MATLAB by MathWorks. The applied methodology is modification of the work published by Smith et al. (2018) which describes an image processing pipeline based on computing the standard deviation through a Z-projection of a stack of images which were acquired in different time points (Smith et al., 2018). Therefore, the resulting image provides data correlated to bacteria motion activity obtained from per-pixel color variation. As a first step, the raw images obtained from the microscope were treated in order to improve their quality and therefore making them more suitable for a posterior accurate image analysis. The contrast of the raw images was adjusted by saturating 1% of bottom and top color values. Finally, a Flat Field Correction (FFC) technique based on gaussian smoothing was applied in order to provide color uniformity against light variations and image artifacts. The image frames were stacked and displaced by applying a computed offset in order to minimize the effects of the media which causes the displacement of all elements in the images through time. Once the images were correctly stacked, the standard deviation through a Z-projection was calculated. The

resulting image stores normalized standard deviation values in each pixel which was correlated to the color variation in time.

4.2.6 Assessment of extracellular polysaccharides (EPS) production

The *Xoo* cells were grown in NGB containing of SA-Ricemate® at concentrations of 0, 50, 100, and 150 ppm with constant shaking at 28 ± 1°C for 72 h. Subsequently, the cells were removed by centrifugation at 12,000 g for 15 min then; the cultures were kept for EPS extraction (Guo, Sagaram, Kim, and Wang, 2010). The EPS were isolated by three volumes of cold 95% ethanol (Vojno, 1998). The precipitated EPS were pelleted by centrifugation at 12,000 g, 4°C for 15 min; then, dried it to a constant weight.

4.2.7 SR-FTIR microspectroscopy measurement

To observe biochemical changes of *Xoo* cells treated and untreated with SA-Ricemate® using SR-FTIR spectroscopy, the *Xoo* suspension (1×10^8 cfu mL^{-1}) was added to NGB amended with 100 ppm final concentration of salicylic acid and incubated at 28 ± 1°C for 24 h. The cell pellets were collected by centrifugation at 8,000 g, 4°C for 10 min; and washed with 0.85% buffered saline (PBS) twice (Sun et al., 2003; Lu et al., 2011). A tiny portion of *Xoo* pellet was placed into barium fluoride windows (BaF2) and subjected to SR-FTIR microscopy. The pelleted cells were dehydrated by vacuum for 48 h and stored in a desiccator until formation of films before being subjected (Eumkeb, Siriwong, and Thumanu, 2012; Prieto-Calvo, Prieto, López, and Alvarez-Ordóñez, 2014). The spectral data were collected by imaging 64 scans in each sample at the beamline 4.1 IR Spectroscopy in the Synchrotron Light Research Institute (SLRI) (Wang et al., 2015). The spectral data were processed by using OPUS 7.2 software (Bruker Ltd., Germany). In order to analyze the biochemical

components of *Xoo* treated and untreated with SA-Ricemate®, the data were analyzed by using PCA and conduced on the spectral range from 3000-2800 cm-1 region of lipid, 1700-1500 cm^{-1} region of protein, and 900-1200 cm^{-1} region of polysaccharide. The original spectra were converted on second derivatives, vector normalized unity to multivariate statistical analysis by using the Unscrambler X 10.5 software and Sigmaplot for graph plotting (Clayton and Thiers, 1966; Le et al., 2017; Thumanu et al., 2017; Durak and Depciuch, 2020).

4.2.8 Statistical analysis

The experiment data were analyzed by one-way ANOVA using SPSS software, version 20. The mean differences were separated by Duncan' s Multiple Range Test at a significance p = 0.05.

4.3 Results

4.3.1 In *vitro* antibacterial activity of SA-Ricemate®

The antibacterial activities of SA-Ricemate® against *Xoo* as shown in Table 4.1 The minimum inhibitory concentration (MIC) of SA-Ricemate® was of concentrations ≥200 ppm when the bacterial growth as observed by turbidity can being inhibited where the minimum bactericidal concentration (MBC) was at 250 ppm. In the same trend, 250 ppm SA-Ricemate® showed significantly clear zone of *Xoo* inhibition when compared with other concentration where 200 ppm salicylic acid was minimum concentration to *Xoo* inhibition (Table 4.1). This indicates that the suitable concentration of SA-Ricemate® to 100% inhibit growth of *Xoo* was at 250 ppm.

Table 4.1 The antibacterial activities of SA-Ricemate® on *Xanthomonas oryzae* pv. *oryzae*.

Treatment	Inhibition zone[1] (mm)	Absorbance 600 nm [2]	Culture turbidity	Cell growth[3] (cfu mL^{-1})	
SA-Ricemate® 300 ppm	16.77 ± 0.42[c]	0.04 ± 0.004	Clear	0	
SA-Ricemate® 250 ppm	14.72 ± 0.51[c]	0.04 ± 0.008	Clear	0	(MBC)
SA-Ricemate® 200 ppm	10.50 ± 1.20[b]	0.08 ± 0.028	Clear	3.30×10^{2}[a]	(MIC)
SA-Ricemate® 150 ppm	0[a]	0.19 ± 0.026	Turbid	1.40×10^{7}[a]	
SA-Ricemate® 100 ppm	0[a]	0.30 ± 0.032	Turbid	2.00×10^{10}[b]	
SA-Ricemate® 50 ppm	0[a]	0.34 ± 0.010	Turbid	2.80×10^{10}[b]	
SA-Ricemate® 0 ppm	0[a]	0.40 ± 0.050	Turbid	3.50×10^{10}[b]	
Copper hydroxide 1000 ppm	21.85 ± 1.96[d]	-	Clear	0	

[1/]Inhibition zone obtained from average of clear zone diameter from five replications on each treatment using paper disc agar diffusion method. [2]The absorbance of *Xoo* was measured by a microplate reader (Bio Tek, USA) at 600 nm. [3]Bacterial count was done at 24 h post incubation using plate count method. The data followed by the same letter are not significantly different at P = 0.05.

4.3.2 Efficacy of the SA-Ricemate® for control the BLB on rice

This experiment confirmed the control efficacy of SA-Ricemate® against BLB when sprayed at SA-Ricemate® 24 h post inoculation of *Xoo*. The results showed that ≥100 ppm SA-Ricemate® can 81.25-100% control the disease. Where the control efficacy calculated from disease severity index showed that ≥250 ppm SA-Ricemate® would 100% control the disease was not significantly different from that of copper hydroxide (Figure 4.1). At 250 ppm SA-Ricemate® was showed the highest efficiency to BLB control on rice leaf and related with in vitro antibacterial activity (Table 4.2).

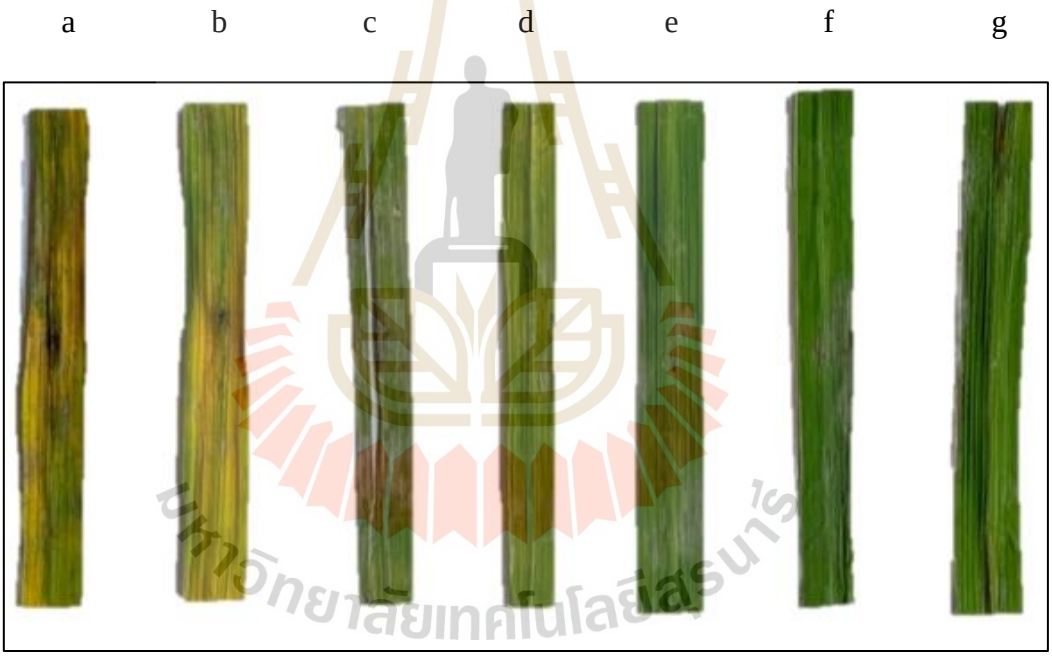


Figure 4.1 Symptoms of bacterial leaf blight on rice (KDML 105) caused by *Xanthomonas oryzae* pv. *oryzae* at 7 days post inoculation andsprayed with 30 mL of SA-Ricemate®. a) 0 ppm, b) with 50 ppm, c) with 100 ppm, d) with 150 ppm, e) with 200 ppm, f) with 250 ppm, and g) with 300 ppm after 24 h *Xoo* inoculation.

Table 4.2 Efficacy of SA-Ricemate® in control the BLB on rice.

Treatment[1/]	7 days post inoculation of *Xanthomonas oryzae* pv. *oryzae*[2/]			
	Disease incidence (%)	**Control efficiency (%)**	**Disease severity (%)**	**Control efficiency (%)**
SA-Ricemate® 300 ppm	0 ± 0^{a}	100 ± 0^{b}	0 ± 0^{a}	100 ± 0^{c}
SA-Ricemate® 250 ppm	0 ± 0^{a}	100 ± 0^{b}	0 ± 0^{a}	100 ± 0^{c}
SA-Ricemate® 200 ppm	12.50 ± 0.29^{a}	87.50 ± 7.22^{b}	9.37 ± 3.81^{b}	89.28 ± 3.81^{b}
SA-Ricemate® 150 ppm	18.75 ± 0.25^{a}	81.25 ± 6.25^{b}	12.50 ± 0.85^{b}	85.71 ± 0.85^{b}
SA-Ricemate® 100 ppm	18.75 ± 0.47^{a}	81.25 ± 11.97^{b}	18.75 ± 4.13^{b}	78.57 ± 4.13^{b}
SA-Ricemate® 50 ppm	100 ± 0^{b}	0 ± 0^{a}	68.75 ± 11.64^{c}	21.43 ± 11.64^{a}
SA-Ricemate® 0 ppm	100 ± 0^{b}	-	87.50 ± 0^{d}	-
Copper hydroxide	0 ± 0^{a}	100 ± 0^{b}	0 ± 0^{a}	100 ± 0^{c}
F-Test	**	**	**	**
CV (%)	17.36	12.85	19.4	17.96

[1/]Rice plants were treated by foliar spays with different concentrations of SA-Ricemate® and water used as the control (0 ppm) at 24 h post inoculation of *Xanthomonas oryzae* pv. *oryzae*. [2]Disease index and disease severity were evaluated at 7 days post inoculation of *X. oryzae* pv. *oryzae*. The data followed by the same letter are not significantly different at P = 0.05.

4.3.3 Assessment of biofilm formation

Biofilm formation produced by *Xoo* in different concentrations of SA-Ricemate® at 0 (untreated), 50, 100, 150, 200, 250, and 300 ppm was evaluated. The result showed that biofilm formation was up to 100% inhibited by the SA-Ricemate®. At concentrations of ≥ 200 ppm (Table 4.3). These results were in the same trend with that of the in vitro antibacterial activity and BLB control efficacy on rice leaf in which 200 ppm SA-Ricemate® performed best. At 200 ppm, SA reduced the MIC and can reduce *Xoo* population to $3.3x10^2$ cfu mL^{-1} (Table 4.1) which was lower than the minimum concentration as $1.0x10^8$ cfu mL^{-1} needed to cause disease on plant resulting from its inability to produce biofilm (Jefferson, 2004). Therefore, the suitable concentration of SA-Ricemate® for inhibiting growth and controlling the BLB disease was 250 ppm.

Table 4.3 Effect of SA-Ricemate® on *Xanthomonas oryzae* pv. *oryzae* biofilm formation.

Treatment	Absorbance 570 nm [1/]	Inhibition rate [2/] (%)
SA-Ricemate® 300 ppm	Clear solution	100 [b]
SA-Ricemate® 250 ppm	Clear solution	100 [b]
SA-Ricemate® 200 ppm	Clear solution	100 [b]
SA-Ricemate® 150 ppm	0.042 ± 0.002	27.84 ± 6.05 [a]
SA-Ricemate® 100 ppm	0.045 ± 0.002	23.86 ± 5.58 [a]
SA-Ricemate® 50 ppm	0.051 ± 0.005	13.07 ± 4.26 [a]
SA-Ricemate® 0 ppm	0.059 ± 0.007	-

[1/] Biofilm formation was measured by the absorbance at 570 nm of biofilm-staining. [2/] The inhibition rate of biofilm formation was calculated by comparing with that of the control. The data followed by the same letter are not significantly different at P = 0.05.

4.3.4 *Xoo* swimming motility assay

The results clearly showed that the SA-Ricemate® was able to inhibit swimming motility of the *Xoo* which was observed on the decreasing diameter of the swimming area (Figure 4.2) in concentrations of SA-Ricemate® in the medium (Table 4.4). The inhibitory concentrations showed a reduction of the swimming motility area of about 10.89-43.10% as average. There was a significant reduction at 100 ppm SA-Ricemate® or above when compared to that of the control, in which ≥200 ppm concentration showed 100% inhibition of swimming motility.

Table 4.4 Quantification of *Xanthomonas oryzae* pv. *oryzae* swimming motility in different concentrations of SA-Ricemate®.

Treatment	Swimming motility area [2/] (mm)	Inhibition rate [3/] (%)
SA-Ricemate® 300 ppm	0	100[c]
SA-Ricemate® 250 ppm	0	100[c]
SA-Ricemate® 200 ppm	0	100[c]
SA-Ricemate® 150 ppm	39.00 ± 0.11	43.10 ± 6.11[b]
SA-Ricemate® 100 ppm	48.33 ± 0.31	29.09 ± 7.42[b]
SA-Ricemate® 50 ppm	61.67 ± 0.20	10.89 ± 3.95[a]
SA-Ricemate® 0 ppm	69.67 ± 0.52	-

[1/] The *Xanthomonas oryzae* pv. *oryzae* cultures grown on swimming medium supplemented with SA-Ricemate® at different concentrations. [2/] The relative swimming motility areas were 24 h measured post inoculation. [3/] The inhibition rate of swimming motility calculated by comparing with that of the control. The data followed by the same letter are not significantly different at P = 0.05.

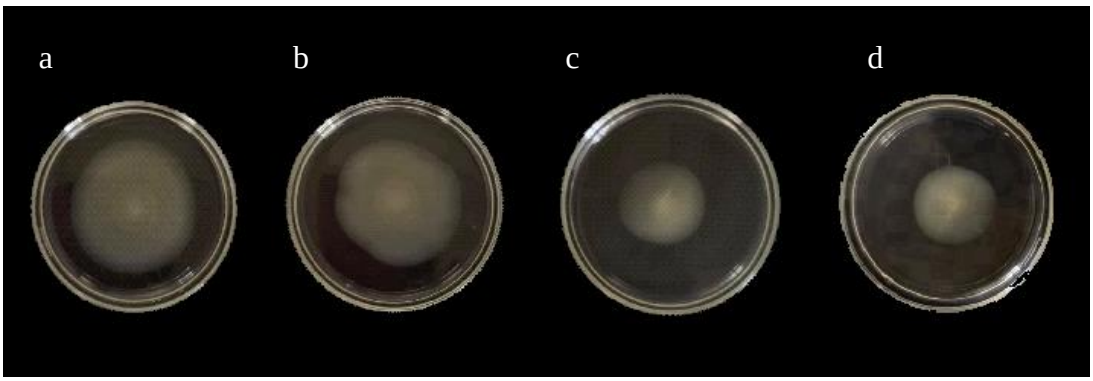


Figure 4.2 Swimming motility of *Xanthomonas oryzae* pv. *oryzae* in the swimming medium containing SA-Ricemate® at different concentrations a) Untreated. b) 50 ppm, c) 100 ppm, and d) 150 ppm.

4.3.5 *Xoo* twitching motility assay

The results showed that SA-Ricemate® decreased the visible twitching motility of *Xoo* observed as inhibition of the halo diameter in the twitching medium supplemented with 50, 100, and 150 ppm of SA-Ricemate® with 5.55-40.00% when compared with the control where ≥200 ppm SA-Ricemate® showed 100% inhibited of twitching motility (Figure 4.3 and Table 4.5). These results suggest that SA-Ricemate® play an important role in *Xoo* twitching motility. Therefore, *Xoo* twitch resulting on twitching medium amended with 100 ppm SA-Ricemate® was next investigated on twitching molarity under light microscope.

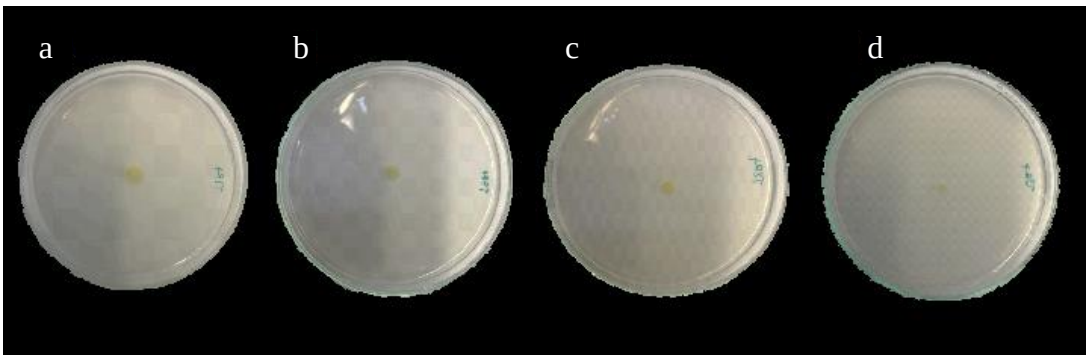


Figure 4.3 Twitching motility of *Xanthomonas oryzae* pv. *oryzae* in the twitching medium amended with of SA-Ricemate® as different concentration a) Untreated, b) 50 ppm, c) 100 ppm, and d) 150.

Table 4.5 Quantification of *Xanthomonas oryzae* pv. *oryzae* twitching motility in different concentrations of SA-Ricemate®.

Treatment	Motility halo [2/] (mm)	Inhibition rate [3/] (%)
SA-Ricemate® 300 ppm	0	100[c]
SA-Ricemate® 250 ppm	0	100[c]
SA-Ricemate® 200 ppm	0	100[c]
SA-Ricemate® 150 ppm	2.70 ± 0.20	40.00 ± 8.35[b]
SA-Ricemate® 100 ppm	3.50 ± 0.25	22.22 ± 8.10[b]
SA-Ricemate® 50 ppm	4.25 ± 0.20	5.55 ± 1.40[a]
SA-Ricemate® 0 ppm	4.50 ± 0.25	-

[1/] The *Xanthomonas oryzae* pv. *oryzae* cultures grown on twitching medium supplemented with SA-Ricemate® at different concentrations. [2/] The relative motility areas were measured 24 h after inoculation. [3/] The inhibition rate of twitching motility was calculated by comparing with that of the control. The data followed by the same letter are not significantly different at P = 0.05.

4.3.6 Quantification and mapping of *Xoo* twitching motility

The results showed that *Xoo* treated with 100 ppm SA-Ricemate® had lower total motility distributions than that of the untreated *Xoo* (Figure 4.4a). The result observed on color-scaled mapping indicated that the SA-Ricemate® reduced the *Xoo*, twitching motility and contributed to inhibit aggregation (Figure 4.4b) while untreated *Xoo* showed ability of twitching motility and cell aggregation (Figure 4.4c). This demonstrates that the *Xoo* bacterium lacked twitching motility due to the SA-Ricemate® treatment.

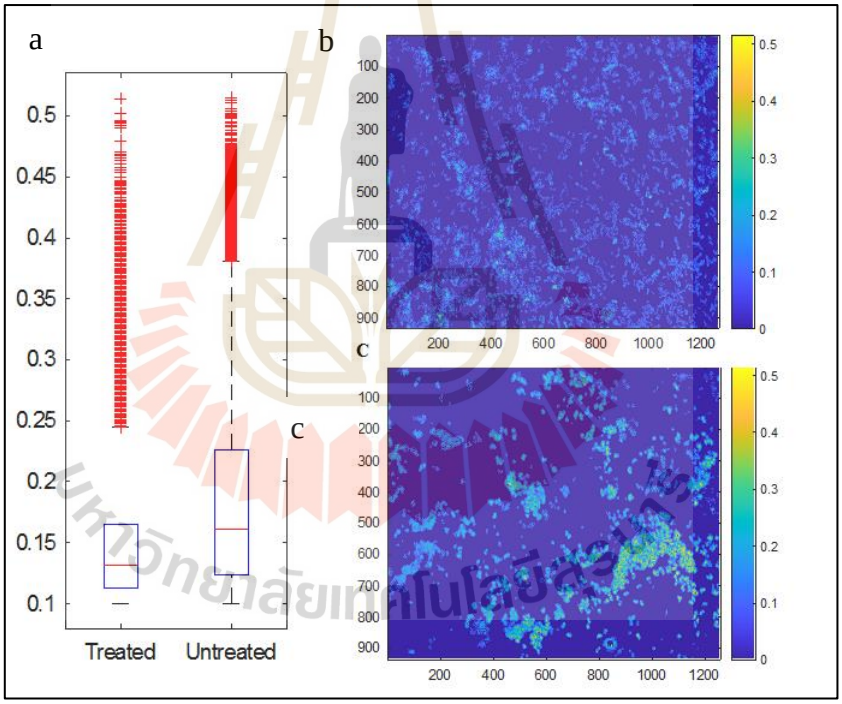


Figure 4.4 Quantification and mapping of *Xoo* twitching motility. a) Comparing of twitching motility distributions between *Xanthomonas oryzae* pv.*oryzae* (*Xoo*) treated with salicylic acid and untreated samples, b) Low twitching motility mapping of *Xoo* treated with 100 ppm SA-Ricemate®, and c) High twitching motility mapping of *Xoo* untreated sample.

4.3.7 Assessment of *Xoo* extracellular polysaccharides (EPS) production

The production of EPS associated with the virulence factors produced in NGB amended with different concentrations of SA-Ricemate® was determined. *Xoo* samples were grown in NGB supplemented with SA-Ricemate® at a concentration of 150 ppm produced less EPS as 0.22 mg mL^{-1} dry weight than untreated control; 0.57 mg mL^{-1} dry weight. Lower concentrations of SA-Ricemate at 100 and 50 ppm produced EPS of 0.27 and 0.46 mg mL^{-1} dry weight, respectively as showed in Table 4.6. SA-Ricemate® concentrations at ≥200 ppm showed 100% inhibition of EPS production. The results indicated that the SA-Ricemate® can inhibit EPS by 100% depending on the concentration.

Table 4.6 Effect of SA-Ricemate® on *Xanthomonas oryzae* pv. *Oryzae* polysaccharides (EPS) production.

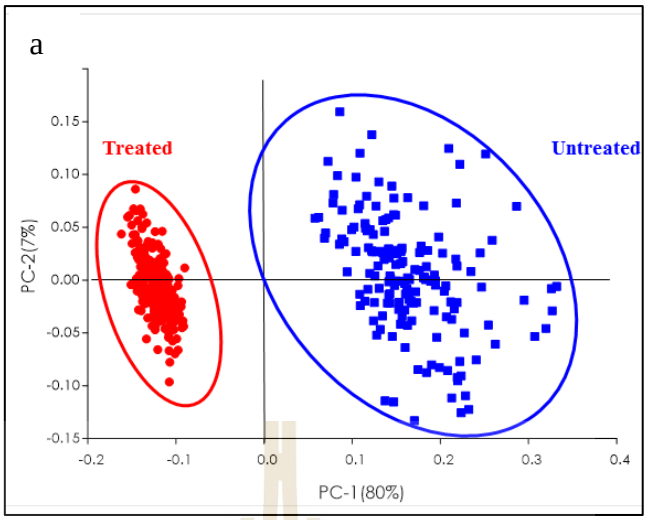
Treatment [1/]	**Extracellular polysaccharides production** [2/] **(mg mL^{-1} dry weight)**	**Inhibition rate** [3/] **(%)**
SA-Ricemate® 300 ppm	0.00	100[c]
SA-Ricemate® 250 ppm	0.00	100[c]
SA-Ricemate® 200 ppm	0.00	100[c]
SA-Ricemate® 150 ppm	0.22 ±0.04[a]	60.04±4.58[b]
SA-Ricemate® 100 ppm	0.27 ±0.03[a]	52.96±6.08[b]
SA-Ricemate® 50 ppm	0.46 ±0.04[b]	18.87±5.61[a]
SA-Ricemate® 0 ppm	0.57 ±0.07[b]	-

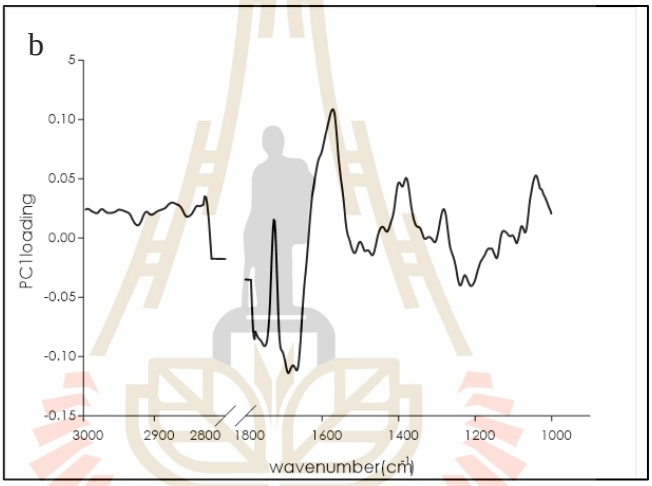
[1/] *Xanthomonas oryzae* pv. *oryzae* was grown in NGB supplemented with salicylic acid at different concentrations at 28°C for 72 h. [2/] Extracellular polysaccharides (EPS) were isolated by ethanol precipitation then dried to a constant weight. [3/]The inhibition rate of EPS

production was compared to that of the untreated. The data followed by the same letter are not significantly different at P = 0.05.

4.3.8 Biochemical change analyses using SR-FTIR microspectroscopy

SR-FTIR spectra were used for investigating biochemical composition changes on bacterial cell. The results showed the components of the *Xoo* cells treated with 100 ppm SA-Ricemate® compared to that of the untreated cell. SA-Ricemate® treated samples were difference in spectra due to showing three distinguishable regions (Figure 4.5). The first region (3000-2800 cm^{-1}) according to bacterial cell membrane including fatty acids with a clearly peak at 2825 cm^{-1} of the untreated sample higher than the treated sample. The second region (1700-1500 cm^{-1}) composed by proteins and peptides with amide group shows a peak at 1655 cm^{-1} from the treated sample higher than the untreated sample. The third region (1300-900 cm^{-1}) involving proteins, nucleic acid, phospholipids, polysaccharides, and carbohydrates of bacterial cell walls shows peaks at 1162 and 1040 cm^{-1} from the untreated sample higher than the treated sample. In addition, the lipid as C–O stretching vibration peak at 1738 cm^{-1} which was monitored in the treated sample, being higher than the untreated sample. This IR band presents an increased in packing of the ester groups.


a
Treated
Untreated
PC-2(7%)
PC-1(80%)
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.2
-0.1
0.0
0.1
0.2
0.3
0.4


b
PC1loading
wavenumber(cm-1)
0.10
0.05
0.00
-0.05
-0.10
-0.15
3000
2900
2800
1800
1600
1400
1200
1000

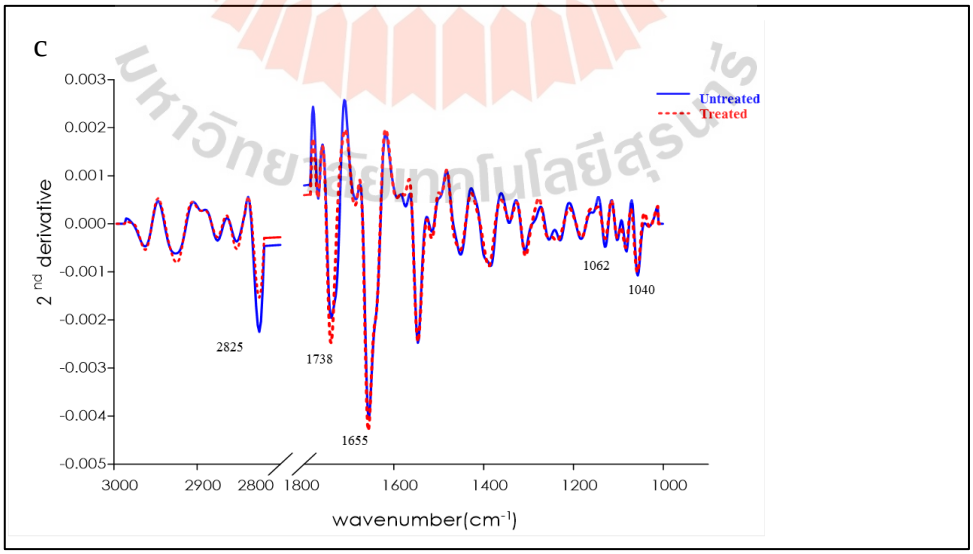

c
Untreated
Treated
2nd derivative
wavenumber(cm-1)
2825
1738
1655
1062
1040
0.003
0.002
0.001
0.000
-0.001
-0.002
-0.003
-0.004
-0.005
3000
2900
2800
1800
1600
1400
1200
1000

Figure 4.5 Principal Component Analysis (PCA) of *Xanthomonas oryzae* pv. *oryzae* (*Xoo)*. a) Principle component analysis (PCA) of *Xoo* treated with SA-Ricemate® and untreated samples, b) loading plots from PCA analysis of treated and untreated group, and c) Average 2nd derivative spectrum of bacterial cell comparison between *Xoo* treated with SA-Ricemate® and untreated samples.

4.4 Discussion

Our experiments demonstrated potent antibacterial capabilities of salicylic acid elicitor (SA-Ricemate®) against *Xoo* pathogenesis on rice plant by working as a strong inhibitory agent when used at high concentrations ≥250 ppm. The results presented in this work agreed with the work by Le et al. (2017) who reported that rice plant treated with salicylic acid affected on *Xoo* grew more slowly and found 50% bacterial population was reduced. Price et al. (2000) reported the ability of salicylic acid to inhibit plant pathogenic bacteria growth due to related compounds to aspirin which cause effects in eukaryotic system. A similar effect was observed by Ntushelo (2017) that determined the use of salicylic acid concentrations of 800 mg L^{-1} or above to completely control the growth of *Pectobacterium carotovorum* whereas being ineffective at lower concentrations (Price, Lee, and Gustafson, 2000; Ntushelo, 2017). The referenced results seem consistent with our experiments when using low concentrations with <200 ppm in which no inhibition zone was observed.

The control effectiveness of the SA-Ricemate® on disease severity treated after with *Xoo* infection was 21.43-100% from low to high concentration solution. In addition, it was observed that SA-Ricemate® at low concentrations (50 and 100 ppm)

can effectively reduce the disease severity of BLB without affecting bacterial population. The results suggested that SA-Ricemate® may contribute to reduce some virulence factor of *Xoo*. This observation was consistent with previous studies in terms of salicylic acid can enhance the inhibit zone of soil born pathogen when compared with that of the control treatment (Sedghi and Gholi, 2014). The observations of Sedghi (2014) reported the use of salicylic acid 0.1 g L^{-1} as a foliar spray on stevia plant in order to increase the percentage of oil, terpenes, and other oil constituents because of effective of reactive oxygen species (ROS) in defense mechanism to against seed borne pathogen of soybean including *Xanthomonas campestris*, *Pseudomonas syringae*, *Rhizoctonia solani*, *Pythium aphanidermatum*, *P. debaryanum*, *P. irregular*, *P. myriotylum*, *P. ultimum*, *Fusarium* spp., *Phomopsis* spp., and *Cercospora kikuchii*. Similarly, Zhang et al. (2020) reported the application of salicylic acid on wild soybean plants before inoculating them with soybean mosaic virus which can enhance the resistance of plants by increasing resistance enzymes as well as reactive oxygen species (ROS), lipid peroxidation, peroxidase (POX), catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX).

To further understand the inhibitory mechanism of SA-Ricemate®. The inhibition of *Xoo* biofilm formation was performed. Biofilm formation is considered as a virulence factor of growth on microbial as a key for the pathogen to survive in environment. The bacteria form biofilms on the surface to protect the cell from toxic agents or other factors such as ultraviolet (UV) radiation, extreme temperature, pH, salinity, and antibiotics that can produce pathogen inhibition. Moreover, biofilm formation helps bacteria on plant invasion by means of using it to create a shelter to increase bacterial cell resistance to antimicrobial or defense compounds produced in

the plant while invading the xylem vessels (Bogino et al., 2013; Núñez et al., 2013; Ham and Kim, 2018). Therefore, biofilm formation reduction can be seen as a potential strategy to control the plant disease. In our study, the biofilm formations were observed on the liquid medium by using crystal violet staining. The SA-Ricemate® treatment presented less crystal violet color intensity in the glass tube than untreated samples indicating low biofilms formation. The biofilm formation on treated and untreated samples determined inhibition rate showing that SA-Ricemate® can reduce biofilm formation around 13.07-100% when compared with that of the untreated samples. This observation is on the same line as the study by Lattab et al. (2017) which reported that incubated *Pseudomonas aeruginosa* with salicylic acid at 4mM showed the biofilm formation was highly inhibited with 62.97%. Shi et al. stated that the absence of *Xoo* biofilm can contribute to increase protection activity (Shi et al., 2015a; Lattab et al., 2017; Sahu, Zheng, and Yao, 2018). This indicating that salicylic acid against BLB by reduced *Xoo* biofilm production because biofilm formation is commonly considered in bacterial attachment to host surface (Crouzet et al., 2014). An important requirement for bacterial attachment is the bacteria having polar flagellum and chemotaxis capability which help microbial cells to get attached to the surface and become to physical forces. The result of increased concentration of salicylic acid caused a reduction on bacterial swimming and twitching motility which may explain the reduction of attached cells. In disease cycle, when attachment step is succeeded, cell division produces bacterium bonds contributing to microcolony formation. This step requires type IV pili which are important for its aggregation on the surface; however, this is compromised by salicylic acid due to bacterial motility reduction resulting in loss of aggregate structures. In our study, the lack of motility

observed in SA-Ricemate® treated samples revealed that the motility area of untreated samples is wider than treated sample and, on the same line, the twitching maps showed low motility on treated sample but not in untreated sample. Swimming motility is a flagellum-dependent movement in aqueous or low-solid media and twitching is a pilus-dependent movement on solid media (Burrows, 2012; Yu et al., 2020). The type IV pilus plays crucial roles in swimming and twitching motility contributing to bacterial surface adhesion and their virulence (Li et al., 2020; Yu et al., 2020). These results are in agreement with the work of Kunin et al. (1995) who explains the salicylate effect in the expression of flagella of *Escherichia coli* and *Pseudomonas cepacian*. They observed that salicylic acid can block the synthesis of flagella on the gene regulation level inhibiting the function of the flagella. Similarly, Bandara et al. (2006) studied the motility of *Pseudomonas aeruginosa* in the presence of 30 mM salicylic acid which were significantly reduced due to lack of flagella and type IV pili. (Kunin, Hua, and Bakaletz, 1995; Zolfaghar, Evans, and Fleiszig, 2003; Bandara et al., 2006). Therefore, this suggests that the flagella or type IV pili in *Xoo* motility ability can lose its functionality by an salicylic acid treatment due to the fact that functional flagellum is necessary for the swimming motility as well as type IV pili is associated on twitching motility.

Extracellular polysaccharide (EPS) was examined reporting that initial functional or structural coherence is involved in biofilm formation. Biofilms are embedded in EPS for protection against dehydration and hydrophobic polypeptide because the ability of EPS to generate a water-saturated matrix throughout the bacterial cell (Esser et al., 2012; Park et al., 2015). The bacterial pathogens invade and grow into the host plant xylem vessels transforming xylem biochemical to nutrients

and metabolism by secreted EPS for surviving causing cell wall degradation enzymes that may cause vessel obstruction (Huang and Allen, 2000; Pinto et al., 2003). While the bacteria is invading the xylem, it produces EPS as a coating for protection from host defense enzymes and also to carry minerals and nutrients near the bacterial cell contributing to plant wilting and damage (Fatima and Kumar, 2015). The secretory capability of EPS involve 12 genes; *gumB to gumM* of gum cluster (Katzen et al., 1998; Vojnovet al., 2001). This is responsible for the polymerization of pentasaccharide and polymers releasing. In addition, *xanA* and *xanB* genes provide nucleotide sugar precursor of EPS biosynthesis (Levander and Rådström, 2001). Thus, inhibition of EPS is important to reduce the symptoms of the disease. As the results showed, the SA-Ricemate® at a concentration of 50 ppm to 300 ppm inhibited the production of EPS at approximately 18-100% when compared with untreated samples. Such results confirmed that salicylic acid can simultaneously reduce virulent factor in terms of biofilm formation and EPS. These results are similar to the work published by Alvarez et al. who reported that the use of salicylic acid 2 mM had an effect on capsular polysaccharide (CP) expression that is a major of *Staphylococcus aureus* virulence factors. The results showed a decrease of CP expression in *S. aureus* caused by the treatment with salicylic acid. Furthermore, Algburi (2018) observed expression of a key producing of extracellular polysaccharide substances (EPS) network produced by *Gardnerella vaginalis* ATCC 14018 can reduce by use of salicylic acid (125 μg mL^{-1}), its inhibit biofilm formation as well as EPS network by inhibiting the bacterial quorum sensing system play a role as communication of triggering pathogen virulence (Alvarez et al., 2010; Algburi et al., 2018).

Moreover, the cell components of *Xoo* which are involved in bacterial pathogenesis and amount of inoculum it produces was examined (Schuster and Coyne, 1974; Aparna et al., 2009). The results from the SR-FTIR spectra obtained for bacterial cells showed that the main components of *Xoo* cell were different in spectral characteristic of bacterial ester functional groups of lipids and carbohydrate. These results provide evidence that the fatty acid (3000-2800 cm^{-1}), nucleic acid and phospholipid (1300-900 cm^{-1}), and the cell wall C-O-C and C-O as carbohydrates (1200-1000 cm^{-1}); shifted to lower lectures than the untreated samples used as control. This suggests that the SA-Ricemate® causes injuries to bacterial cells due to the peak of spectra representing bacterial cell membrane and phospholipid polysaccharides (Kamnev, 2008; Kochan et al., 2018). These affirmations agree with the results from Alvarez et al. (2010) that described a stress treatment for Salmonella enterica in which visible spectral changes were observed afterwards as a lower peak at 1,171 cm^{-1} and 1,084 cm^{-1}. Such spectral lectures indicated that the cell membrane was damaged or was changed in bacterial membrane compositions. Similarly, Sahu et al. (2018) reported changes in absorbance as a negative peak of carboxylic acid O-H at 2989 cm^{-1}, C-N at 1953 cm^{-1}, and fluoroalkanes at 1071-1177 cm^{-1} after a niclosamide based treatment (Alvarez and Prieto, 2010; Sahu et al., 2018). The conclusions from the previous mentioned studies converge on the affirmation that the salicylic acid has a damage effect to bacterial cell membrane caused by cell wall component deterioration (Grogan, 2012). The cell wall is an important structure that regulates interactions between plants and pathogens (Gupta et al., 2015) acting as a function that pathogens use to recognize the host (Grogan, 2012; Miedes et al., 2014). *Xanthomonas* spp. are often referred as biotrophic bacteria (Kraepiel and Barny,

2016), salicylic acid disrupts the cell unity that leads enzyme degradation during the infection process to utilize the nutrients from the host plant and activates plant immune to combat biotrophs (Salomon and Sessa, 2012; Li et al., 2019). For example, Rowlett et al. (2017) found that variation of phospholipids in Escherichia coli degrade cellular envelope structure and serve to bacteria as an ability to form biofilms and provide bacteria stability. Furthermore, the participating phospholipids are contributing to the design of structural, signaling, and metabolic pathways involved in bacterial adaptation whereas lipid domain in membrane is related with bacteria cell regulation (Barák and Muchová, 2013; Rowlett et al., 2017). Baxter et al. (2015) explained the importance of the phospholipids as a key factor on setting cellular processes to facilitate the host–microbe interactions as well as hydrolysis of host membranes (Bellincampi, Cervone, and Lionetti, 2014; Baxter, Hulett, and Poon, 2015). This supports our spectra results as the peak observed related to phospholipids and the lipids group that showed lower signals in samples treated with salicylic acid. In contrast, 1738–C=O stretching vibrations (crocetin and amino acids), 1655 =C–H stretching vibrations (groups of crocetin), and amide IO–H bending vibrations in water (Li, Lantz, and Du, 2019) shift higher than lectures from untreated samples. This may be involved with the degradation of proteins that are constituent of cell membrane (Podolsky, 1953). Protein degradation plays an important role in cellular functionality since damaged proteins are removed from the bacterial cell to avoid toxicity by degradation (Schrader, Harstad, and Matouschek, 2009; Babst, 2014). Amino acids are represented by regulated membrane protein degradation and also play a role in cell adaptation in starvation (Jones et al., 2012; Jin, Kiral, and Hiesinger, 2018). Moreover, Raivio (2018) describes small proteins defined as amino acids

which are prevalent in bacteria that secreted in the cytoplasmic membrane to keep the function of large protein complexes (Raivio, 2018). This suggests that, when the bacteria are disturbed by salicylic acid, the cells must protect themself by secreting amino acid to balance while protein in cell walls are being degraded by salicylic acid. These results are on the same line as in the work of Wang (2017) and Yin (2019) who reported that the preservation of cell integrity when lack of Mg2+ in *Escherichia coli*. 31 amino acid MgtS protein interacts with MgtA which contributes to increase intracellular Mg2+ levels and maintains the cell by small proteins that regulate transporters cell membrane (Wang et al., 2017; Yin et al., 2019).

In conclusion, the results of this work indicated that SA-Ricemate® had ability to perform antibacterial activities against *Xoo* the cause of bacterial leaf blight on rice plant with direct inhibitory effect (Figure 4.6). The experiments confirmed that SA-Ricemate® was effective in reducing the disease severity by inhibiting the virulence factors of *Xoo* such as biofilm formation, extracellular polysaccharides, and bacterial motility. This treatment caused damages to the bacterial cell membrane which contributed to suppressing communication abilities and inhibiting the motility gene associated with the flagella and type IV pili resulting in a weaker pathogenicity.

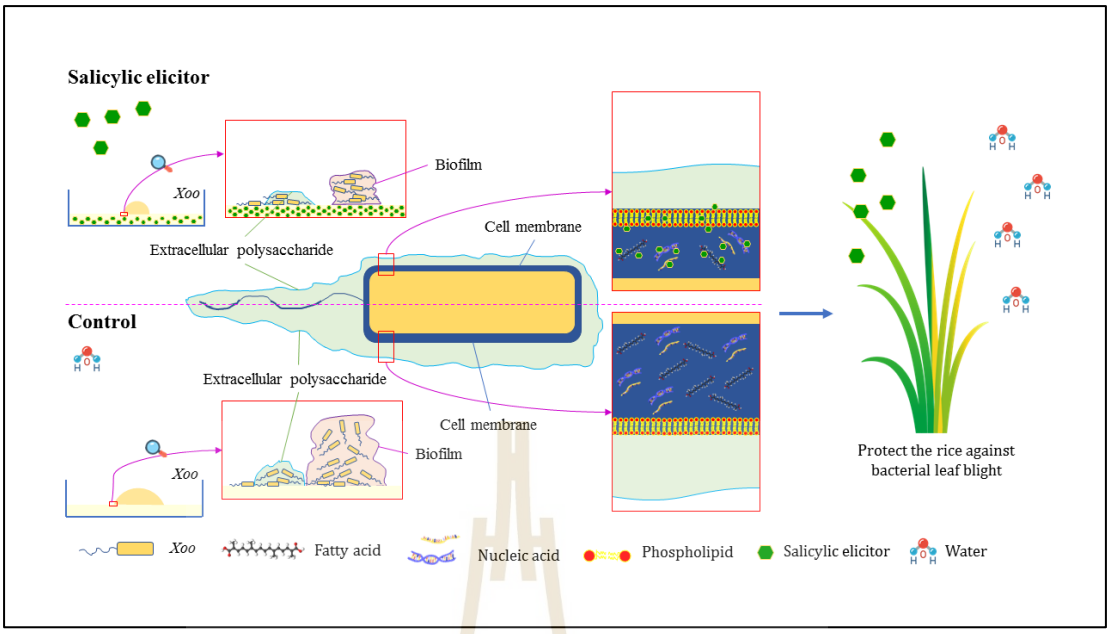


Figure 4.6 Model describing of *Xanthomonas oryzae* pv. *oryzae* virulence factor disruption by SA-Ricemate® leading to weaker pathogenesis on rice plant.

4.5 References

Algburi, A., Zehm, S., Netrebov, V., Weeks, R., Zubovskiy, K., and Chikindas, M. L. (2018). Benzoyl peroxide inhibits quorum sensing and biofilm formation by gardnerella vaginalis 14018. **Infect. Dis. Obstet. Gynecol.** 2018: 1426109-1426109.

Alvarez-Ordóñez, A., and Prieto, M. (2010). Changes in ultrastructure and fourier transform infrared spectrum of *salmonella enterica serovar typhimurium* cells after exposure to stress conditions. **Appl. Environ. Microbiol.** 76 (22): 7598-7607.

Alvarez, L. P., Barbagelata, M. S., Gordiola, M., Cheung, A. L., Sordelli, D. O., and Buzzola, F. R. (2010). Salicylic acid diminishes *Staphylococcus aureuscapsular* polysaccharide type 5 expression. **Infect. Immun.** 78 (3): 1339-1344.

Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. **J. Antimicrob. Chemother.** 48: 5-16.

Aparna, G., Chatterjee, A., Sonti, R. V., and Sankaranarayanan, R. (2009). A cell wall-degrading esterase of *Xanthomonas oryzae* requires a unique substrate recognition module for pathogenesis on rice. **Plant Cell.** 21 (6): 1860-1873.

Athinuwat, D., Brooks, S., Burr, T. J., and Prathuangwong, S. (2018). Flagella and pili of *Xanthomonas axonopodis* pv. Glycines are associated with motility, biofilm formation and virulence on soybean. **J. Phytopathol.** 166 (7-8): 590-600.

Auer, G. K., and Weibel, D. B. (2017). Bacterial cell mechanics. **Biochemistry.** 56 (29): 3710-3724.

Babst, M. (2014). Quality control at the plasma membrane: One mechanism does not fit all. **J. Cell Biol.** 205 (1): 11-20.

Bae, N., Park, H.-J., Park, H., Kim, M., Do, E., and Han, S.-W. (2018). Elucidating functions of fleq in *Xanthomonas oryzae* pv. *oryzae* by comparative proteomic and phenotypic analyses. **Int. J. Mol. Sci.** 19(10).

Bandara, M., Zhu, H., Sankaridurg, P., and Willcox, M. (2006). Salicylic acid reduces the production of several potential virulence factors of *Pseudomonas aeruginosa* associated with microbial keratitis. **Investig. Ophthalmol. Vis. Sci.** 47: 4453-4460.

Bandara, M. B. K., Zhu, H., Sankaridurg, P. R., and Willcox, M. D. P. (2006). Salicylic acid reduces the production of several potential virulence factors of *Pseudomonas aeruginosa* associated with microbial keratitis. **Investig. Ophthalmol. Vis. Sci.** 47 (10): 4453-4460.

Barák, I., and Muchová, K. (2013). The role of lipid domains in bacterial cell processes. **Int. J. Mol. Sci.** 14 (2): 4050-4065.

Batoni, G., Maisetta, G., and Esin, S. (2016). Antimicrobial peptides and their interaction with biofilms of medically relevant bacteria. **Biophysica Acta.** 1858 (5): 1044-1060.

Baxter, A. A., Hulett, M. D., and Poon, I. K. H. (2015). The phospholipid code: A key component of dying cell recognition, tumor progression and host-microbe interactions. **Cell Death Differ.** 22 (12): 1893-1905.

Bellincampi, D., Cervone, F., and Lionetti, V. (2014). Plant cell wall dynamics and wall-related susceptibility in plant-pathogen interactions. **Front. Plant Sci.** 5: 228-228.

Bogino, P. C., Oliva, M. d. l. M., Sorroche, F. G., and Giordano, W. (2013). The role of bacterial biofilms and surface components in plant-bacterial associations. **Int. J. Mol. Sci..** 14 (8): 15838-15859.

Boling, A. A., Bouman, B. A. M., Tuong, T. P., Konboon, Y., and Harnpichitvitaya, D. (2011). Yield gap analysis and the effect of nitrogen and water on photoperiod-sensitive jasmine rice in north-east thailand. **Wagen. J. Life Sci.** 58 (1): 11-19.

Bosund, I. (1963). The action of benzoic and salicylic acids on the metabolism of microorganisms. In C. O. Chichester, E. M. Mrak, and G. F. Stewart (Eds.). **Advances in food research** (pp. 331-353). Bjuv: Elsevier.

Burrows, L. L. (2012). *Pseudomonas aeruginosa* twitching motility: Type IV pili in action. **Annu. Rev. Microbiol.** 66 (1): 493-520.

Buttner, D., and Bonas, U. (2010). Regulation and secretion of *Xanthomonas* virulence factors. **FEMS Microbiol. Rev.** 34 (2): 107-133.

Cattò, C., James, G., Villa, F., Villa, S., and Cappitelli, F. (2018). Zosteric acid and salicylic acid bound to a low density polyethylene surface successfully control bacterial biofilm formation. **Biofouling.** 34 (4): 440-452.

Chithrashree, Udayashankar, A. C., Chandra Nayaka, S., Reddy, M. S., and Srinivas, C. (2011). Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv. *Oryzae*. **Biol. Control.** 59 (2): 114-122.

Chow, S., Gu, K., Jiang, L., and Nassour, A. (2011). Salicylic acid affects swimming, twitching and swarming motility in *Pseudomonas aeruginosa*, resulting in decreased biofilm formation. **JEMI.** 15: 22-29.

Clayton, A. W., and Thiers, R. E. (1966). Direct spectrophotometric determination of salicylic acid, acetylsalicylic acid, salicylamide, caffeine, and phenacetin in tablets or powders. **J. Pharm.Sci.** 55 (4): 404-407.

Crouzet, M., Le Senechal, C., Brözel, V. S., Costaglioli, P., Barthe, C., Bonneu, M., . . . Vilain, S. (2014). Exploring early steps in biofilm formation: Set-up of an experimental system for molecular studies. **BMC Microbiol.** 14: 253-253.

De la Fuente-Núñez, C., Reffuveille, F., Fernández, L., and Hancock, R. E. W. (2013). Bacterial biofilm development as a multicellular adaptation: Antibiotic resistance and new therapeutic strategies. **Curr. Opin. Microbiol.** 16 (5): 580-589.

De Pinto, M. C., Lavermicocca, P., Evidente, A., Corsaro, M. M., Lazzaroni, S., and De Gara, L. (2003). Exopolysaccharides produced by plant pathogenic bacteria affect ascorbate metabolism in *Nicotiana tabacum*. **Plant Cell Physiol.** 44 (8): 803-810.

Durak, T., and Depciuch, J. (2020). Effect of plant sample preparation and measuring methods on ART-FTIR spectra results. **Environ. Exp. Bot.** 169: 103915.

Esser, K., Kadereit, J. W., Lüttge, U., and Runge, M. (2012). **Progress in botany: Genetics cell biology and physiology systematics and comparative morphology ecology and vegetation science**. Berlin: Springer.

Eumkeb, G., Siriwong, S., and Thumanu, K. (2012). Synergistic activity of luteolin and amoxicillin combination against amoxicillin-resistant *Escherichia coli* and mode of action. **J. Photochem. Photobiol.** 117: 247-253.

Faize, L., and Faize, M. (2018). Functional analogues of salicylic acid and their use in crop protection. **Agronomy.** 8 (1): 5.

Fatima, U., and Senthil-Kumar, M. (2015). Plant and pathogen nutrient acquisition strategies. **Front. Plant Sci.** 6: 750-750.

Ferreira, D., Falahati, F., Linden, C., Buckley, R. F., Ellis, K. A., Savage, G., . . . Westman, E. (2017). A ‘disease severity index’ to identify individuals with subjective memory decline who will progress to mild cognitive impairment or dementia. **Sci. Rep.** 7: 44368.

Flemming, H.-C., and Wingender, J. (2010). The biofilm matrix. **Nat. Rev. Microbiol.** 8: 623.

Grogan, D. W. (2012). Chapter 50 - physiology of prokaryotic cells. In N. Sperelakis (Ed.), **Cell physiology** (pp. 891-906). San Diego: Academic Press.

Guo, Y., Sagaram, U. S., Kim, J.-s., and Wang, N. (2010). Requirement of the galu gene for polysaccharide production by and pathogenicity and growth in planta of xanthomonas citri subsp. Citri. **Appl. Environ. Microbiol.** 76 (7): 2234-2242.

Gupta, R., Lee, S. E., Agrawal, G. K., Rakwal, R., Park, S., Wang, Y., and Kim, S. T. (2015). Understanding the plant-pathogen interactions in the context of proteomics-generated apoplastic proteins inventory. **Front. Plant Sci.** 6: 352-352.

Ham, Y., and Kim, T.-J. (2018). Nitrogen sources inhibit biofilm formation of *Xanthomonas oryzae* pv. *oryzae*. **J. Microbiol. Biotech.** 28 (12): 2071-2078.

Huang, Q., and Allen, C. (2000). Polygalacturonases are required for rapid colonization and full virulence of *Ralstonia solanacearum* on tomato plants. **Physiol. Mol. Plant Pathol.** 57: 77-83.

Hull, R. (2014). Plant to plant movement. In **Plant virology** (pp. 669-751). Boston: Academic Press.

Jefferson, K. K. (2004). What drives bacteria to produce a biofilm?. **FEMS Microbio. Lett.** 236 (2): 163-173.

Jia, Y., Valent, B., and Lee, F. (2003). Determination of host responses to magnaporthe grisea on detached rice leaves using a spot inoculation method. **PLANT DIS.** 87: 129-133.

Jin, E. J., Kiral, F. R., and Hiesinger, P. R. (2018). The where, what, and when of membrane protein degradation in neurons. **Dev. Neurobiol.** 78 (3): 283-297.

Jones, C. B., Ott, E. M., Keener, J. M., Curtiss, M., Sandrin, V., and Babst, M. (2012). Regulation of membrane protein degradation by starvation-response pathways. **Traffic.** 13 (3): 468-482.

Justin Koilpillai, Y. (2014). Antimicrobial activity study of flavonoids and salicylic acid extracted from tagetes erecta linn.

Kamnev, A. A. (2008). FTIR spectroscopic studies of bacterial cellular responses to environmental factors, plant-bacterial interactions and signalling. **Spectroscopy.** 22: 862085.

Katzen, F., Ferreiro, D. U., Oddo, C. G., Ielmini, M. V., Becker, A., Pühler, A., and Ielpi, L. (1998). *Xanthomonas campestris* pv. *Campestris gum* mutants: Effects on xanthan biosynthesis and plant virulence. **J. Bacteriol. Res.** 180 (7): 1607-1617.

Ke, Y., Hui, S., and Yuan, M. (2017). *Xanthomonas oryzae* pv. *oryzae* inoculation and growth rate on rice by leaf clipping method. **Bio. protocol.** 7 (19): e2568.

Kerchove, A. J. d., and Elimelech, M. (2008). Bacterial swimming motility enhances cell deposition and surface coverage. **Environ. Sci. Technol.** 42 (12): 4371-4377.

Kochan, K., Perez-Guaita, D., Pissang, J., Jiang, J.-H., Peleg, A. Y., McNaughton, D., . . . Wood, B. R. (2018). In vivo atomic force microscopy-infrared spectroscopy of bacteria. **J. R. Soc. Interface.** 15 (140): 20180115.

Kraepiel, Y., and Barny, M.-A. (2016). Gram-negative phytopathogenic bacteria, all hemibiotrophs after all? **Mol. Plant Pathol.** 17 (3): 313-316.

Kunin, C. M., Hua, T. H., and Bakaletz, L. O. (1995). Effect of salicylate on expression of flagella by *Escherichia coli* and proteus, providencia, and *Pseudomonas* spp. **Infect Immun.** 63 (5): 1796-1799.

Lattab, A., Rachid, D., Arabi, A., Hichem, D., and Com, A. y. (2017). Effect of salicylic acid on biofilm formation and on some virulence factors in *Pseudomonas aeruginosa*. 10: 60-71.

Le Thanh, T., Thumanu, K., Wongkaew, S., Boonkerd, N., Teaumroong, N., Phansak, P., and Buensanteai, N. (2017). Salicylic acid-induced accumulation of biochemical components associated with resistance against *Xanthomonas oryzae* pv. *oryzae* in rice. **J. Plant Interact.** 12 (1): 108-120.

Levander, F., and Rådström, P. (2001). Requirement for phosphoglucomutase in exopolysaccharide biosynthesis in glucose- and lactose-utilizing *Streptococcus thermophilus*. **Appl. Environ Microbiol.** 67 (6): 2734-2738.

Li, H., Lantz, R., and Du, D. (2019). Vibrational approach to the dynamics and structure of protein amyloids. **Molecules.** 24 (1): 186.

Li, N., Han, X., Feng, D., Yuan, D., and Huang, L.-J. (2019). Signaling crosstalk between salicylic acid and ethylene, jasmonate in plant defense: Do we understand what they are whispering? **Int. J. Mol. Sci.** 20 (3): 671.

Li, Y., Yan, Y., Deng, S., Zhang, C., Haq, F., Chen, T., . . . Chen, G. (2020). The *Xanthomonas oryzae* pv. *oryzae* type IV pilus alignment subcomplex protein piln contributes to regulation of bacterial surface-associated behaviours and T3SS system. **Plant Pathol.** 69 (4): 744-755.

Lim, S.-H., So, B.-H., Wang, J.-C., Song, E.-S., Park, Y.-J., Lee, B.-M., and Kang, H.-W. (2008). Functional analysis of pilq gene in xanthomanas oryzae pv. Oryzae, bacterial blight pathogen of rice. **J. Microbiol.** 46 (2): 214-220.

Lu, X., Rasco, B. A., Jabal, J. M. F., Aston, D. E., Lin, M., and Konkel, M. E. (2011). Investigating antibacterial effects of garlic (*Allium sativum*) concentrate and garlic-derived organosulfur compounds on campylobacter jejuni by using fourier transform infrared spectroscopy, raman spectroscopy, and electron microscopy. **Appl. Environ. Microbiol.** 77 (15): 5257-5269.

Miedes, E., Vanholme, R., Boerjan, W., and Molina, A. (2014). The role of the secondary cell wall in plant resistance to pathogens. **Front. Plant Sci.** 5: 358-358.

Minamino, T., Imada, K., and Namba, K. (2008). Molecular motors of the bacterial flagella. **Curr. Opin. Struct. Biol.** 18 (6): 693-701.

Mizobuchi, R., Sato, H., Fukuoka, S., Tsushima, S., Imbe, T., and Yano, M. (2013). Identification of qRBS1, a QTL involved in resistance to bacterial seedling rot in rice. **Theor. Appl. Genet.** 126 (9): 2417-2425.

Niba, E. T. E., Naka, Y., Nagase, M., Mori, H., and Kitakawa, M. (2007). A genome-wide approach to identify the genes involved in biofilm formation in *E. Coli*. **DNA Res.** 14 (6):237-246-46.

Ntushelo, K. (2017). Effect of salicylic acid on the growth and chemical responses of pectobacterium carotovorum subsp. Carotovorum. **Pak. J. Biol. Sci.** 20: 278-288.

O'Toole GA. and Kolter, R. (1998). Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: A genetic analysis. **Mol. Microbiol.** 28 (3): 449-461.

Park, H.-S., Ham, Y., Shin, K., Kim, Y.-S., and Kim, T.-J. (2015). Sanitizing effect of ethanol against biofilms formed by three gram-negative pathogenic bacteria. **Curr. Microbiol.** 71 (1): 70-75.

Podolsky, R. J. (1953). Protein degradation in bacteria. **Arch. Biochem.** 45 (2): 327-340.

Price, C. T. D., Lee, I. R., and Gustafson, J. E. (2000). The effects of salicylate on bacteria. **Int. J. Biochem. Cell Biol.** 32 (10): 1029-1043.

Prieto-Calvo, M., Prieto, M., López, M., and Alvarez-Ordóñez, A. (2014). Effects of high hydrostatic pressure on *Escherichia coli* ultrastructure, membran eintegrity and molecular composition as assessed by FTIR spectroscopy and microscopic imaging techniques. **Molecules.** 19 (12): 21310-21323.

Raivio, T. L. (2018). Regulation of proteolysis in the gram-negative bacterial envelope. **J. Bacteriol.** 200 (3): e00639-00617.

Rann, V., Anusontpornperm, S., Thanachit, S., and Sreewongchai, T. (2016). Response of kdml105 and rd41 rice varieties grown on a typic natrustalf to granulated pig manure and chemical fertilizers. **Agric. Nat. Resour.** 50 (2): 104-113.

Rowlett, V. W., Mallampalli, V. K. P. S., Karlstaedt, A., Dowhan, W., Taegtmeyer, H., Margolin, W., and Vitrac, H. (2017). Impact of membrane phospholipid alterations in *Escherichia coli* on cellular function and bacterial stress adaptation. **J. Bacteriol.** 199 (13): e00849-00816.

Sahu, S. K., Zheng, P., and Yao, N. (2018). Niclosamide blocks rice leaf blight by inhibiting biofilm formation of *Xanthomonas oryzae*. **Front. plant Sci.** 9: 408-408.

Salomon, D., and Sessa, G. (2012). **21 biotechnological strategies for engineering plants with durable resistance to fungal and bacterial pathogens**. In A. Altman and P. M. Hasegawa (Eds.) Plant biotechnology and agriculture (pp. 329-342). San Diego: Academic Press.

Sandle, T. (2016). **14 - antibiotics and preservatives**. In T. Sandle (Ed.), Pharmaceutical microbiology (pp. 171-183). Oxford: Woodhead Publishing.

Schmidt, H., and Hensel, M. (2004). Pathogenicity islands in bacterial pathogenesis. **Clin. Microbiol. Rev.** 17 (1): 14-56.

Schrader, E. K., Harstad, K. G., and Matouschek, A. (2009). Targeting proteins for degradation. **Nat Chem Biol.** 5 (11): 815-822.

Schuster, M. L., and Coyne, D. P. (1974). Survival mechanisms of phytopathogenic bacteria. **Ann. Rev. Phytopathol.** 12 (1): 199-221.

Sedghi, M., and Gholi-Toluie, S. (2014). Influence of salicylic acid on the antimicrobial potential of stevia (*stevia rebaudiana bertoni*, *asteraceae*) leaf extracts against soybean seed-borne pathogens. **Trop. J. Pharm. Res.** 12: 1035.

Seligy, V. L., and Rancourt, J. M. (1999). Antibiotic mic/mbc analysis of *Bacillus*-based commercial insecticides: Use of bioreduction and DNA-based assays. **J. Ind. Microbiol. Biotechnol.** 22 (6): 565-574.

Shaheen, R., Zahid Sharif, M., Amrao, L., Zheng, A., Manzoor, M., Majeed, D., . . . Ali, A. (2019). Investigation of bacterial leaf blight of rice through various

detection tools and its impact on crop yield in Punjab, Pakistan. **Pak. J. Bot.** 51.

Shi, L., Li, P., Wang, W., Gao, M., Wu, Z., Song, X., and Hu, D. (2015a). Antibacterial activity and mechanism of action of sulfone derivatives containing 1,3,4-oxadiazole moieties on rice bacterial leaf blight. **Molecules.** 20 (7): 11660-11675.

Shi, L., Li, P., Wang, W., Gao, M., Wu, Z., Song, X., and Hu, D. (2015b). Antibacterial activity and mechanism of action of sulfone derivatives containing 1,3,4-oxadiazole moieties on rice bacterial leaf blight. **Molecules.** 20 (7): 11660.

Shukla, S. K., Ye, Z., Sandberg, S., Reyes, I., Fritsche, T. R., and Keifer, M. (2017). The nasal microbiota of dairy farmers is more complex than oral microbiota, reflects occupational exposure, and provides competition for *Staphylococci*. **PLoS ONE.** 12 (8): e0183898.

Smith, B., Li, J., Metruccio, M., Wan, S., Evans, D., and Fleiszig, S. (2018). Quantification of bacterial twitching motility in dense colonies using transmitted light microscopy and computational image analysis. **Bio-protocol.** 8 (8): e2804.

Sun, Q., Wu, W., Qian, W., Hu, J., Fang, R., and He, C. (2003). High-quality mutant libraries of *Xanthomonas oryzae* pv. *oryzae* and *X. Campestris* pv. *campestris* generated by an efficient transposon mutagenesis system. **FEMS Microbiol. Lett.** 226 (1): 145-150.

Thumanu, K., Wongchalee, D., Sompong, M., Phansak, P., Le Thanh, T., Namanusart, W., . . . Buensanteai, N. (2017). Synchrotron-based FTIR

microspectroscopy of chili resistance induced by *Bacillus subtilis* strain D604 against anthracnose disease. **J. Plant Interact.** 12 (1): 255-263.

Trunk, T., Khalil, H. S., and Leo, J. C. (2018). Bacterial autoaggregation. **AIMS Microbiol.** 4 (1): 140-164.

Vicario, J. C., Dardanelli, M. S., and Giordano, W. (2014). Swimming and swarming motility properties of peanut-nodulating rhizobia. **FEMS Microbiol. Lett.** 362 (2): 1-6.

Vojnov, A. A., Slater, H., Daniels, M. J., and Dow, J. M. (2001). Expression of the gum operon directing xanthan biosynthesis in *Xanthomonas campestris* and its regulation in planta. **Mol. Plant Microbe. Interact.** 14 (6): 768-774.

Vojnov AA, Z. A., Dow JM, Daniels MJ, Dankert MA. (1998). Evidence for a role for the gumb and gumc gene products in the formation of xanthan from its pentasaccharide repeating unit by *Xanthomonas campestris*. **Microbiology.** 144 (6): 1487-1493.

Walters, D., Newton, A. C., and Lyon, G. (2008). **Induced resistance for plant defence: A sustainable approach to crop protection**. New Jersey: John Wiley & Sons.

Wang, H., Yin, X., Wu Orr, M., Dambach, M., Curtis, R., and Storz, G. (2017). Increasing intracellular magnesium levels with the 31-amino acid mgts protein. **Proc. Natl. Acad. Sci.** 114 (22): 5689-5694.

Wang, M., Lu, X., Yin, X., Tong, Y., Peng, W., Wu, L., . . . Zhang, J. (2015). Synchrotron radiation-based fourier-transform infrared spectromicroscopy for characterization of the protein, peptide distribution in single microspheres. **Acta. Pharm. Sinica B.** 5 (3): 270-276.

Weller-Stuart, T., Toth, I., De Maayer, P., and Coutinho, T. (2017). Swimming and twitching motility are essential for attachment and virulence of pantoea ananatis in onion seedlings. **Mol. Plant. Pathol.** 18 (5): 734-745.

Xu, J., Audenaert, K., Hofte, M., and De Vleesschauwer, D. (2013). Abscisic acid promotes susceptibility to the rice leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae* by suppressing salicylic acid-mediated defenses. **PLoS One.** 8 (6): e67413.

Xu, J., Zhou, L., Venturi, V., He, Y.-W., Kojima, M., Sakakibari, H., . . . De Vleesschauwer, D. (2015). Phytohormone-mediated interkingdom signaling shapes the outcome of rice *Xanthomonas oryzae* pv. *oryzae* interactions. **Plant Biol.** 15: 10.

Yadeta, K. A., and J. Thomma, B. P. H. (2013). The xylem as battleground for plant hosts and vascular wilt pathogens. **Front. Plant Sci.** 4: 97.

Yaron, S., and Römling, U. (2014). Biofilm formation by enteric pathogens and its role in plant colonization and persistence. **Microb. Biotechnol.** 7 (6): 496-516.

Yin, X., Wu Orr, M., Wang, H., Hobbs, E. C., Shabalina, S. A., and Storz, G. (2019). The small protein mgts and small rna mgrr modulate the pita phosphate symporter to boost intracellular magnesium levels. **Mol. Microbiol.** 111 (1): 131-144.

Yu, C., Nguyen, D.-P., Ren, Z., Liu, J., Yang, F., Tian, F., . . . Chen, H. (2020). The rpon2-pilrx regulatory system governs type iv pilus gene transcription and is required for bacterial motility and virulence in *Xanthomonas oryzae* pv. *oryzae*. **Mol. Plant Pathol.** 21 (5): 652-666.

Yuan, M., Chu, Z., Li, X., Xu, C., and Wang, S. (2010). The bacterial pathogen *Xanthomonas oryzae* overcomes rice defenses by regulating host copper redistribution. **The Plant Cell.** 22 (9): 3164-3176.

Zolfaghar, I., Evans, D. J., and Fleiszig, S. M. J. (2003). Twitching motility contributes to the role of pili in corneal infection caused by *Pseudomonas aeruginosa*. **Infect. Immun.** 71 (9): 5389-5393.

CHAPTER V

EFFICACY OF SALICYLIC ACID ELICITOR ON INCREASING RICE HEALTH AGAINST BACTERIAL LEAF BLIGHT DISEASE AND YIELD IMPROVEMENT

ABSTRACT

Salicylic acid elicitor (SA) is an effective inducer for plant defense activation against diseases and plant growth promotion. In this research, rice treated with SA formula as SA-Ricemate® to enhance plant defense mechanism against bacterial leaf blight disease (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). The results found that the application of SA-Ricemate® at the concentrations of more than 100 ppm can reduce BLB disease severity up to 42-70% under greenhouse conditions. SA-Ricemate® treatment also increased H_2O_2 contents in rice over non-treated with 39-61% within 24 hours after *Xoo* inoculation leading to an induction of plant resistance. Besides, malondialdehyde in rice treated with SA-Ricemate® showed an increase of 50-65% when compared with non-treated control. The differential production of these defense components was faster and distinct when SA-Ricemate® treated rice was infected with *Xoo* indicating induced resistance. Moreover, the induced resistance by SA-Ricemate® elicitor at concentration more than 50 ppm was also related with a significant increase on accumulation of total chlorophyll content in

rice leaves at 2.53-2.73 mg g^{-1} of fresh weight, suggesting that innate immunity is triggered by these elicitor. In field conditions, the foliar sprayed with SA-Ricemate® significantly reduced BLB disease severity with 37-78% and improved yield by 25-69% in all two consecutive crop seasons during Aug-Nov 2017-2018. Therefore, this work recommended the utilization of SA-Ricemate® as a green and safe-chemical elicitor to control BLB disease and improve rice productivity.

Keywords

Bacterial leaf blight; induce resistance; rice production; salicylic acid

5.1 Introduction

Rice (*Oryza sativa*) is one of the most important crops in the world. Efficiency on rice production can be negatively affected by several factors such as poor soil quality, nutrient deficiency, pests, and diseases, leading to reduction of rice yield (Keeratipipatpong, 2010; FAO, 2015; Kawasaki and Herath, 2018; Timsina et al., 2018). One of the most important disease in rice production system is bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) which is widely present in South-East Asia and Japan with averaged losses of 20-50% (Son, 1993, Noh et al., 2007; Walters, Newton, and Lyon, 2008; Singh et al., 2015 Chiawpanit, Arayaskul, and Lithanatudom, 2018). In Thailand, BLB is considered one of the most serious rice diseases because of the favorable climate conditions. (Kawasaki, 2010; Sekhar, 2018). Therefore, reducing severity of BLB is an essential target to improve rice production in regional Thailand. The main current methods used to control this pathogen are based on synthetic chemical products such as copper hydroxide, copper oxychloride, copper sulfate, etc. However, they such components potentially have

harmful effects from synthetic chemical residues to human and the environment. Moreover, these chemical pesticides cannot effectively reduce the spread of BLB due to their non-systemic chemicals and their high cost of application at field level. Therefore, it is important to continue researching for effective green and safe agrochemicals to control the BLB. There are other control methods based on biotic and abiotic inducers to activate plant defense mechanisms that have been documented and used such as plant growth promoting rhizobacteria (PGPR), oligosaccharides, polymers of carbohydrate, derivatives of lipids, green agrochemical, and chemical plant hormones (Shibuya and Minami, 2001; Trouvelot et al., 2014; Zhou and Wang, 2018). In the recent years, abiotic elicitors have been extensively investigated as a mean to control plant diseases based on the induced resistance (IR) concept including induced systemic resistance (ISR) and systemic acquired resistance (SAR) (Buensanteai, Yuen, and Prathuangwong, 2008; Aranega-Bou et al., 2014). IR contributes plants to develop innate defenses against plant pathogens involving multiple biochemical mechanisms which can be activated by plant elicitors/ activators/ inducers (Liyanage et al., 2017). An important characteristic of IR is the priming on plants such as foliar application or seed priming that enables rapid expression of defense responses upon pathogen infection (Aranega et al., 2014; Le et al., 2017). Previous studies reported that the plant resistance can be induced by several elicitors or inducers such as acibenzolar-S-methyl, benzo-123-thiadiazole-7-carbothionic acid, ascorbic acid, oligosaccharides, jasmonic acid, phosphoric acid, vitamin B1, β-aminobutyric acid, salicylic acid, etc. (Thakur and Sohal, 2013; Chakraborty et al, 2016; Kukawka, et al., 2018). Salicylic acid (SA) is a phenolic compound that acts by signaling to acquire systemic resistance as plant defense

against several pathogens (Rivas and Plasencia, 2011; Dempsey and Klessig, 2017). Furthermore, SA plays an essential role as plant hormone in plant growth and development and as plant elicitor in plant innate immunity (Hayat, Khan, and Alyemeni, 2013). The SA response in plants immune is involved in the oxidative burst and the induction of the reactive oxygen species (ROS). The oxidative burst response to SA was reported in several plants such as potato, rice, tobacco by increase of hydrogen peroxide (H_2O_2) production (Kauss, 1995; Leon, Lawton, and Raskin, 1996; Fauth et al., 1996), which is similar to the effects of the chitooligosaccharides (Malerba and Cerana, 2016). The increase of the H_2O_2 levels led to an expression of glucanase and chitinase transcripts in rice which is involved in induction of plant resistance (Anuratha et al., 2006). It was suggested that H_2O_2 production under induced resistance caused functional formation and reinforcement of plant structural tissues (Bacete et al., 2018). Malondialdehyde (MDA) is a marker of oxidative lipid injury involved with programed cell death (PCD) which concentration varies in response to biotic and abiotic stresses (Zoeller, 2012; Liu et al., 2018). Moreover, the application of SA causes chloroplast derived retrograde signals such as chlorophyll synthesis, encoded genes for photosynthesis, and important mediators of plant immunity (Nomura et al., 2012; Khan et al., 2015).

Moreover, the application of SA causes chloroplast derived retrograde signals such as chlorophyll synthesis, encoded genes for photosynthesis, and important mediators of plant immunity

The application of salicylic acid has been researched as a resistance elicitor against plant pathogen in greenhouse conditions based on the induced resistance concept whereas the SA has never been evaluated on rice fields neither developed into

a commercial formulation in Thailand. The aim of this work was to investigate the resistance mechanism induced by an abiotic elicitor formulation based on SA-Ricemate® (SA combined with inert-ingredients) against BLB on rice under greenhouse and field conditions. Hence, the efficacy of SA-Ricemate® elicitor formulate on stimulating hydrogen peroxide (H_2O_2), malondialdehyde (MDA), chlorophyll and reduction of BLB severity under greenhouse conditions as well as growth parameters, reduction of BLB, and rice yield of 2 consecutive rice seasons during Aug-Nov 2017 and Aug-Nov 2018 were evaluated.

5.2 Materials and methods

5.2.1 Elicitor preparation

The exogenous salicylic acid elicitor (SA-Ricemate®) prototype is a product of the Bioactive Agro Industry Co., Ltd.; developed at the PPB Laboratory, Suranaree University of Technology, Thailand.

5.2.2 Rice cultivar, bacterial strains and culture conditions

A homogenous sample of Thai jasmine rice (*Oryza sativa*) seeds of susceptible BLB cultivar Khao Dawk Mali 105 (KDML 105) were obtained from the Rice Seed Center, Thailand. The *Xoo* SUT1-121 virulence strain was used in this research. It was originally provided by Prae Rice Research Institute. The bacterial culture was grown in nutrient glucose broth (NGB) at 28±1 °C then, applied 180 rpm constant shaking for 48 h (Buensanteai, Yuen, and Prathuangwong, 2008; Krishnan et al., 2014; Le et al., 2017).

5.2.3 The assessment of SA-Ricemate® against BLB under greenhouse conditions

The *Xoo* SUT1-121 strain was inoculated on rice plants by scissors dip technique (Ke, Hui, and Yuan, 2017; Le et al., 2017). Rice seeds cv. KDML 105 were soaked in water for 24 h before planting. The seedlings were transferred into 30-cm-diameter plant pots that contained 5 kg of Suranaree University of Technology farm soil. The pots were kept in greenhouse conditions under 12 hours of photoperiod and 60-75% of humidity. The rice plants were sprayed with 30 ml of the SA-Ricemate® at 15, 30, and 45 days after sowing, with a range of various concentrations including 50, 100, 150, 200, 250, and 300 ppm; two positive control as a copper hydroxide, and commercial elicitor; active ingredient of chitooligosaccharide and an untreated as a negative control. The suspension of *Xoo* as $1x10^8$ cfu mL^{-1} was inoculated on the top-leaves by cutting the leaf tip; then, the plants were covered with plastic bags and incubated at 28ºC for 24 h (Chithrashree et al., 2011; Xu et al., 2013; Ke et al., 2017; Le et al., 2017).

5.2.4 Assessment on reduction of BLB severity

The BLB disease severity was estimated within an interval of 7 days post inoculation by using the method of IRRI (IRRI, 2002). The percentage of disease severity was calculated according to the following formula equation (1). The reduction of disease severity was calculated using the formula equation (2) presented by Ferreira et al. and Le et al. (Ferreira et al., 2017; Le et al., 2017).

$$\text{Disease severity (\%)} = \left(\frac{\sum_{i=1}^{n} r_i}{n \times m} \right) \times 100 \qquad (1)$$

Where ‘r’ is the set of numerical ratings, ‘n’ is the total of evaluations per sample, and ‘m’ is the maximum value used for the evaluations.

$$\text{Reduction on disease severity (\%)} = \frac{DSn - Dst}{DSn} \times 100 \quad (2)$$

Where ‘DSn’ is the calculated disease severity from untreated samples and ‘DSt’ is the calculated disease severity from elicitor-treated samples.

5.2.5 Determination of hydrogen peroxide (H_2O_2)

Rice leaf tissues; 0.5 g from each treatment were soaked in liquid nitrogen and homogenized with 5 mL of 0.1% trichloroacetic acid then centrifuged at 14000 x g for 15 min under 4°C. A dose of the supernatant; 0.5 mL was mixed with 0.5 mL of 10 mM potassium phosphate buffer, pH 7.0, and 1 mL of 1M KI. The absorbance was quantified by a microplate reader (Bio-Tek, USA) at 390 nm. The H_2O_2 content was calculated based on a standard curve (Sahebani and Hadavi, 2009).

5.2.6 Determination of malondialdehyde (MDA)

Rice leaf tissues; 0.5 g from each treatment were soaked in liquid nitrogen and homogenized with 5 mL of 5% trichloroacetic acid, then centrifuged at 4000 x g for 10 min. A dose of the supernatant; 2 mL was mixed with 2 mL of 0.67% barbituric acid, then the mixture was boiled for 30 min at 100°C. Next, the mixture was centrifuged at 3000 x g for 10 min. The absorbance was quantified by a microplate reader (Bio-Tek, USA). The MDA content was calculated according to the following formula presented in a research of Draper and Hadley (Draper and Hadley, 1990).

$$\text{MDA } (\mu M \cdot L^{-1}) = 6.45(A_{532} - A_{600}) - 0.56A_{450} \quad (3)$$

Where A450, A532, and A600 are the absorbance values at the wavelengths 450, 532, and 600 nm, respectively.

5.2.7 Determination of chlorophyll content

Rice leaf tissues; 0.1 g were homogenized with 5 mL of acetone and ethanol (4:1). The extraction was put in the dark conditions for 3 h then centrifuged at 4000 x g for 5 min. The supernatant was quantified by a microplate reader (Bio-Tek, USA) at 645 and 663 nm. The chlorophyll content was calculated according to the following formulas proposed by Aminot and Rey (Aminot and Rey, 2002).

$$\text{Chlorophyll a } (mg \cdot L^{-1}) = 0.0127\ A_{663} - 0.00269\ A_{645} \quad (4)$$

$$\text{Chlorophyll b } (mg \cdot L^{-1}) = 0.0229 A_{645} - 0.00468\ A_{663} \quad (5)$$

$$\text{Total chlorophyll content} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad (6)$$

5.2.8 The assessment of SA-Ricemate® against BLB under field conditions

5.2.8.1 Field arrangement, cultivation, and spray of elicitors

This experiment consisted in the application of Randomized Complete Block Design with 4 treatments including SA-Ricemate®, commercial elicitor; chitooligosaccharide, copper hydroxide 77% WP, and water as untreated control with 4 replications and 2 consecutive crops during Aug-Nov 2017-2018. All experiments were carried out at the paddy field in Khok Sung, Ubolratana district, Khon Kaen province, Thailand (DMS: 16° 40’ 38.96” N102° 41, 57.23”E). The treatments and

replications were separated by 1 m buffer strips. The individual plot size was 81 m^2; 9 m length and 9 m wide. The planting spaces were 25 cm x 25 cm row to row with 32 rows and 32 seedlings per row in each plot (IRRI, 2002; Department of Agriculture, 2009). The margin rows were considered as a border row to avoid possible sample contamination. The BLB disease severity, the growth parameters, and yield production were assessed by quadrants (1 m^2) as representative data for each treatment plot (IRRI, 2002; Office of Agricultural Economics, 2003; FAO, 2014).

The seedlings for transplanting were prepared in a small plot as a seed bed. The seed bed was prepared with farm soil and decomposed by 0.5 kg m^{-2}, 10 cm in deep. The rice seeds cv. KDML 105 were soaked for 24 h and sown in seed beds at 50 g m^{-2}. The 20 days old seedlings were uprooted and arranged for replanting at 3 stems per hill. The field trial was ploughed crisscross twice by using a tractor at 7 days before transplanting. Decomposition at 500 kg rai^{-1} (0.4 acre) was applied 3 day before transplanting. The fertilizers used in the experiments were based on nitrogen, phosphorus, and potassium following the treatment basis recommended by the Land Development Department (LDD), Ministry of Agriculture and Cooperatives, Thailand (LDD, 2017). The 16-20-0 fertilizers formula were applied on the field at 10 days after transplanting (tilling stage) at a dose of 30 kg rai^{-1} and 0-0-60 fertilizer formula at a dose of 10 kg rai^{-1}. The 46-0-0 fertilizer formula was applied at 50 days after transplanting (panicle initiation stage) at 3 kg rai^{-1} in crop 1 and crop 2. Crop 3 was applied a double dose of fertilizer including 16-20-0, 0-0-60, and 46-0-0 to contribute plant susceptibility to disease. Hand weeding was performed for weed control. Besides, golden apple snails were removed by hand.

The application of the elicitor was done by spraying to rice foliage (20 L rai^{-1}) three times at 15, 30, and 45 days post transplanting. Water was used as a negative control treatment (untreated) whereas the commercial elicitor as well as chitooligosaccharide and copper hydroxide served as positive controls.

5.2.8.2 Disease severity, growth parameters, and BLB disease severity

The potential of the elicitors to control BLB was evaluated against natural infection of *Xoo*. Disease severity on rice plants was evaluated four times from 45 days post transplanting. Percentage of disease severity and disease reduction were calculated using the equations (1) and (2), which slightly modified from Le et al. (2017).

The plant parameters were observed in term of plant height, number of tillers per hill, number of seeds per panicle, 1000-grain weigh, and total grain yield. The data of grain yield was calculated and reported as kg rai^{-1} according to the following formula (7) presented in a research of Regalado and Ramos (Regalado and Ramos, 2018).

$$\text{Grain yield (rai}^{-1}) = \frac{\text{Y x (100-MC)}}{\text{(100-14) A}} \text{ X } 1600 \qquad (7)$$

Where ‘MC’ is the moisture content of grain (%), ‘Y’ is the net plot yield, and ‘A’ is the plot area.

5.2.9 Statistical analysis

The experimental data was analyzed by one-way ANOVA using the SPSS software version 20. The mean differences were separated by Duncan' s Multiple Range Test at a significance of P = 0.05.

5.3 Results

5.3.1 The efficacy of SA-Ricemate® against BLB under greenhouse conditions

The application of SA-Ricemate® at 100 ppm or higher concentrations significantly reduces BLB disease severity when used as a foliage treatment at 15, 30, and 45 days post sowing. BLB disease reduction of rice plants treated with 100 ppm was 42.86, 57.14, and 50.00% at 7, 14, and 21 days post spraying, respectively. Whereas, the protective activity of SA-Ricemate® at 150, 200, 250, and 300 ppm show non-significant reduction of BLB disease severity when compared with the 100 ppm treatment (Table 5.1).

Table 5.1 Effectiveness of SA-Ricemate® on the disease severity of BLB in rice cv. KDML 105 caused by *Xoo* strain SUT1-121.

Treatment	Disease severity (%) [2/]			Control efficiency (%) [3/]		
	7 DPI[4/]	14 DPI[4/]	21 DPI[4/]	7 DPI	14 DPI	21 DPI
SA-Ricemate® 50 ppm	18.75 ± 3.61^{abc}	31.25 ± 3.61^{b}	59.37 ± 10.67^{b}	14.29	28.57	13.64
SA-Ricemate® 100 ppm	12.50 ± 5.10^{ab}	18.75 ± 3.60^{a}	34.37 ± 3.12^{a}	42.86	57.14	50
SA-Ricemate® 150 ppm	12.50 ± 0.00^{ab}	15.62 ± 3.12^{a}	28.12 ± 3.12^{a}	42.86	64.29	59.09
SA-Ricemate® 200 ppm	12.50 ± 0.00^{ab}	15.62 ± 3.12^{a}	28.12 ± 3.13^{a}	42.86	64.29	59.09
SA-Ricemate® 250 ppm	12.50 ± 0.00^{ab}	15.62 ± 3.12^{a}	28.12 ± 3.14^{a}	42.86	64.29	59.09
SA-Ricemate® 300 ppm	12.50 ± 0.00^{ab}	12.5 ± 0.00^{a}	25.00 ± 0.00^{a}	42.86	71.43	63.64
Chitooligosaccharide-commercial	9.37 ± 3.12^{a}	15.62 ± 5.98^{a}	28.12 ± 5.98^{a}	57.14	64.29	59.09
Copper hydroxide 1000 ppm	6.25 ± 3.61^{a}	9.37 ± 3.12^{a}	21.87 ± 5.98^{a}	71.43	78.57	68.18
Untreated (control)	21.87 ± 3.12^{c}	43.75 ± 8.07^{c}	68.75 ± 11.96^{b}	-	-	-
F-test	*	*	*			
CV (%)	42.76	39.38	35.75			

[1/] Rice plants were sprayed every 15 day intervals, three times with SA-Ricemate® at different concentrations and rice leaves were inoculated with *Xoo* SUT1-121strain at 50 days post sowing. [2/] Disease severity was examined at 7, 14, 21 days post inoculation. Each value represents a mean of five replicates. [3/] The control efficiency of BLB calculated by compared with the untreated. [4]The meaning of the different letters indicate significant difference via Duncan's multiple range test at P = 0.05

5.3.2 The change of hydrogen peroxide (H_2O_2)

The presence of H_2O_2 in rice plants was increased at 24 h post inoculation by all treatments as shown in Table 5.2. The H_2O_2 contents in rice plants treated with SA-Ricemate® at 100, 150, 200, 250, and 300 ppm and with commercial elicitor were increased with 39.31, 46.48, 44.25, 54.36, 61.10, and 62.84%, respectively. Whereas, the treatment of copper hydroxide comparing with untreated control had an H_2O_2 increase of 12.68% against the 9.63% from the untreated control.

Table 5.2 Effectiveness of SA-Ricemate® in H_2O_2 content in rice cv. KDML 105 against by *Xoo* strain SUT1-121.

Treatment [1/]	Hydrogen peroxide (µmol g^{-1} of fresh weight)		Increase of H_2O_2 activity (%) [2/]
	Pre inoculation	Post inoculation 24h	
SA-Ricemate® 50 ppm	9.34 ± 0.38[a]	11.21 ± 0.27[a]	20.07 ± 2.25[a]
SA-Ricemate® 100 ppm	10.22 ± 0.25[a]	14.24 ± 0.06[b]	39.31 ± 3.60[b]
SA-Ricemate® 150 ppm	10.12 ± 0.54[a]	14.83 ± 0.97[bc]	46.48 ± 5.99[bc]
SA-Ricemate® 200 ppm	10.21 ± 0.85[a]	14.74 ± 1.61[bc]	44.25 ± 6.20[bc]
SA-Ricemate® 250 ppm	9.98 ± 1.04[a]	15.40 ± 1.52[bcd]	54.36 ± 4.75[cd]
SA-Ricemate® 300 ppm	11.01 ± 0.20[a]	17.75 ± 0.09[d]	61.10 ± 3.80[d]
Chitooligosaccharide-commercial	10.63 ± 0.23[a]	17.31 ± 0.33[cd]	62.84 ± 4.96[d]
Copper hydroxide 1000 ppm	9.27 ± 0.25[a]	10.44 ± 0.25[a]	12.68 ± 0.86[a]
Untreated (control)	9.26 ± 0.18[a]	10.15 ± 0.02[a]	9.63 ± 1.94[a]
F-test	**	**	**
CV (%)	7.27	9.9	18.62

[1/] Rice plants were sprayed every 15 day intervals, three times with SA-Ricemate® at different concentrations and rice leaves were inoculated with *Xoo* SUT1-121 strain at 50 days post sowing. [2/]The increase of H_2O_2 was calculated by the difference with pre inoculation samples. Each value represents a

mean of five replications. The meaning of the different letters indicated significant difference via Duncan's multiple range test at P = 0.05.

5.3.3 The change of malondialdehyde (MDA)

Treated plants experienced an increase on MDA contents in rice plant after 24 h from the time point of inoculation. Rice plants treated with commercial elicitor and SA-Ricemate® at 100, 150, 200, 250, and 300 ppm showed significantly higher increase of MDA with 69.69, 60.98, 54.59, 50.58, 65.04, and 53.06% respectively in comparison with the untreated samples; 33.01%. However, the MDA increase in rice treated with SA-Ricemate® at 50 ppm was showed 40.23% MDA accumulation, considering it non-significant in comparison with the untreated whereas copper hydroxide showed significantly lower MDA with 23.75% (Table 5.3).

Table 5.3 Effectiveness of SA-Ricemate® based on the changes of MDA content in rice cv. KDML 105 infected by *Xoo* strain SUT1-121.

Treatment [1/]	Malondialdehyde (MDA) (µmol g^{-1})		Increase of MDA Activity (%) [2/]
	Pre inoculation	Post inoculation 24h	
SA-Ricemate® 50 ppm	2.40 ± 0.24^{a}	3.39 ± 0.03^{ab}	40.23 ± 3.93^{b}
SA-Ricemate® 100 ppm	2.31 ± 0.12^{a}	3.72 ± 0.11^{b}	60.98 ± 3.90^{c}
SA-Ricemate® 150 ppm	2.32 ± 0.18^{a}	3.59 ± 0.14^{b}	54.59 ± 5.75^{c}
SA-Ricemate® 200 ppm	2.51 ± 0.27^{ab}	3.78 ± 0.07^{b}	50.58 ± 5.11^{c}
SA-Ricemate® 250 ppm	2.91 ± 0.17^{ab}	4.81 ± 0.14^{c}	65.04 ± 6.85^{c}
SA-Ricemate® 300 ppm	3.04 ± 0.25^{ab}	4.66 ± 0.13^{c}	53.06 ± 1.05^{c}
Chitooligosaccharide-commercial	3.08 ± 0.42^{ab}	5.23 ± 0.31^{c}	69.69 ± 2.70^{c}
Copper hydroxide 1000 ppm	3.20 ± 0.16^{b}	3.96 ± 0.65^{b}	23.75 ± 3.77^{a}
Untreated (control)	2.25 ± 0.24^{a}	3.00 ± 0.36^{a}	33.01 ± 8.77^{b}
F-test	**	**	**
CV(%)	9.99	8.07	22.44

[1/]Rice plants were sprayed every 15 day intervals, three times with SA-Ricemate® at different concentrations and rice leaves were inoculated with *Xoo* SUT1-121 strain at 50 days post sowing. [2/]The increase of MDA was calculated by the difference with pre-inoculated samples. Each value represents a mean of five replications. The meaning of the different letters indicate significant difference via Duncan's multiple range test at P = 0.05.

5.3.4 The change of chlorophyll content

After rice plants being sprayed with the elicitor three times, the total chlorophyll content in rice leaves treated with SA-Ricemate® at 50, 100, 150, 200, 250 ppm, and commercial elicitor chlorophyll content significantly increased with 2.65, 2.66, 2.73, 2.62, 2.53, and 2.29 mg g^{-1} of fresh weight, respectively when compared with untreated samples. The treatment with copper hydroxide and 300 ppm

of SA-Ricemate® showed non-significantly content when compared with untreated (Table 5.4).

Table 5.4 Effectiveness of SA-Ricemate® based on the accumulation of chlorophyll content in rice cv. KDML 105 infected by *Xoo* strain SUT1-121.

Treatment [1/]	Chlorophyll a	Chlorophyll b	Total chlorophyll
	(mg g^{-1} of fresh weight) [2/]		
SA-Ricemate® 50 ppm	1.91 ± 0.04[b]	0.73 ± 0.05[c]	2.65 ± 0.09[c]
SA-Ricemate® 100 ppm	1.94 ± 0.03[b]	0.72 ± 0.05[c]	2.66 ± 0.08[c]
SA-Ricemate® 150 ppm	1.95 ± 0.03[b]	0.77 ± 0.05[c]	2.73 ± 0.08[c]
SA-Ricemate® 200 ppm	1.91 ± 0.02[b]	0.71 ± 0.05[c]	2.62 ± 0.07[c]
SA-Ricemate® 250 ppm	1.83 ± 0.12[b]	0.71 ± 0.05[c]	2.53 ± 0.16[b]
SA-Ricemate® 300 ppm	1.54 ± 0.03[a]	0.55 ± 0.05[a]	2.09 ± 0.07[a]
Chitooligosaccharide-commercial	1.65 ± 0.03[a]	0.63 ± 0.05[b]	2.29 ± 0.08[b]
Copper hydroxide 1000 ppm	1.58 ± 0.04[a]	0.59 ± 0.05[a]	2.17 ± 0.09[a]
Untreated (control)	1.60 ± 0.02[a]	0.60 ± 0.06[ab]	2.20 ± 0.08[a]
F-test	**	**	**
CV (%)	4.71	15.97	6.47

[1/]Rice plants were sprayed every 15 day intervals, three times with SA-Ricemate® at different concentrations. [2/]The chlorophyll was examined at pre inoculation and 50 days post sowing. Each value represents a mean of five replications. The meaning of the different letters indicated significant difference via Duncan's multiple range test at P = 0.05.

5.3.5 The efficacy of SA-Ricemate® against BLB under field condition

5.3.5.1 The reduction of disease severity

The results of the first crop are summarized in Table 5.5. The application of SA-Ricemate® at 100 ppm concentration significantly reduces BLB disease severity when used as a foliage treatment at 15, 30, and 45 days post

transplant with 100 ppm was 78, 68, 52, and 37% respectively whereas commercial elicitor showed BLB reduction with 64, 77, 59, and 50% respectively; and copper hydroxide showed BLB reduction with 86, 86, 59, and 53% at 7, 14, 21, and 28 days after the third spraying, respectively.

The results of the second crop are summarized in Table 5.6. SA-Ricemate® at 100 ppm concentration significantly reduces BLB disease severity when used as a foliage treatment at 15, 30, and 45 days post transplant. Disease reduction of rice plants treated with 100 ppm was 39, 45, 53, and 37% respectively; commercial elicitor as 44, 57, 53, and 49% respectively; and copper hydroxide as 50, 55, 57, and 39% at 7, 14, 21, and 28 days after the third spraying, respectively.

The results of the third crop are summarized in Table 5.7. SA-Ricemate® at 100 ppm concentration significantly reduces BLB disease severity when used as a foliage treatment at 15, 30, and 45 days post-transplant. Disease reduction of rice plants treated with 100 ppm was 50, 41, 42, and 38% respectively; commercial elicitor as 35, 45, 40, and 41% respectively; and copper hydroxide as 45, 47, 43, and 43% at 7, 14, 21, and 28 days after the third spraying, respectively.

5.3.5.2 The efficacy of SA-Ricemate® in plant parameters and yield

The foliar applications of SA-Ricemate® on the paddy field; crop 1during Aug-Nov 2017 were stimulated plant parameters and yield for KDML 105 rice as showed in Table 5.8. The data indicated that the rice plant samples treated with SA-Ricemate® had significantly higher with 108 and 116 cm at 45 and 75, DPT respectively; followed by commercial elicitor with 104 and 116 cm. The highest number of tillers per hill was obtained from rice plant treated with SA-Ricemate®

with 14.4 tillers per hill followed by the commercial elicitor treatment with 14.2 tillers per hill, copper hydroxide treatment with 13.8 tillers per hill and the untreated with 11.2 tillers per hill. The highest number of panicles per hill was obtained from rice plant treated with copper hydroxide with 12.8 panicles per hill followed by the commercial elicitor treatment with 12.2 panicles per hill, then SA-Ricemate® treatment with 11.6 panicle per hill, and the untreated with 10.2 panicle per hill. Greater number of filled grains per panicle was counted for each treated sample. SA-Ricemate® treated samples generated 154 grains per panicle followed by copper hydroxide treated samples with 147 grains per panicle, commercial elicitor treated samples with 145 grains per panicle, and untreated samples with 138 grains per panicle. A 1000-grain weight was revealed that the commercial elicitor showed highest result with 28.44 g followed by SA-Ricemate® treatment with 28.09 g, copper hydroxide treatment with 27.30 g, and untreated with 25.56 g. The highest grain yield was collected from the samples treated with SA-Ricemate® with 570 kg rai^{-1} (0.4 acre) followed by copper hydroxide treatment with 525 kg rai^{-1},then the commercial elicitor treatment with 501 kg rai^{-1} and untreated samples with 454 kg rai^{-1}.

The foliar applications of SA-Ricemate® on the paddy field; crop 2 during Aug-Nov 2018 at location 1 were stimulated plant parameters and yield for KDML 105 rice as showed in Table 5.9. The data indicated that the effect of SA-Ricemate® treatment in plant height was significantly the highest at 45 DPT with 113 cm followed by commercial elicitor treatment with 111 cm. Furthermore, the commercial elicitor treated samples showed the highest height at 75 and 105 DPT with 122 cm and 146 cm, respectively followed by the SA-Ricemate® treated

samples with 120 and 143 cm. The highest number of tillers per hill was obtained from the commercial elicitor treatment with 16.0 tillers per hill followed by the SA-Ricemate® with 14.8 tillers per hill, copper hydroxide with 14.0 tillers per hill and the untreated with 11.8 tillers per hill. The highest number of panicles per hill was obtained from the SA-Ricemate® treatment with 14.0 panicle per hill followed by the copper hydroxide treatment with 13.6 panicle per hill, then the commercial elicitor treatment with 13.4 panicle per hill and the untreated samples with 11.0 panicle per hill. The commercial elicitor treatment delivered the highest value on grains per panicle with 163 grains per panicle followed by SA-Ricemate® with 149 grains per panicle, copper hydroxide with 145 grains per panicle, and untreated samples with 132 grains per panicle. A 1000-grain weight was revealed that the SA-Ricemate® treatment showed highest result with 27.66 g followed by commercial elicitor treatment with 27.59 g, copper hydroxide treatment with 27.00 g, and untreated with 26.43 g. The larger yield was collected in SA-Ricemate® treated samples with 760 kg rai^{-1} (0.4 acre) followed by commercial elicitor samples with 737 kg rai^{-1}, copper hydroxide treated samples with 698 kg rai^{-1}, and untreated samples with 566 kg rai^{-1}.

The foliar applications on paddy field; crop 2 during Aug-Nov 2018 at location 2 of elicitors were stimulated plant parameters and yield for KDML 105 rice as showed in Table 5.10. The data suggested that the SA-Ricemate® treatment provided the plant highest at 45 DPT with 116 cm, followed by copper hydroxide treatment with 115 cm, untreated with 115 cm, and commercial elicitor treatment with 114 cm. At 75 DPT, the commercial elicitor treated samples was highest with 127 cm followed by copper hydroxide treatment with 125 cm, SA-Ricemate® with 123 cm, and untreated with 117 cm. At 105 DPT, copper hydroxide treated samples

had the highest height with 149 cm followed by SA-Ricemate® with 145 cm, commercial elicitor treatment 138 cm, and untreated 128 cm. The highest number of tillers per hill was obtained from rice plant treated with commercial elicitor with 17.8 tillers per hill followed by copper hydroxide treatment with 16.6 tillers per hill, SA-Ricemate® treatment with 16.40 tillers per hill, and untreated with 13.8 tillers per hill. The highest number of panicles per hill was obtained from SA-Ricemate® treated samples with 12.4 panicle per hill followed by commercial elicitor treated samples with 11.0 panicle per hill, copper hydroxide with 10.2 panicle per hill, and untreated samples with 11.0 panicle per hill. The highest number of filled grains per panicle was recorded in commercial elicitor samples with 151 grains per panicle followed by SA-Ricemate® treated samples with 142 grains per panicle, copper hydroxide with 137 grains per panicle, and untreated samples with 116 grains per panicle. The 1000-grain weight test observed the highest measurement in commercial elicitor treated samples with 28.70 g followed by SA-Ricemate® treated samples with 27.38 g, copper hydroxide with 25.38 g, and untreated with 23.75 g. The highest grain yield was recorded from samples treated with SA-Ricemate® with 627 kg rai^{-1} (0.4 acre) followed by commercial elicitor with 607 kg rai^{-1}, copper hydroxide with 501 kg rai^{-1},and untreated with 371 kg rai^{-1}.

Table 5.5 Efficacy of the elicitors to control BLB on rice cv. KDML 105 in the first crop season, during August-November 2017.

Treatment [1/]	Disease severity (%) [2/]				Disease reduction (%) [3/]			
	52 DPT	59 DPT	66 DPT	73 DPT	52 DPT	59 DPT	66 DPT	73 DPT
SA-Ricemate®	3.13 ± 2.08[a]	7.29 ± 1.04[a]	13.54 ± 4.17[a]	20.83 ± 6.34[a]	78.53 ± 2.77	68.19 ± 5.96	51.86 ± 14.83	37.50 ± 13.24
Chitooligosaccharide-commercial	5.21 ± 2.073[a]	5.21 ± 2.08[a]	11.46 ± 2.08[a]	16.67 ± 4.54[a]	64.26 ± 8.33	77.26 ± 5.99	59.26 ± 5.01	49.98 ± 9.10
Copper hydroxide 77%WP	2.08 ± 1.04[a]	3.13 ± 1.08[a]	11.46 ± 1.80[a]	15.63 ± 2.04[a]	85.73 ± 7.34	86.34 ± 8.31	59.26 ± 10.32	53.10 ± 6.95
Untreated (control)	14.58 ± 1.82[b]	22.92 ± 2.76[b]	28.13 ± 2.08[b]	33.33 ± 4.07[b]	-	-	-	-
F-test	*	*	*	*				
CV (%)	43.3	36.26	29.03	35.41				

[1/] Rice plants treated with the elicitors by foliar spaying at 15, 30, and 45 days post transplanting (DPT). [2/] Disease severity was evaluated at 52, 59, 66, and 73 DPT. [3/]The control efficiency of BLB was calculated by comparing with untreated. Each value represents a mean of four replicate. The mean in the column followed by the same letter is not significantly different according to Duncan's multiple range test at P = 0.05.

Table 5.6 Efficacy of the elicitors to control BLB on rice cv. KDML 105 in the second crop season, location1, during August-November 2018.

Treatment [1/]	Disease severity (%) [2/]				Disease reduction (%) [3/]			
	52 DPT	59 DPT	66 DPT	73 DPT	52 DPT	59 DPT	66 DPT	73 DPT
SA-Ricemate®	8.59 ± 2.67[a]	17.97 ± 1.49[a]	18.75 ± 4.59[a]	28.13 ± 3.37[a]	38.88 ± 10.75	44.88 ± 14.74	52.94 ± 14.95	36.8 ± 18.47
Chitooligosaccharide-commercial	7.81 ± 2.01[a]	14.84 ± 2.34[a]	18.75 ± 3.34[a]	22.66 ± 2.34[a]	44.44 ± 17.34	57.77 ± 8.00	52.94 ± 5.51	49.12 ± 7.21
Copper hydroxide 77% WP	7.03 ± 2.70[a]	15.63 ± 4.42[a]	17.19 ± 2.01[a]	27.34 ± 2.26[a]	50.00 ± 16.66	55.55 ± 20.92	56.86 ± 13.62	38.59 ± 7.61
Untreated (control)	14.06 ± 1.96[b]	35.16 ± 6.03[b]	39.84 ± 7.14[b]	44.53 ± 7.25[b]	-	-	-	-
F-test	**	**	*	*				
CV (%)	50.44	38.18	47.33	28.57				

[1/] Rice plants treated with the elicitors by foliar spaying at 15, 30, and 45 days post transplanting (DPT). [2/] Disease severity was evaluated at 52, 59, 66, and 73 DPT. [3/]The control efficiency of BLB was calculated by comparing with untreated. Each value represents a mean of four replicate. The mean in the column followed by the same letter is not significantly different according to Duncan's multiple range test at P = 0.05.

Table 5.7 Efficacy of the elicitors to control BLB on rice cv. KDML 105 in the second crop season, location2, during August-November 2018.

Treatment [1/]	Disease severity (%) [2/]				Disease reduction (%) [3/]			
	52 DPT	**59 DPT**	**66 DPT**	**73 DPT**	**52 DPT**	**59 DPT**	**66 DPT**	**73 DPT**
SA-Ricemate®	7.81 ± 2.71	23.44 ± 2.01[a]	27.34 ± 3.22[a]	30.47 ± 2.95[a]	50.00 ± 17.97	41.17 ± 10.24	41.66 ± 4.54	38.09 ± 5.98
Chitooligosaccharide-commercial	10.16 ± 2.34	21.88 ± 2.85[a]	28.13 ± 2.85[a]	28.91 ± 3.46[a]	35.00 ± 11.02	45.09 ± 9.43	40.00 ± 6.39	41.26 ± 6.96
Copper hydroxide 77% WP	8.59 ± 3.22	21.09 ± 6.67[a]	26.56 ± 2.99[a]	28.13 ± 6.62[a]	45.00 ± 20.23	47.05 ± 18.31	43.33 ± 7.07	42.85 ± 16.51
Untreated (control)	15.63 ± 2.85	39.84 ± 4.83[b]	46.88 ± 2.21[b]	49.22 ± 3.22[b]	-	-	-	-
F-test	ns	*	**	*				
CV (%)	55.11	25.07	22.17	23.64				

[1/] Rice plants treated with the elicitors by foliar spaying at 15, 30, and 45 days post transplanting (DPT). [2/] Disease severity was evaluated at 52, 59, 66, and 73 DPT. [3/]The control efficiency of BLB was calculated by comparing with untreated. Each value represents a mean of four replicate. The mean in the column followed by the same letter is not significantly different according to Duncan's multiple range test at P = 0.05.

Table 5.8 Efficacy of the elicitors to growth parameters and yield component of rice plant cv. KDML 105 in the first crop season, during August-November 2017.

Treatment [1/]	Plant height (cm)			Tiller (per hill)	Panicle (per hill)	Grain (per panicle)	1000 grain weight (g)	Grain yield (kg rai^{-1})	Yield improve (%)
	45 DPT	75 DPT	105 DPT						
SA-Ricemate®	108 ± 1.67^{c}	116 ± 0.73^{b}	133 ± 3.38^{ab}	14.4 ± 0.68^{b}	11.6 ± 0.68^{b}	154 ± 4.62	28.09 ± 0.64^{ab}	570 ± 13.52^{c}	25.55
Chitooligosaccharide-commercial	104 ± 1.90^{c}	116 ± 1.03^{b}	138 ± 0.92^{b}	14.2 ± 0.37^{b}	12.2 ± 0.37^{b}	145 ± 5.30	28.44 ± 0.77^{b}	501 ± 14.35^{b}	10.35
Copper hydroxide 77% WP	90 ± 1.62^{a}	107 ± 3.00^{a}	131 ± 2.42^{ab}	13.8 ± 0.58^{b}	12.8 ± 0.20^{b}	147 ± 7.10	27.30 ± 1.03^{ab}	525 ± 10.13^{b}	15.64
Untreated (control)	96 ± 1.69 ^{b}	110 ± 3.07^{ab}	125 ± 4.83^{a}	11.2 ± 0.66^{a}	10.2 ± 0.37^{a}	138 ± 9.54	25.56 ± 1.03^{a}	454 ± 12.00^{a}	-
F-test	**	**	**	*	*	ns	*	*	
CV (%)	3.87	4.49	5.44	9.8	11.6	10.42	7.22	13.06	

[1/] Rice plants treated with the elicitors by foliar spaying at 15, 30, and 45 days post transplanting (DPT). The mean in the column followed by the same letter is not significantly different according to Duncan's multiple range test at P = 0.05.

Table 5.9 Efficacy of the elicitors to growth parameters and yield component of rice plant cv. KDML 105 in the second crop season at location 1 during August-November 2018.

Treatment [1/]	Plant height (cm)			Tiller (per hill)	Panicle (per hill)	Grain (per panicle)	1000 grain weight (g)	Grain yield (kg rai^{-1})	Yield improve (%)
	45 DPT	75 DPT	105 DPT						
SA-Ricemate®	113 ± 1.02^{b}	120 ± 1.69^{b}	143 ± 2.03^{b}	14.8 ± 0.37^{b}	14.0 ± 0.32^{b}	149 ± 11.78^{ab}	27.66 ± 0.84	760 ± 15.29^{b}	34.27
Chitooligosaccharide-commercial	111 ± 3.67^{ab}	122 ± 1.93^{b}	146 ± 3.00^{b}	16.0 ± 0.83^{b}	13.4 ± 0.68^{b}	163 ± 8.25^{b}	27.59 ± 0.47	737 ± 17.46^{b}	30.21
Copper hydroxide 77%WP	106 ± 2.97^{ab}	117 ± 1.22^{ab}	142 ± 2.60^{b}	14.0 ± 0.63^{b}	13.6 ± 0.51^{b}	145 ± 7.48^{ab}	27.00 ± 0.43	698 ± 7.22^{b}	23.32
Untreated (control)	103 ± 2.76^{a}	113 ± 2.16^{a}	126 ± 2.65^{a}	11.80 ± 0.73^{a}	11.0 ± 1.00^{a}	132 ± 6.60^{a}	26.43 ± 0.68	566 ± 4.87^{a}	-
F-test	*	*	**	**	**	*	ns	*	
CV (%)	5.74	3.38	4.16	10.54	11.6	13.26	5.17	9.94	

[1/] Rice plants treated with the elicitors by foliar spaying at 15, 30, and 45 days post transplanting (DPT). Each value represents a mean of four replicate. The mean in the column followed by the same letter is not significantly different according to Duncan's multiple range test at P = 0.05.

Table 5.10 Efficacy of the elicitors to growth parameters and yield component of rice plant cv. KDML 105 in the second crop season at location 2 during August-November 2018.

Treatment [1/]	Plant height (cm)			Tiller (per hill)	Panicle (per hill)	Grain (per panicle)	1000 grain weight (g)	Grain yield (kg rai^{-1})	Yield improve (%)
	45 DPT	75 DPT	105 DPT						
SA-Ricemate®	116 ± 1.28	123 ± 2.11^{b}	145 ± 2.31^{bc}	16.40 ± 0.75^{b}	12.4 ± 0.74^{b}	142 ± 7.22^{b}	27.38 ± 1.26^{bc}	627±15.47^{b}	69.00
Chitooligosaccharide-commercial	114 ± 0.20	127 ± 3.12^{b}	138 ± 5.23^{ab}	17.80 ± 0.2^{b}	11.0 ± 0.63^{b}	151 ± 7.79^{b}	28.70 ± 0.94^{c}	607±15.59^{b}	63.61
Copper hydroxide 77%WP	115 ± 2.11	125 ± 2.17^{b}	149 ± 2.70^{c}	16.60 ± 0.75^{b}	10.2 ± 0.97^{ab}	137 ± 10.30^{ab}	25.38 ± 0.72^{ab}	501±20.54^{ab}	35.04
Untreated (control)	115 ± 1.88	117 ± 2.42^{a}	128 ± 2.29^{a}	13.80 ± 0.20^{a}	8.4 ± 0.75^{a}	116.2 ± 6.79^{a}	23.75 ± 1.01^{a}	371±9.35^{a}	
F-test	ns	*	*	**	*	*	*	*	
CV (%)	3.01	4.41	5.35	7.58	16.7	13.29	8.52	16.56	

[1/] Rice plants treated with the elicitors by foliar spaying at 15, 30, and 45 days post transplanting (DPT). Each value represents the mean of four replications. The mean in the column followed by the same letter is not significantly different according to Duncan's multiple range test at P = 0.05.

5.4 Discussion

Rice plants treated with SA-Ricemate® by foliar application as it is described in this study showed significant reduction of BLB disease severity >50% in greenhouse conditions and >30% in field trials. Data obtained from multiple experiments such as hydrogen peroxide (H_2O_2), malondialdehyde (MDA), chlorophyll, growth parameters, and yield; stated that the SA-Ricemate® has an important role in bioproductivity defense in which acts as phytohormones in rice plant. The evidence of SA in plant defense response was previously appreciated in several research works. The elicitor is applied by priming on foliage that pushes the host plants to engage a process of protection from pathogen infection by triggering bio-signals that causes systemic acquired resistance (SAR) with the induced resistance (IR) concept (Gao, et al., 2015; Kumar and Almomin, 2018; Tripathi, Raikhy, and Kumar, 2019). The application of SA in other studies reported that the inducer can activate plant immunity by increasing resistance to fungal, bacterial, and viral pathogens in several plants such as tomato, rice, wheat, etc. (Makandar et al., 2012; Hussain, Hamid, and Ghazanfar, 2015; Le et al., 2017; Ueda, et al., 2019). The concentration of SA-Ricemate® determines the effectiveness of the treatment for disease control having demonstrated in greenhouse condition that doses of 100 ppm to 300 ppm are an optimal concentration range for BLB. Therefore, the dose of SA-Ricemate® elicitor is an important metric to stimulate plant defense enzymes. The obtained results are in accordance with Ghazanfar et al. (2019) who found out that 6 mM of SA showed promising anti-fungal activity by inhibiting mycelium growth of postharvest tomato diseases as well as sour rot, pink mold rot, and soft rot. However, doses of 2 and 4 mM were considered not effective for disease control (Ghazanfar et

al., 2019). War et al. (2011) reported that chickpea (*Cicer arietinum* L.) responded to a SA treatment at 1.5 mM with higher induction of plant defense enzymes as a POD, PPO, H_2O_2, and defense proteins activities more than an usage of SA at 1 and 2 mM. These results indicated that SA at 1.5 mM could be utilized for plant defense induction (War et al., 2011).

Hydrogen peroxide (H_2O_2) as a reactive oxygen species is the earliest response from plant-pathogen interactions in plant cells (Bolwell, 1999; Bastas, 2014). Hydrogen peroxide acts as a local signal for hypersensitive response (HR) contributing to a rapid localized cell death (program cell death; PCD) localized in pathogen infected parts, then leads to defense enzymes synthesis activating the defensive genes (Alvarez et al., 1998; Kuźniak and Urbanek, 2000). Moreover, H_2O_2 is also involved in reinforcement processes of plant cell wall, xylem, and cell wall structure (Dempsey and Klessig, 1995; Kuźniak and Urbanek, 2000; Verma and Sharma, 2010). Our results revealed that the accumulation of H_2O_2 significantly increases when using 100 ppm or above of SA-Ricemate® with 39-61% at 24 h post inoculation. This result suggests that the elicitor of SA-Ricemate® triggers an internal signal involved in oxidative bursts required for inducing defense-related genes. Hao et al. (2014) reported that defense induction of *Salvia miltiorrhiza* by SA contributed to enhance the production of H_2O_2. Similarly, Deenamo et al. (2018) stated that treating priming rubber tree seedlings with 5 mM of SA before inoculation of *Phytophthora palmivora* caused a significant increase in H_2O_2 content with 9.44-fold which possibly triggered IR to terminate the invading pathogen (Hao et al., 2014; Deenamo et al., 2018). In addition, Niu and Liao (2016) described that H_2O_2 production not only contributed to stress-triggered cell death, it also is involved in plant development and

abiotic responses (Niu and Liao, 2016).

Malondialdehyde (MDA) is one of the secondary lipid peroxidation products. It has been widely used as a biomarker for cell membrane damage (Corbineau et al., 2002; Gaweł et al., 2004). Numerous researches indicated that hypersensitive response (HR) is related to MDA accumulation. In this study, the observed MDA levels at 24 h post inoculation is higher in 100, 150, 200, 250 and 300 ppm of SA-Ricemate® treatments when compared with untreated. This suggests that superoxide radicals was accumulated in rice plant on SA-Ricemate® treated samples which increase the processes of lipid peroxidation that can potentially destroy cell membranes during the infection process leading to cell death and notable symptoms of hypersensitive response. These results are in agreement with the work presented by Singh and Upadhyay (2014) who also found lipid peroxidation (MDA) in tomato plants treated with fusaric acid to induce the cell death which was significantly increased after 8 h of treatment and peaked at 48 h (Singh and Upadhyay, 2014). Similarly, Zhang et al. (2015) reported that the foliage application of 100 mM of glycerol enhanced disease resistance in cacao by stimulating the levels of pathogenesis-related (*PR*) genes and increased the level of MDA content.

Chlorophyll is the primary pigment used in photosynthesis to convert light energy into chemical energy providing plant growth (Pallardy, 2008). Hence, raising the chlorophyll content will enhance photosynthesis. Salicylic acid is also involved in the regulation of photosynthesis. Several previous studies demonstrated that the application of exogenous SA influenced plant growth and photosynthesis (Pancheva, Popova, and Uzunova, 1996; Khan, Prithiviraj, and Smith, 2003). In an earlier study, Nazar et al. (2015) described the application of SA to plants with and without stress

which developed a significant increase on photosynthetic parameters and chlorophyll contents by synthesis of proline, nitrate reductase activity ATP-sulfurylase, and inhibition of aminocyclopropane carboxylic acid synthase (Nazar et al., 2015). In our results, after rice plants were sprayed with 50, 100, 150, 200, and 250 ppm of SA-Ricemate® elicitor 3 times, the chlorophyll content was significantly increased (chlorophyll a, chlorophyll b, and total chlorophyll) when compared with untreated. These results are in accordance with the work published by Razmi et al. (2017) who found that foliar application of SA on soybean plants at 0.4 mM significantly increased the chlorophyll content with 15% chlorophyll a and 19% chlorophyll b in comparison to the control. The work presented by El-Gamal (2010) reported that the application of 500 μM of SA as seed priming on wheat (*Triticum aestivum*) increased chlorophyll concentration, CO_2 fixation, and phosphoenolpyruvate carboxylase activity regardless of cadmium toxicity (Moussa and El-Gamal, 2010). Therefore, we consider that our greenhouse experiments demonstrated that the elicitor of SA-Ricemate® at 100, 150, 200, and 250 ppm has an effective control on the BLB and the photosynthesis capability.

To further determine the effectiveness of SA-Ricemate® elicitor to control BLB, growth parameters, and yield, three consecutive experiments were carried out on field trials. Salicylic acid has long been recognized as a regulative agent of plant defense responses based on the activation of systemic acquired resistance (SAR) in plants (Métraux, 2013; Furniss and Spoel, 2015; Palmer, Shang, and Fu, 2017). In addition, several studies have supported the fact that SA priming on plant can reduce pathogen attack intensity (Aranega-Bou et al., 2014; Dempsey and Klessig, 2017). In our study, the application of SA-Ricemate® at 100 ppm significantly reduced disease

severity of BLB with 35-86% compared to untreated. This observation was consistent with the findings of Sood et al. (2013) who reported that the application of 50 mg of SA have a positive effect on sheath blight disease by accumulation of defense related enzymes including phenylalanine ammonia lyase, superoxide dismutase, chitinase, β-1,3-glucanase, and phenols in rice leaves cv. Pusa Basmati I (Sood, Sohal, and Lore, 2013). Similarly, Hadi and Balali reported that the use of SA at 0.2 mM reduced the number of potato infected spots caused by *Rizoctonia solani* (Hadi and Balali, 2010). In our study, the application of the salicylic elicitor at 100 ppm significantly increased plant height in comparison with the untreated samples. This observed improvement was not significantly different from the application of a commercial elicitor treatment; chitooligosaccharide. This may be due to the effect of SA-Ricemate® on growth stages of rice. Our result confirms the findings of Sadeghipour (2012) who described a pre-treatment for common bean (*Phaseolus vulgaris*) by soaking with 0.25-0.75 mM of SA leading to increase plant height under water stress (Sadeghipour, 2012). Gorni and Pacheco (2016) also found that 0.5 mM of SA gave a significantly increase of 83.11% on the dry mass of *Achillea millefolium* roots (Gorni and Pacheco, 2016). Similarly, Parashar et al. (2014) reported that 0.01 mM of SA applied on *Brassica juncea* plants increases dry mass of root and shoot with 26% and 51%, respectively (Parashar et al., 2014). It is well known that SA also promotes division and elongation of plant cells (Hayat et al., 2005; Ahmad et al., 2014). The SA also act as a stimulant of plant cells to stabilize plants under environmental stresses such as salted, drought, extreme temperature, and heavy element (Hayat and Ahmad, 2007b; Maruri-López et al., 2019). It is also important in plant physiological that contributes to an increase of nucleic acids and amino acids leading to speed up the accumulation of photosynthetic

pigment and yield increase (Hayat and Ahmad, 2007a; Mousavi et al., 2009; Saleh and Abdulsattar, 2019). Our results on field experimentation demonstrated that SA-Ricemate® can effectively enhance yield component as tillers per hill, panicles per hill, grains per panicle, and 1000-grain weight with an overall crop yield increased with 25-69%. The two main different functions of salicylic acid are plant defense and growth promotion which led to a general increase of rice production (An and Mou, 2011; Hayat et al., 2013). Reddy (1979) described a linear relationship between severity of BLB disease at the stage of plant growth and grain yield. It has been stated that an increase of BLB severity has a reduction effect in terms of grain yield, 1,000 kernel weight, and grain fertility (Reddy, 1979). Similar to Noh et al. (2007), the authors reported the decrease in rice yield and brown head rice rate more than 50 % were observed at 29% disease infected on leaf area (Noh et al., 2007). Moreover, the effects of SA as a plant growth regulator act directly in the metabolic pathways as response to abiotic stress including salinity, UV-B radiation, extreme temperatures, and drought via SA-mediated (Khan et al., 2015). Under salt stress, The expression of CtPAL (phenylalanine ammonia-lyase) and CtCHS (chalcone synthase) enzymes related with the applied SA, CtPAL and CtCHS were higher for both salt stress tolerance and pathogen resistance (Dehghan et al., 2014). Similar to Palma et al., (2013) reported that pretreatment with 0.1 and 0.5mM SA were contributed to against salt stress in root nodules of *Medicago sativa* resulted in the plant growth and photosynthetic capacity (Palma et al., 2013). This process may be caused by SA signaling to other hormones such as jasmonic acid, ethylene, and auxin which have influence in plant growth and development under stress condition (Hayat et al., 2013; Li, 2017). The previous observation is in agreement with the work presented by

Kareem et al. (2019) which reported that the exogenous application of SA at 1.44 mM has the ability to promote shoot growth, final wheat biomass, and yield components (spike drying weight, grain dry weight, and 1000 grain dry weight) (Kareem, Rihan, and Fuller, 2019). It is also suggested that a pre-treatment based on SA-Ricemate® (salicylic acid) can increase the productivity of rice by improving tolerance to biotic and abiotic stress.

5.5 References

Alvarez, M. a. E., Pennell, R. I., Meijer, P.-J., Ishikawa, A., Dixon, R. A., and Lamb, C. (1998). Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. **Cell.** 92 (6): 773-784.

An, C., and Mou, Z. (2011). Salicylic acid and its function in plant immunityf. **J. Integr. Plant Biol.** 53 (6): 412-428.

Anuratha, C., Cole, K., Mew, T., and Muthukrishnan, S. (2006). Induction of chitinases and β-l,3-glucanases in rhizoctonia solani-infected rice plants: Isolation of an infection-related chitinase cdna clone. **Physiol. Plantarum.** 97: 39-46.

Aminot, A., and Rey, F. (2002). Standard procedure for the determination of chlorophyll a by spectroscopic methods. **Techniques Mar. Environ. Sci.** 28.

Aranega-Bou, P., de la O Leyva, M., Finiti, I., García-Agustín, P., and González-Bosch, C. (2014). Priming of plant resistance by natural compounds. Hexanoic acid as a model. **Front. Plant Sci.** 5: 488-488.

Bacete, L., Mélida, H., Miedes, E. and Molina, A. (2018). Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses. **Plant J.** 93:

614-636.

Bastas, K. K. (2014). Importance of reactive oxygen species in plant-pathogen interactions. **Selcuk Jour. Agri. Food Sci.** 28: 11-26.

Bolwell, G. P. (1999). Role of active oxygen species and no in plant defence responses. **Curr. Opin. Plant Biol.** 2 (4): 287-294.

Buensanteai, N., Yuen, G., and Prathuangwong, S. (2008). The biocontrol bacterium bacillus amyloliquefaciens kps46 produces auxin, surfactin and extracellular proteins for enhanced growth of soybean plant. **Thai J. Agric. Sci.** 41(3-4): 101-116.

Chakraborty, N., Ghosh, S., Chandra, S., Sengupta, S., and Acharya, K. (2016). Abiotic elicitors mediated elicitation of innate immunity in tomato: An ex vivo comparison. **Physiol Mol Biol Plants.** 22 (3): 307-320.

Chiawpanit, C., Arayaskul, N., and Lithanatudom, P. (2018). phenotypic classification of bacterial isolates derived from lesion of the bacterial leaf blight of rice cultivated in Chiang Mai. **Proc. MST International Conference, 30th January – 2nd February 2018, Chiang Mai, Thailand.**

Chithrashree, Udayashankar, A. C., Chandra Nayaka, S., Reddy, M. S., and Srinivas, C. (2011). Plant growth-promoting rhizobacteria mediate induced systemic resistance in rice against bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*. **Biol. Control.** 59 (2): 114-122.

Corbineau, F., Gay-Mathieu, C., Vinel, D., and Côme, D. (2002). Decrease in sunflower (helianthus annuus) seed viability caused by high temperature as related to energy metabolism, membrane damage and lipid composition. **Physiol. Plant.** 116 (4): 489-496.

Deenamo, N., Kuyyogsuy, A., Khompatara, K., Chanwun, T., Ekchaweng, K., and Churngchow, N. (2018). Salicylic acid induces resistance in rubber tree against *Phytophthora palmivora*. **Int. J. Mol. Sci.** 19 (7): 1883.

Dehghan, S., Sadeghi, M., Pöppel, A., Fischer, R., Lakes-Harlan, R., Kavousi, H. R., . . . Rahnamaeian, M. (2014). Differential inductions of phenylalanine ammonia-lyase and chalcone synthase during wounding, salicylic acid treatment, and salinity stress in safflower, carthamus tinctorius. **Biosci. Rep.** 34 (3).

Dempsey, D. M. A., and Klessig, D. F. (1995). Signals in plant disease resistance. **Bulletin de l'Institut Pasteur.** 93 (3): 167-186.

Dempsey, D. M. A., and Klessig, D. F. (2017). How does the multifaceted plant hormone salicylic acid combat disease in plants and are similar mechanisms utilized in humans? **BMC Biol.** 15 (1): 23.

Department of Agriculture. (2009). **The statistical techniques for standard of agricultural research** [On-line]. Available: http://www.doa.go.th/research /attachment.php?aid=2160.

Draper, H. H., and Hadley, M. (1990). Malondialdehyde determination as index of lipid peroxidation. **Meth. Enzymol.** 186: 421-431.

Fauth M, Merten A, Hahn MG, Jeblick W, Kauss H. (1996). Competence for elicitation of H_2O_2 in hypocotyls of cucumber is induced by breaching the cuticle and is enhanced by salicylic acid. **Plant Physiol**. 110 (2): 347–354.

Ferreira, D., Falahati, F., Linden, C., Buckley, R. F., Ellis, K. A., Savage, G., . . . Westman, E. (2017). A ‘disease severity index’ to identify individuals with subjective memory decline who will progress to mild cognitive impairment or

dementia. **Sci. Rep.** 7: 44368.

Food and Agriculture Organization of the United Nations. (2014). **Crop cutting experiments in Thailand** [On-line]. Available: http://www.fao.org/asiapacific/events/detail-events/en/c/957/

Food and Agriculture Organization of the United Nations. (2015). **Total production for rice in Thailand** [On-line]. Available: http://www.fao.org/statistics/en/

Furniss, J. J., and Spoel, S. H. (2015). Cullin-ring ubiquitin ligases in salicylic acid-mediated plant immune signaling. **Front. Plant Sci.** 6: 154.

Gao, Q.-M., Zhu, S., Kachroo, P., and Kachroo, A. (2015). Signal regulators of systemic acquired resistance. **Front. Plant Sci.** 6 (228).

Gaweł, S., Wardas, M., Niedworok, E., and Wardas, P. (2004). Malondialdehyde (MDA) as a lipid peroxidation marker. **Wiad Lek.** 57 (9-10): 453-455.

Ghazanfar, M., Raza, W., Iqbal, Z., Ahmad, S., and Qamar, M. (2019). Antifungal activity by resistance inducing salicylic acid and plant extracts against postharvest rots of tomato. **Int. J. Biosci.** 14: 264-274.

Gorni, P., and Pacheco, A. (2016). Growth promotion and elicitor activity of salicylic acid in *Achillea millefolium* L. **Afr. J. Biotechnol.** 15: 657-665.

Hadi, M. R., and Balali, G. (2010). The effect of salicylic acid on the reduction of *Rizoctonia solani* damage in potato. **American-Eurasian J. Agric. & Environ. Sci.** 7: 492-496.

Hao, W., Guo, H., Zhang, J., Hu, G., Yao, Y., and Dong, J. (2014). Hydrogen peroxide is involved in salicylic acid-elicited rosmarinic acid production in *Salvia miltiorrhiza* cell cultures. **Sci. World J.** 2014: 843764.

Hayat, S., and Ahmad, A. (2007a). **Salicylic acid: A plant hormone.** Netherlands:

Springer.

Hayat, S., and Ahmad, A. (2007b). **Salicylic acid: A plant hormone.** Netherlands: Springer.

Hayat, S., Khan, N. A., and Alyemeni, M. (2013). **Salicylic acid: Plant growth and development.** Netherlands: Springer.

Hussain, M., Hamid, M. I., and Ghazanfar, M. U. (2015). Salicylic acid induced resistance in fruits to combat against postharvest pathogens: A review. **Arch. Phytopathol. Prot.** 48 (1): 34-42.

Kareem, F., Rihan, H., and Fuller, M. P. (2019). The effect of exogenous applications of salicylic acid on drought tolerance and up-regulation of the drought response regulon of iraqi wheat. **J. Crop Sci. Biotechnol.** 22 (1): 37-45.

Kauss H, and Jeblick W. (1995). Pretreatment of parsley suspension cultures with salicylic acid enhances spontaneous and elicited production of H_2O_2. **Plant Physiol**. 108 (3): 1171–1178.

Kawasaki, J. (2018). **Thailand's Rice Farmers Adapt to Climate Change** [On-line]. Available: https://ourworld.unu.edu/en/climate-change-adaptation-Thailand

Kawasaki, J., and Herath, S. (2018). Impact assessment of climate change on rice production in khon kaen province, Thailand. **J. ISSAAS.** 17 (2) :14-28.

International Rice Research Institute; IRRI. (2002). **Standard evaluation system for rice**. (2002). [On-line]. Available: http://www.knowledgebank.irri.org/ /rice-standard-evaluation-system.pdf

Ke, Y., Hui, S., and Yuan, M. (2017). *Xanthomonas oryzae* pv. *oryzae* inoculation and growth rate on rice by leaf clipping method. **Bio-protocol.** 7 (19): e2568.

Keeratipipatpong, W. (2010). **Disease threat to rice crop.** [On-line]. Available:

https://www.bangkokpost.com/learning/learning-news/208868/disease-threat-to-rice-crop.

Khan, M. I. R., Fatma, M., Per, T. S., Anjum, N. A., and Khan, N. A. (2015). Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. **Front. Plant Sci.** 6: 462-462.

Khan, W., Prithiviraj, B., and Smith, D. L. (2003). Photosynthetic responses of corn and soybean to foliar application of salicylates. **J. Plant Physiol.** 160 (5): 485-492.

Krishnan, N., Gandhi, K., Peeran, M. F., Muthurajan, R., Kuppusamy, P., and Thiruvengadam, R. (2014). Management of bacterial leaf blight disease in rice with endophytic bacteria. **World Appl. Sci. J.** 28(12): 2229-2241.

Kumar, V., and Almomin, S. (2018). Plant defense against pathogens: The role of salicylic acid. **Res. J. Biotechnol.** 13: 97-103.

Kukawka, R., Czerwoniec, P., Lewandowski, P., Pospieszny, H., and Smiglak, M. (2018). New ionic liquids based on systemic acquired resistance inducers combined with the phytotoxicity reducing cholinium cation. **New J. Chem.** 42.

Kuźniak, E., and Urbanek, H. (2000). The involvement of hydrogen peroxide in plant responses to stresses. **Acta Physiol. Plant.** 22 (2): 195-203.

Land Development Department, Ministry of Agriculture and Cooperatives. (2017). **LDD Soil Guide** [On-line]. Available: https://www.ldd.go.th/

Le Thanh, T., Thumanu, K., Wongkaew, S., Boonkerd, N., Teaumroong, N., Phansak, P., and Buensanteai, N. (2017). Salicylic acid-induced accumulation of biochemical components associated with resistance against *Xanthomonas oryzae* pv. *oryzae* in rice. **J. Plant Interact.** 12 (1): 108-120.

Leon J, Lawton MA, Raskin I. (1995). Hydrogen peroxide stimulates salicylic acid biosynthesis in tobacco. **Plant Physiol.** 108 (4): 1673–1678.

Li, C. (2017). **Hormone metabolism and signaling in plants.** San Diego: Academic Press.

Liyanage, S., Dassanayake, R. S., Bouyanfif, A., Rajakaruna, E., Ramalingam, L., Moustaid-Moussa, N., and Abidi, N. (2017). Optimization and validation of cryostat temperature conditions for trans-reflectance mode FTIR microspectroscopic imaging of biological tissues. **MethodsX.** 4: 118-127.

Makandar, R., Nalam, V. J., Lee, H., Trick, H. N., Dong, Y., and Shah, J. (2012). Salicylic acid regulates basal resistance to fusarium head blight in wheat. **MPMI.** 25 (3): 431-439.

Malerba, M., & Cerana, R. (2016). Chitosan effects on plant systems. **Int. J. Mol. Sci.** 17 (7): 996.

Maruri-López, I., Aviles-Baltazar, N. Y., Buchala, A., and Serrano, M. (2019). Intra and extracellular journey of the phytohormone salicylic acid. **Front. Plant Sci.** 10 (423).

Métraux, J. P. (2013). Systemic acquired resistance. In S. Maloy and K. Hughes (Eds.), **Brenner's encyclopedia of genetics** (2 nd., pp. 627-629). San Diego: Academic Press.

Nomura, H., Komori, T., Uemura, S. et al. (2012). Chloroplast-mediated activation of plant immune signaling in *Arabidopsis*. **Nat. Commun.** 3: 926.

Mousavi, K., Seyed, M., Khara, J., and Heidari, R. (2009). Effect of salicylic acid on photosynthetic pigment content in ocimum bacilicum under UVC radiation stress. **J. Cell Mol. Res.** 1: 57-60.

Moussa, H., and El-Gamal, S. (2010). Effect of salicylic acid pretreatment on cadmium toxicity in wheat. **Biol. Plant.** 54: 315-320.

Nazar, R., Umar, S., Khan, N. A., and Sareer, O. (2015). Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline accumulation and ethylene formation under drought stress. **S. Afr. J. Bot.** 98: 84-94.

Niu, L., and Liao, W. (2016). Hydrogen peroxide signaling in plant development and abiotic responses: Crosstalk with nitric oxide and calcium. **Front. Plant Sci.** 7 (230).

Noh, T.-H., Lee, D.-K., Park, J.-C., Shim, H.-K., Choi, M.-Y., Kang, M.-H., and Kim, J.-D. (2007). Effects of bacterial leaf blight occurrence on rice yield and grain quality in different rice growth stage. **Res. Plant Dis.** 13: 20-23.

Office of Agricultural Economics. (2003). **Crop cutting survey** [On-line]. Available: http://www3.oae.go.th/ crop-cutting-rice56-57.pdf.

Pallardy, S. G. (2008). Photosynthesis. In S. G. Pallardy (Ed.), **Physiology of woody plants** (3th, pp. 107-167). San Diego: Academic Press.

Palma, F., López-Gómez, M., Tejera, N. A., and Lluch, C. (2013). Salicylic acid improves the salinity tolerance of medicago sativa in symbiosis with sinorhizobium meliloti by preventing nitrogen fixation inhibition. **Plant Sci.** 208: 75-82.

Palmer, I. A., Shang, Z., and Fu, Z. Q. (2017). Salicylic acid-mediated plant defense: Recent developments, missing links, and future outlook. **Front. Biolo.** 12 (4): 258-270.

Pancheva, T. V., Popova, L. P., and Uzunova, A. N. (1996). Effects of salicylic acid

on growth and photosynthesis in barley plants. **J. Plant Physiol.** 149 (1): 57-63.

Parashar, A., Yusuf, M., Fariduddin, Q., and Ahmad, A. (2014). Salicylic acid enhances antioxidant system in *Brassica juncea* grown under different levels of manganese. **Int. J. Biol. Macromol.** 70: 551-558.

Reddy, A. P. K. (1979). Relationship of bacterial leaf blight severity to grain yield of rice. **Phytopathology.** 69: 967-969.

Regalado, M. J., and Ramos, P. (2018). Field testing of a rice crop postharvest management protocol for reduced postproduction losses and improved product quality. **Rice-Based Biosystems J.** 4: 31.

Rivas-San Vicente, M., and Plasencia, J. (2011). Salicylic acid beyond defence: Its role in plant growth and development. **J. Exp. Bot.** 62 (10): 3321-3338.

Sadeghipour, O. (2012). Impact of exogenous salicylic acid application on some traits of common bean (*Phaseolus vulgaris* L.) under water stress conditions. **Int. J. Agric.** 4: 685-690.

Sahebani, N., and Hadavi, N. (2009). Induction of H_2O_2 and related enzymes in tomato roots infected with root knot nematode (*M. Javanica*) by several chemical and microbial elicitors. **Biocontrol Sci. Technol.** 19 (3): 301-313.

Saleh, A., and Abdulsattar, I. (2019). Review on the role of salicylic acid in plants. **Physiol. Mol. Plant Pathol.** 59 (5): 223-233.

Sekhar, C.S.C. (2018). **Climate change and rice economy in Asia: Implications for trade policy.** Rome, FAO. 62 pp.

Singh, A., Dharmraj, E., Nayak, R., Singh, P. K., and Singh, N. (2015). Identification of bacterial leaf blight resistance genes in wild rice of eastern india. **Turk J**

Bot. 39: 1060-1066.

Singh, V. K., and Upadhyay, R. S. (2014). Fusaric acid induced cell death and changes in oxidative metabolism of *Solanum lycopersicum* L. **Bot. Stud.** 55 (1): 66.

Son T.M. (1993) Breeding rice cultivars resistant to bacterial leaf blight (*Xanthomonas oryzae*) in Vietnam. Ccurrent plant science and biotechnology in agriculture. Dordrecht: Springer.

Sood, N., Sohal, B. S., and Lore, J. S. (2013). Foliar application of benzothiadiazole and salicylic acid to combat sheath blight disease of rice. **Rice Sci.** 20 (5): 349-355.

Thakur, M., and Sohal, B. S. (2013). Role of elicitors in inducing resistance in plants against pathogen infection: A review. **ISRN Biochem.** 2013: 762412-762412.

Timsina, J., Wolf, J., Guilpart, N., van Bussel, L. G. J., Grassini, P., van Wart, J., . . . Ittersum, M. K. (2018). Can bangladesh produce enough cereals to meet future demand? **Agric. Syst.** 163: 36-44.

Tripathi, D., Raikhy, G., and Kumar, D. (2019). Chemical elicitors of systemic acquired resistance salicylic acid and its functional analogs. **Curr. Plant Biol.** 17: 48-59.

Trouvelot, S., Héloir, M.-C., Poinssot, B., Gauthier, A., Paris, F., Guillier, C., . . . Adrian, M. (2014). Carbohydrates in plant immunity and plant protection: Roles and potential application as foliar sprays. **Front. Plant Sci.** 5: 592-592.

Ueda, H., Kugimiya, S., Tabata, J., Kitamoto, H., and Mitsuhara, I. (2019). Accumulation of salicylic acid in tomato plant under biological stress affects oviposition preference of *Bemisia tabaci*. **J. Plant Interact.** 14 (1): 73-78.

Verma, G., and Sharma, S. (2010). Role of H_2O_2 and cell wall monoamine oxidases in germination of *Vigna radiata* seeds. **Indian J. Biochem. Biophys.** 47 (4): 249-253.

Walters, D., Newton, A. C., and Lyon, G. (2008). **Induced resistance for plant defence: A sustainable approach to crop protection.** New Jersey: John Wiley & Sons.

War, A. R., Paulraj, M. G., War, M. Y., and Ignacimuthu, S. (2011). Role of salicylic acid in induction of plant defense system in chickpea (*Cicer arietinum* l.). **Plant Signal Behav.** 6 (11): 1787-1792.

Xu, J., Audenaert, K., Hofte, M., and De Vleesschauwer, D. (2013). Abscisic acid promotes susceptibility to the rice leaf blight pathogen *Xanthomonas oryzae* pv. *oryzae* by suppressing salicylic acid-mediated defenses. **PLoS One.** 8 (6): e67413.

Zhou, M., and Wang, W. (2018). Recent advances in synthetic chemical inducers of plant immunity. **Front. Plant Sci.** 9 (1613).

Zoeller, M., Stingl, N., Krischke, M., Fekete, A., Waller, F., Berger, S., & Mueller, M. J. (2012). Lipid profiling of the *Arabidopsis* hypersensitive response reveals specific lipid peroxidation and fragmentation processes: biogenesis of pimelic and azelaic acid. **Plant physiol.** 160 (1): 365–378.

CHAPTER VI

OVERALL DISCUSSION AND CONCLUSION

6.1 Overall discussion

Efficacy of salicylic acid elicitor to induce systemic resistance against bacterial leaf blight on rice, there are two main objectives in this study: (1) to evaluate the role of abiotic elicitor SA-Ricemate® formula based on salicylic acid (SA) for inducing systemic resistance against bacterial leaf blight of rice under greenhouse and field conditions; (2) to examine the biology of *Xoo* during pathogenesis under SA-Ricemate® elicitor treated conditions. The concentration of SA-Ricemate® determined the efficiency of the treatment on controlling the diseases stating that 100 ppm is an optimal concentration for controlling BLB. The use of lower concentrations than 100 ppm is considered non-effective against rice BLB. The process of inducing resistance (IR) provides protection in distant parts of rice plants via signaling transduction pathway. SA signaling pathway can be triggered by exogenous SA which increases disease resistance. This pathway is related to systemic acquired resistance (SAR) that can occur by the accumulation of endogenous SA which is activated after plant pathogen infection. The results showed that the accumulation of endogenous SA content in treated rice plants after infection with *Xoo* was higher than the non-treated by approximately 50%. An elevated concentration of endogenous SA was similarly observed in tomato and citrus plants after a treatment with the elicitor ASM 1 mM for

Tomato Virus and Citrus Viroid (Lopez-Gresa et al., 2016).The H_2O_2 accumulation was significantly increased at 24 h post inoculation when using 100 ppm or more ofSA-Ricemate® by 39-61%. Hydrogen peroxide acts as a local signal for hypersensitive response (HR) which contributes to a rapid localized cell death (program cell death; PCD) that occurs at the pathogen infected spots leading to defense signaling, enzymes synthesis, and activation (Alvarez et al., 1998; Kuźniak and Urbanek, 2000).

Malondialdehyde (MDA) is one of the secondary lipid peroxidation products that has been widely used as a biomarker to determine the damage degree of cell membrane (Corbineau et al., 2002; Gaweł et al., 2004). Numerous researches indicated that hypersensitive response (HR) is related to MDA accumulation. In this study, MDA level at 24 h post inoculation showed higher values when using ≥100 ppm of SA-Ricemate® when compared with untreated.

Furthermore, raising the chlorophyll contents can enhance plant photosynthesis systems. Several previous studies demonstrated that the application of exogenous SA has an effect on plant growth and photosynthesis (Pancheva, Popova, and Uzunova, 1996; Khan, Prithiviraj, and Smith, 2003). In our results, after rice plants were sprayed with 50, 100, 150, 200, and 250 ppm of SA-Ricemate® 3 times, the total chlorophyll content was significantly increased when compared with untreated. These results are in accordance with Razmi et al. (2017) who found that foliar application on soybean plants of 0.4 mM SA significantly increased the chlorophyll content with 15% of chlorophyll *a* and 19% of chlorophyll *b* compared to control.

The association of the biomolecular structures and their intensity from the

average spectra suggested higher accumulations of lipids and proteins. Lipids are the major part of a plant cell membrane and plays different roles in the cellular system such as energy storage, protection, communication, structural support, and also hydrocarbon as a monomer providing a prevention of water loss, protect host cells and nutrients of plant and coat plant leaf surface to against pathogen attract. Zhang et al. (2015) reported that phospholipids phosphatidic acid (PA) belongs to the membrane lipid bilayer which acts as signaling immunity and is related to ROS activity and SA accumulation. Similarly, Gao et al. (2017) reported that lipids and lipid metabolites are important in rice plants in order to fight against rice bacteria blight and rice blast (Zhang and Xiao, 2015; Gao et al., 2017). Moreover, resistance defense enzymes including beta-glucanases and chitinases are part of the pathogenesis-related (*PR*) proteins that have important roles in plant cells protecting them from pathogen infections (Buensanteai et al., 2012; Thakur and Sohal, 2013; Thumanu et al., 2015; Thumanu et al., 2017). When plants detect an attack by insects, fungi, bacteria, or virus-viroid; Beta-1,3-glucanase or chitinase activates plant defenses against pathogens infections as reported by Anita et al. (2014) who state that systemic induced resistance in rice against rice root knot can occur by increasing chitinase enzyme activity (Leubner-Metzger and Meins, 2000; Wu et al., 2001; Anita and Samiyappan, 2012). Amide I and amide II associated with secondary proteins are important amino acids which are involved in disease resistance. L-phenylalanine is a precursor that contributes to plant defense metabolites by phenylpropanoid and lignin pathway. Furthermore, polysaccharide as carbohydrates or sugars groups are necessary in order to supply the energy to defenses and can be used as regulation signals for defense genes that can be helpful to controlling plant diseases (Bolton,

2009; Buensateai et al., 2012; Trouvelot et al., 2014). During infection, the plants modify or change their sugar source and activate their defense responses as well as increase PR proteins to combat pathogens (Tauzin and Giardina, 2014; Zhao et al., 2018).

MIC experiments performed in this work demonstrated potent antibacterial capabilities of SA-Ricemate® against *Xoo* working as a strong inhibitory agent when used at high concentrations ≥200 ppm. The results presented in this work are in agreement with the work presented by Price et al. (2000). The authors reported that salicylic acid has the ability to inhibit bacterial growth due to having related compounds to aspirin which cause effects in the eukaryotic system. In addition, bacterial persistence on host surface is considered as a virulence strategy and an important factor during the stages of pathogenic infection (Pfeilmeier, Caly, and Malone, 2016).

Biofilm formation is considered as a virulence factor of growth on microbial as a key for the pathogen to survive in the environment. Therefore, biofilm formation reduction can be seen as a potential strategy to control the plant disease. In our study it is observed that SA-Ricemate® can reduce biofilm formation between 13% to 100% when comparing with untreated samples.

The extracellular polysaccharides (EPS) were examined reporting that initial functional and structural coherence is involved in biofilm formation. As the results showed, the SA-Ricemate® inhibited the production of EPS at approximately 18% to 100% when comparing with untreated samples. Such results confirmed that SA-Ricemate® can simultaneously reduce the virulence factor in terms of biofilm formation and EPS.

The lack of motility observed in SA-Ricemate® treated samples revealed that the motility area of untreated samples was wider than the area observed in the treated samples and, on the same line, the twitching maps showed low motility on treated samples. These results are in agreement with the work of Kunin et al. (1995). The authors explained the salicylate effect as the expression of flagella of *Escherichia coli* and *Pseudomonas cepacian*.

Moreover, the results from the SR-FTIR spectra obtained for bacterial cells showed that the main components of *Xoo* cells were different in the spectra of bacterial ester functional groups of lipids and carbohydrates. These results provide evidence that the fatty acid (3000-2800 cm^{-1}), nucleic acid and phospholipid (1300-900 cm^{-1}), and the cell wall C-O-C and C-O as carbohydrates (1200-1000 cm^{-1}) shifted to lower lectures than the untreated samples used as control. This observation suggests that the SA-Ricemate® caused injuries to bacterial cells observed as the peak of spectra representing bacterial cell membrane and phospholipid polysaccharides (Kamnev, 2008; Kochan et al., 2018).

To further determine the effectiveness of SA-Ricemate® elicitor to control BLB and yield improvement, disease severity and growth parameters were analyzed in two consecutive experiments carried out on field trials at Khon Kaen province, Thailand. In our study, the application of the SA-Ricemate® at 100 ppm significantly reduced disease severity of BLB with a control effectiveness of 35% to 86% compared to untreated. This observation was consistent with the findings of Sood et al. (2013) who reported that the application of 50 mg of SA have a positive effect on sheath blight disease by accumulation of defense related enzymes including phenylalanine ammonia lyase, superoxide dismutase, chitinase, β-1,3-glucanase, and

phenols in rice leaves cv. Pusa Basmati I (Sood, Sohal, and Lore, 2013). Similarly, Hadi and Balali (2010) reported that the use of SA at 0.2 mM reduced the number of potato infected spots caused by *Rizoctonia solani* (Hadi and Balali, 2010). Moreover, the application of the SA-Ricemate® elicitor at 100 ppm significantly increased plant height in comparison with the untreated samples. This observed improvement was not significantly different from the samples treated with a commercial elicitor based on chitooligosaccharide. This may be due to the SA-Ricemate® effect on growth stages of rice which acts as a plant hormone. These results are in accordance with the findings of Sadeghipour (2012) who described a pre-treatment for common bean (*Phaseolus vulgaris*) by soaking with 0.25-0.75 mM of SA leading to an increase of plant height under water stress (Sadeghipour, 2012). Gorni and Pacheco (2016) also found that 0.5 mM of SA gave a significant increase of 83.11% on the dry mass of *Achillea millefolium* (*Asteraceae*) roots (Gorni and Pacheco, 2016). Similarly, Parashar et al. (2014) reported that 0.01 mM of SA applied on *Brassica juncea* plants increases dry mass of root and shoot by 26% and 51%, respectively (Parashar et al., 2014). It is well known that SA also promotes division and elongation of plant cells (Hayat et al., 2005; Ahmad et al., 2014). The SA also acts as a stimulant of plant cells to stabilize plants under environmental stresses such as drought, extreme temperature, and heavy element (Hayat and Ahmad, 2007b; Maruri-López et al., 2019). It is also important in plant physiology that theses treatments contribute to an increase of nucleic acids and amino acids speeding up the accumulation of photosynthetic pigment which led to an increase of yield (Hayat and Ahmad, 2007a; Mousavi et al., 2009; Saleh and Abdulsattar, 2019). The results on field experimentation demonstrated that SA-Ricemate® can effectively enhance yield in tillers per hill,

panicles per hill, grains per panicle, and 1000-grain weight with an overall crop yield improved by 25-69%.

As previously mentioned, the two main functions of salicylic acid are plant defense and growth promotion which lead to a general increase of rice production (An and Mou, 2011; Hayat et al., 2013). Reddy (1979) described a linear relationship between severity of BLB disease at the stage of plant growth and grain yield. It has been stated that an increase of BLB severity has a reduction effect in terms of grain yield, 1,000-grain weight, and fertility (Reddy, 1979). Similar to Noh et al. (2007), the authors reported the decrease in rice yield and brown head rice rate more than 50 % were observed at 29% disease infected on leaf area (Noh et al., 2007). Moreover, the effects of SA as a plant growth regulator act directly in the metabolic pathways as response to abiotic stress. This process may be caused by SA signaling to other hormones such as jasmonic acid, ethylene, and auxin which influence plant growth and development (Hayat et al., 2013; Li, 2017). These results are in agreement with the work presented by Kareem et al. (2019) which reported that the exogenous application of SA at 1.44 mM has the ability to promote shoot growth, final wheat biomass, and yield components (spike drying weight, grain dry weight, and 1000-grain dry weight) (Kareem, Rihan, and Fuller, 2019). It is also suggested that a pre-treatment based on SA-Ricemate® can improve the productivity of rice by improving tolerance to biotic and abiotic stress.

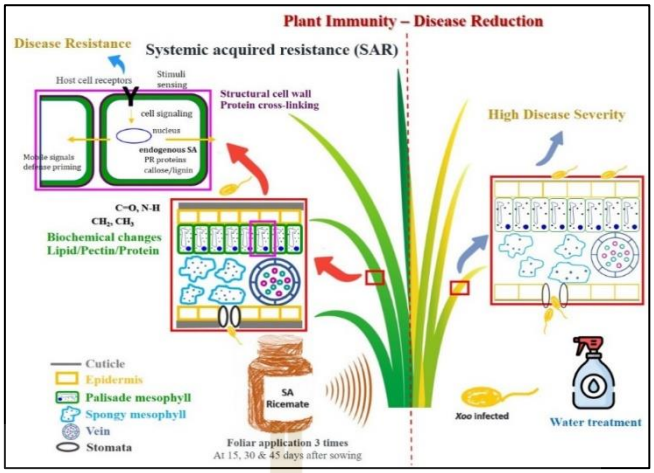


Figure 6.1 Model depicting the mechanism of induced resistance in rice plant against *Xanthomonas oryzae* after being treated with SA-Ricemate® elicitor. Briefly, the molecule of SA-Ricemate® would be attached by a plant receptor changing the receptor conformation which results in activating kinases and ion channels. Activated effectors continue to transfer the signals of SA molecules to secondary messengers which can amplify the signals and send them to other reactions. Following pathogen recognition and signal transduction, a series of defense reactions are triggered such as the activation of signal transduction pathways, ion fluxes increase, ROS, phosphorylation, phytoalexin, and secondary metabolite pathway activation. This inducer can create long distance signals and stimulate defense genes. After the activation, treated rice plants can quickly recognize this infection of *Xoo* leading to stimulation of HR and cell death which isolate the pathogen infection. The SA-Ricemate® treated rice plant can protect cell walls producing more phytoalexins and systemic signals which stimulate defense genes to synthesize many kinds of defense products to help the plant to stop invasion of *Xoo*.

6.2 Conclusion

The results of this work demonstrated the applicability of SA-Ricemate® as a safe, effective, and environmental friendly elicitor to control the BLB of rice by its ability to perform antibacterial activities against *Xoo* caused by bacterial leaf blight of rice with direct inhibitory and induced resistance mechanisms in plant defense. The experiments confirmed that SA-Ricemate® was effective in reducing the disease severity by inducing plant defense including endogenous salicylic acid, H_2O_2, MDA, and protein and lipid compounds. Furthermore, SA-Ricemate® can inhibit the virulence factors of *Xoo* such as biofilm formation, extracellular polysaccharides, and bacterial motility which contribute on suppressing communication abilities resulting in a weaker pathogenicity.

6.3 Suggestion

Although the study has significantly advanced the mechanistic understanding of induced resistance against BLB in rice, several aspects still deserve further experimental investigation. Firstly, more studies are needed to fully disentangle the mechanism of induced resistance of SA-Ricemate® induced BLB resistance in rice. Second, the effectiveness of SA-Ricemate® inducer on induced resistance to control BLB in another rice cultivar should be studied for commercial brand registered. In addition, the SA-Ricemate® inducer effects on natural microorganisms and bioproducts should be addressed for crop production.

6.4 Application

The recommended application of the SA-Ricemate® elicitor is performed by spraying to rice foliage application for 20 L rai^{-1}; at the concentration of 100 ppm, three times at 15, 30, and 45 days post transplanting.

6.5 References

Alvarez, M. a. E., Pennell, R. I., Meijer, P.-J., Ishikawa, A., Dixon, R. A., and Lamb, C. (1998). Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. **Cell.** 92 (6): 773-784.

An, C., and Mou, Z. (2011). Salicylic acid and its function in plant immunity. **J. Integrat. Plant Biol.** 53 (6): 412-428.

Anita, B., and Samiyappan, R. (2012). Induction of systemic resistance in rice by pseudomonas fluorescens against rice root knot nematode meloidogyne graminicola. **J. Biopest.** 5: 53-59.

Bolton, M. D. (2009). Primary metabolism and plant defense—fuel for the fire. **Mol. Plant-Microb. Interact.** 22 (5): 487-497.

Buensanteai, N., Thumanu, K., Sompong, M., Athinuwat, D., and Prathuangwong, S. (2012). The FTIR spectroscopy investigation of the cellular components of cassava after sensitization with plant growth promoting rhizobacteria, *Bacillus subtilis* CaSut007. **Afr. J. Microbiol. Res.** 6(3):603-610.

Corbineau, F., Gay-Mathieu, C., Vinel, D., and Côme, D. (2002). Decrease in sunflower (*Helianthus annuus*) seed viability caused by high temperature as related to energy metabolism, membrane damage and lipid composition. **Physiol. Plantarum.** 116 (4): 489-496.

Gao, M., Yin, X., Yang, W., Lam, S. M., Tong, X., Liu, J., . . . He, Z. (2017). Gdsl lipases modulate immunity through lipid homeostasis in rice. **PLoS Pathog.** 13 (11): e1006724-e1006724.

Gaweł, S., Wardas, M., Niedworok, E., and Wardas, P. (2004). Malondialdehyde (MDA) as a lipid peroxidation marker. **Wiadomosci lekarskie.** 57 (9-10): 453-455.

Gorni, P., and Pacheco, A. (2016). Growth promotion and elicitor activity of salicylic acid in *Achillea millefolium* l. **Afr. J. Microbiol.** 15: 657-665.

Hadi, M. R., and Balali, G. (2010). The effect of salicylic acid on the reduction of *Rizoctonia solani* damage in potato. **American-Eurasian J. Agric. & Environ. Sci.** 7: 492-496.

Hayat, S., and Ahmad, A. (2007a). **Salicylic acid: A plant hormone.** Netherlands: Springer.

Hayat, S., and Ahmad, A. (2007b). **Salicylic acid: A plant hormone.** Netherlands: Springer.

Hayat, S., Khan, N. A., and Alyemeni, M. (2013). **Salicylic acid: Plant growth and development.** Netherlands: Springer.

Kamnev, A. A. (2008). FTIR spectroscopic studies of bacterial cellular responses to environmental factors, plant-bacterial interactions and signalling. **Spectroscopy.** 22: 862085.

Kareem, F., Rihan, H., and Fuller, M. P. (2019). The effect of exogenous applications of salicylic acid on drought tolerance and up-regulation of the drought response regulon of Iraqi wheat. **J. Crop Sci. Biotechnol.** 22 (1): 37-45.

Khan, W., Prithiviraj, B., and Smith, D. L. (2003). Photosynthetic responses of corn and soybean to foliar application of salicylates. **J Plant Physiol.** 160 (5): 485-492.

Kochan, K., Perez-Guaita, D., Pissang, J., Jiang, J.-H., Peleg, A. Y., McNaughton, D., . . . Wood, B. R. (2018). In vivo atomic force microscopy-infrared spectroscopy of bacteria. **J. R. Soc. Interface.** 15 (140): 20180115.

Kuźniak, E., and Urbanek, H. (2000). The involvement of hydrogen peroxide in plant responses to stresses. **Acta Physiol. Plant.** 22 (2): 195-203.

Leubner-Metzger, G., and Meins Jr, F. (2000). Sense transformation reveals a novel role for class I β-1,3-glucanase in tobacco seed germination. **The Plant J.** 23 (2): 215-221.

Li, C. (2017). **Hormone metabolism and signaling in plants.** San Diego: Academic Press

Lopez-Gresa, M. P., Lisón, P., Yenush, L., Conejero, V., Rodrigo, I., and Bellés, J. M. (2016). Salicylic acid is involved in the basal resistance of tomato plants to citrus exocortis viroid and tomato spotted wilt virus. **PLoS One.** 11 (11): e0166938-e0166938.

Maruri-López, I., Aviles-Baltazar, N. Y., Buchala, A., and Serrano, M. (2019). Intra and extracellular journey of the phytohormone salicylic acid. **Front. Plant Sci.** 10 (423).

Mousavi, K., Seyed, M., Khara, J., and Heidari, R. (2009). Effect of salicylic acid on photosynthetic pigment content in *Ocimum bacilicum* under UV-C radiation stress. **J. Cell Mol. Res.** 1: 57-60.

Noh, T.-H., Lee, D.-K., Park, J.-C., Shim, H.-K., Choi, M.-Y., Kang, M.-H., and Kim,

J.-D. (2007). Effects of bacterial leaf blight occurrence on rice yield and grain quality in different rice growth stage. **Res. Plant Dis.** 13: 20-23.

Pancheva, T. V., Popova, L. P., and Uzunova, A. N. (1996). Effects of salicylic acid on growth and photosynthesis in barley plants. **J. Plant Physiol.** 149 (1): 57-63.

Parashar, A., Yusuf, M., Fariduddin, Q., and Ahmad, A. (2014). Salicylic acid enhances antioxidant system in brassica juncea grown under different levels of manganese. **Int. J. Biol. Macromol.** 70: 551-558.

Pfeilmeier, S., Caly, D. L., and Malone, J. G. (2016). Bacterial pathogenesis of plants: Future challenges from a microbial perspective: Challenges in bacterial molecular plant pathology. **Mol. Plant Pathol.** 17 (8): 1298-1313.

Reddy, A. P. K. (1979). Relationship of bacterial leaf blight severity to grain yield of rice. **Phytopathology.** 69: 967-969.

Sadeghipour, O. (2012). Impact of exogenous salicylic acid application on some traits of common bean (*Phaseolus vulgaris* L.) under water stress conditions. **Int. J. Agric..** 4: 685-690.

Saleh, A., and Abdulsattar, I. (2019). Foliar application of benzothiadiazole and salicylic acid to combat sheath blight disease of rice. **Rice Science.** 20 (5): 349-355.

Tauzin, A. S., and Giardina, T. (2014). Sucrose and invertases, a part of the plant defense response to the biotic stresses. **Front. Plant Sci.** 5: 293-293.

Thakur, M., and Sohal, B. S. (2013). Role of elicitors in inducing resistance in plants against pathogen infection: A review. **ISRN Bio. Chem.** 2013: 762412.

Thumanu, K., Sompong, M., Phansak, P., Nontapot, K., and Buensanteai, N. (2015). Use of infrared microspectroscopy to determine leaf biochemical composition of cassava in response to *Bacillus subtilis* CaSut007. **J. Plant Interact.** 10 (1): 270-279.

Thumanu, K., Wongchalee, D., Sompong, M., Phansak, P., Le Thanh, T., Namanusart, W., Buensanteai, N. (2017). Synchrotron-based ftir microspectroscopy of chili resistance induced by *Bacillus subtilis* strain D604 against anthracnose disease. **J. Plant Interact.** 12 (1): 255-263.

Trouvelot, S., Héloir, M.-C., Poinssot, B., Gauthier, A., Paris, F., Guillier, C., . . . Adrian, M. (2014). Carbohydrates in plant immunity and plant protection: Roles and potential application as foliar sprays. **Front. Plant Sci.** 5: 592-592.

Wu, C. T., Leubner-Metzger, G., Meins, F., Jr., and Bradford, K. J. (2001). Class I beta-1,3-glucanase and chitinase are expressed in the micropylar endosperm of tomato seeds prior to radicle emergence. **Plant physiol.** 126 (3): 1299-1313.

Zhang, Q., and Xiao, S. (2015). Lipids in salicylic acid-mediated defense in plants: Focusing on the roles of phosphatidic acid and phosphatidylinositol 4-phosphate. **Front. Plant Sci.** 6: 387-387.

Zhao, D., You, Y., Fan, H., Zhu, X., Wang, Y., Duan, Y., Chen, L. (2018). The role of sugar transporter genes during early infection by root-knot nematodes. **Int. J. Mol. Sci.** 19 (1): 302.

APPENDIX

I. CHEMICALS

NGB medium

+ Beef extract 3g

+ Peptone 5g

+ Water 1L

+ Glucose 2.5g

NGA medium

+ Beef extract 3g

+ Peptone 5g

+ Glucose 2.5g

+ Agar 15g

+ Water 1L

Swimming medium

+ Tryptone 10g

+ Agar 3 g

+ Water 1L

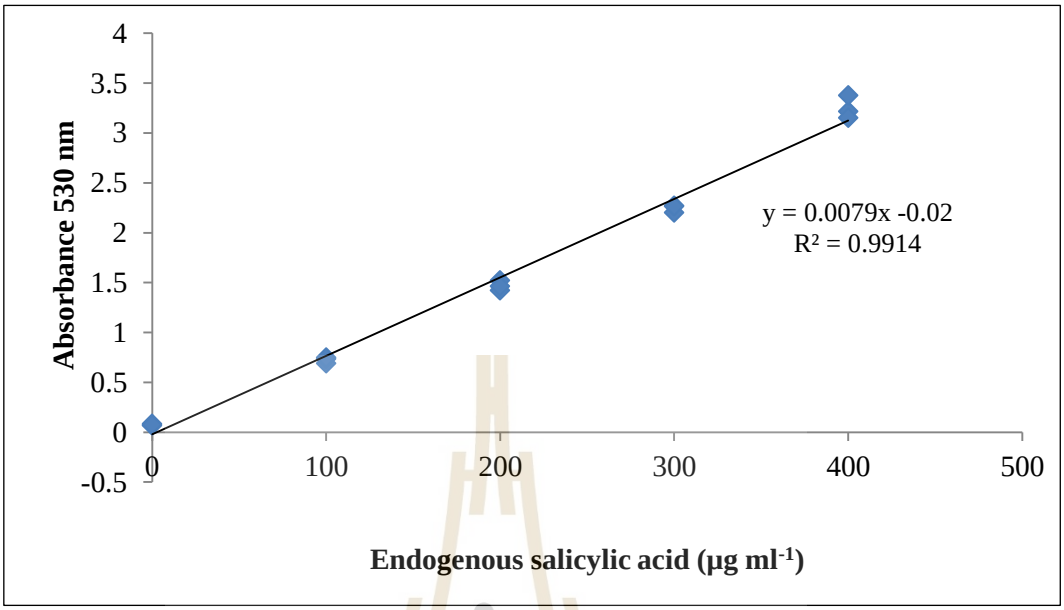
Twitching medium

+ Tryptone 4g

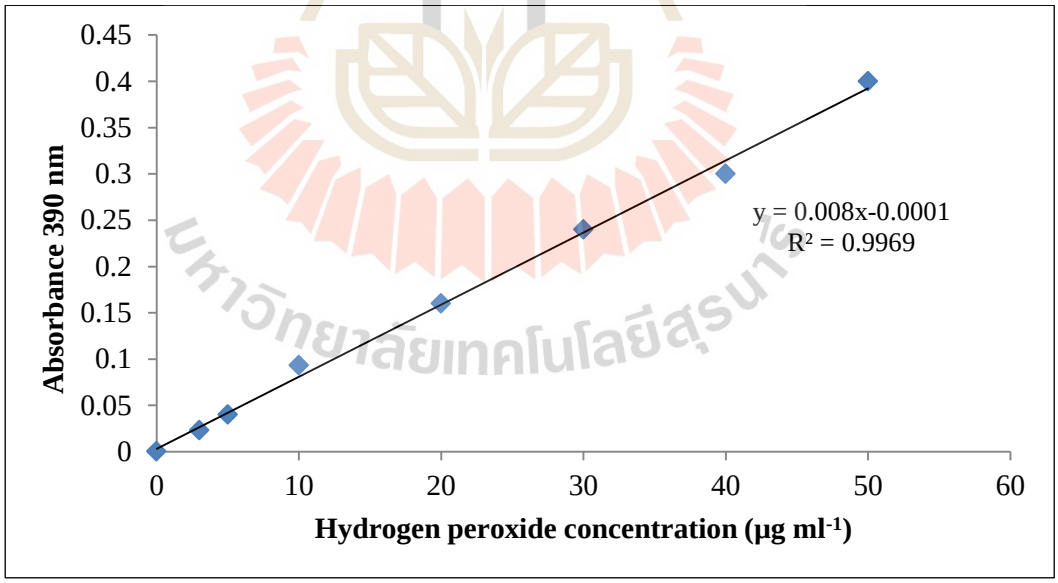
+ Agar 10 g

+ Water 1L

II. Biochemical compound stand curve

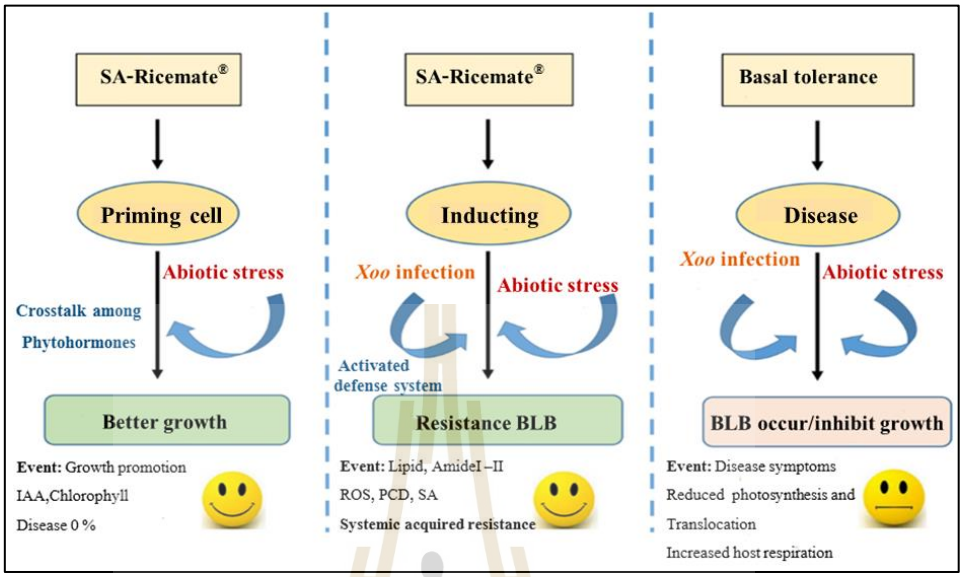


Attach figure 1. Standard curve of endogenous salicylic acid



Attach figure 2. Standard curve of hydrogen peroxide

III. MECHANISMS OF INDUCED RESISTANCE IN RICE CELLS



Attach figure 3. Resistance inductor treatment; SA-Ricemate® applied as a preventive treatment, plant can activate several mechanisms of defense that are usually sufficient to control the disease.

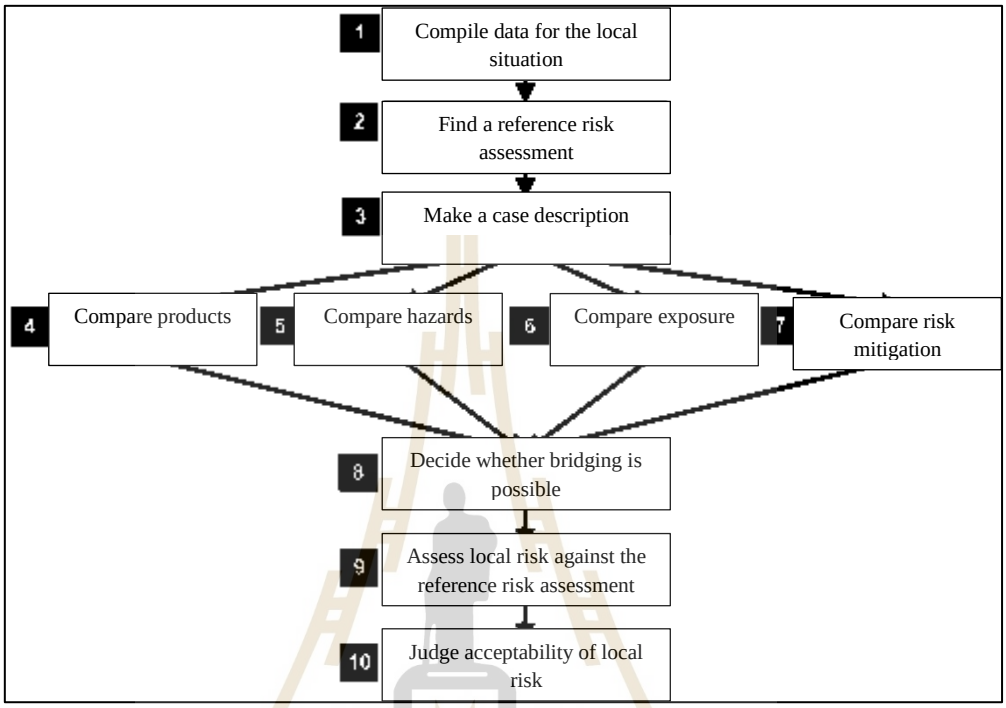
IV. COSTS OF PRACTICAL TO CONTROLLING THE BLB

Attached table 1 Costs of the controlling disease in rice production

Items	Cost[1/]		
	Copper hydroxide	**Commercial elicitor**	**SA-Ricemate®**
Protection product	30.00	60.00	3.60
Labor	150.00	150.00	150.00
Total	180.00	210.00	153.60

[1/]Units are in Thai baht (THB) per rai, in conventional rice farming, expense for labor was accounted for 3 times sprayed. The cost of pesticide was higher than that under used of SA-Ricemate®.

V. REGISTRATION OF SALICYLIC ACID IN COMMERCIAL SALICYLIC ACIDS



Attach figure 4. Schematic process and steps of assessment the product

BIOGRAPHY

Miss Wannaporn Thepbandit was born on September 28, 1987 in Bangkok, Thailand. She graduated with a Bachelor of Science from Thammasat University, Thailand in 2010. She achieved the Master's degree in Organic Farming Management from Thammasat University, Thailand in 2013. In 2015, she was accepted for a Ph.D. program under the supervision of Asst. Prof. Dr. Natthiya Buensanteai at the School of Crop Production Technology, Institute of Agricultural Technology, Suranareee University of Technology, Thailand. This program was supported by the Research and Researchers for Industries (RRI) and The Thailand Research Fund (TRF) under grant [PHD58I0071]. At the last year of Ph.D. program, she was an exchange student at Cornell University, New york, USA, from February 2019 to December 2019. Moreover, she presented her doctoral research work at the annual meeting of the American Phytopathological Society (APS) in Cleveland, Ohio, USA.