DEVELOPMENT OF METHODS FOR RESISTANT RICE VARIETIES SELECTION TO FEEDING BEHAVIOR OF BROWN PLANTHOPPER (*Nilaparvata lugens* (Stål))

NAPATSON CHANSAWANG

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การพัฒนาวิธีการคัดเลือกพันธุ์ข้าวต้านทานต่อพฤติกรรมการกินของเพลี้ยกระโดดสีน้ำตาล (*Nilaparvata lugens* (Stål))

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตร วิทยาศาสตรมหาบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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เพลี้ยกระโดดสีน้ำตาล, *Nilaparvata lugens* (Stål) เป็นหนึ่งในแมลงศัตรูข้าวที่สำคัญชนิดหนึ่งในภูมิภาคเอเชีย การเข้าทำลายของเพลี้ยกระโดดสีน้ำตาล *N. lugens* ทำให้ต้นข้าวเกิดอาการไหม้ (hopper burn) และยังเป็นแมลงพาหะในการถ่ายทอดเชื้อไวรัสเข้าสู่ต้นข้าว ซึ่งเป็นการขัดขวางการเจริญเติบโตของข้าว ส่งผลให้ผลผลิตของข้าวลดลง ซึ่งวิธีการควบคุมและป้องกันกำจัดเพลี้ยกระโดดสีน้ำตาล *N. lugens* มีทั้งการใช้และไม่ใช้สารเคมี อย่างไรก็ตามการควบคุมที่หลากหลายยังมีข้อจำกัดในการปฏิบัติ โดยการสร้างพันธุ์ข้าวต้านทานต่อเพลี้ยกระโดดสีน้ำตาล เป็นแนวทางป้องกันที่ดีวิธีหนึ่ง ซึ่งมีการวิจัยทดสอบหาข้าวพันธุ์ต้านทานด้วยวิธีการที่แตกต่างหลากหลาย ซึ่งแต่ละวิธีที่ใช้ในการศึกษาวิจัยมีข้อดีและข้อจำกัดแตกต่างกัน ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อ 1) ศึกษาพฤติกรรมการกินด้วยกระแสไฟฟ้า (AC-DC EPG) ซึ่งยังไม่พบการศึกษาในเพลี้ยกระโดดสีน้ำตาล *N. lugens* ด้วยเทคนิคดังกล่าว 2) พัฒนาวิธีการคัดเลือกพันธุ์ข้าวต้านทานต่อพฤติกรรมการกินของเพลี้ยกระโดดสีน้ำตาล *N. lugens* การศึกษาพฤติกรรมการกินด้วยกระแสไฟฟ้า ได้ผลการทดลองเป็นลักษณะกราฟ 7 ลักษณะ ที่สัมพันธ์กับการกินของแมลง ดังนี้ 1) กราฟ Nl1 และ 2) กราฟ Nl2 ทั้งสองกราฟเป็นการดูดกินของเพลี้ยกระโดดสีน้ำตาล *N. lugens* ที่ในชั้นผนังเซลล์พืช 3) กราฟ Nl3 เป็นกิจกรรมการปล่อยน้ำลายเพลี้ยกระโดดสีน้ำตาล *N. lugens* ขณะดูดกินในชั้นของท่อลำเลียง 4) กราฟ Nl4 ที่เกิดการกินในชั้นของท่อลำเลียงอาหาร เพลี้ยกระโดดสีน้ำตาล *N. lugens* ดูดกินน้ำเลี้ยง 5) กราฟ Nl5 เป็นกราฟที่ยังไม่สามารถระบุกิจกรรมและตำแหน่งได้ชัดเจน (unknown) เกิดขึ้นภายหลังจาก Nl4 6) กราฟ pd คือ การดูดกินของเพลี้ยกระโดดสีน้ำตาล *N. lugens* ที่ภายในเซลล์มีชีวิตของพืช (living cells) และ 7) กราฟ NP เพลี้ยกระโดดสีน้ำตาล *N. lugens* ไม่ดูดกินเนื้อเยื่อพืช สำหรับการประเมินวิธีการคัดเลือกพันธุ์ข้าวต้านทานต่อเพลี้ยกระโดดสีน้ำตาล *N. lugens* มีการทดลอง 6 การทดลอง ได้แก่ 1) การตรวจวัดพื้นที่มูลหวาน 2) การนับจำนวนรอยเจาะ 3) ประเมินการเจริญเติบโตและการรอดชีวิตของแมลง 4) การประเมินความชอบต่อการเข้าหาพืชของแมลง 5) การทดสอบการดูดกินของแมลง (EPG) และ 6) การประเมินขนที่ผิวใบข้าว จากนั้นนำผลการทดสอบ มาวิเคราะห์ ประเมินความต้านทานของพันธุ์ โดยการวิเคราะห์ความสัมพันธ์ของพารามิเตอร์ในแต่ละวิธีการทดสอบ และจัดจำแนกกลุ่มพันธุ์ด้วยโปรแกรม Origin 2023b พบว่า วิธีการทดสอบที่ใช้ในการคัดเลือกพันธุ์ข้าวต้านทาน แตกต่างอย่างมีนัยสำคัญทางสถิติ 28 จาก 32 พารามิเตอร์

ซึ่งนำมาใช้ในการจัดระดับความต้านทานของพันธุ์ข้าวทั้ง 8 พันธุ์ได้ เป็น 5 กลุ่ม ตามระดับความต้านทานของข้าว ได้แก่ กลุ่มที่ 1 ต้านทาน ได้แก่ พันธุ์ KDML105 และพันธุ์ PTB33 กลุ่มที่ 2 ค่อนข้างต้านทาน ได้แก่ พันธุ์ Rathu Heenati และพันธุ์ Babawee กลุ่มที่ 3 ค่อนข้างอ่อนแอ คือ TN1 กลุ่มที่ 4 พันธุ์อ่อนแอ ได้แก่ พันธุ์ Mudgo และพันธุ์ UBN03078-101-342-4-148, และกลุ่มที่ 5 พันธุ์อ่อนแอมาก คือพันธุ์ ASD7 จากการศึกษาวิจัยนี้ ได้ลักษณะกราฟที่เกิดขึ้นจากการศึกษาการดูดกินของเพลี้ยกระโดดสีน้ำตาล *N. lugens* ด้วยเทคนิค AC-DC EPG เป็นการศึกษาครั้งแรก สามารถนำไปเป็นต้นแบบในการใช้ต่อยอดงานวิจัยเกี่ยวกับพฤติกรรมการกินของเพลี้ยกระโดดสีน้ำตาลต่อข้าวสายพันธุ์อื่น ๆ และการศึกษาการถ่ายทอดเชื้อสาเหตุโรคของเพลี้ยกระโดดสีน้ำตาลวิธีการคัดเลือกพันธุ์ต้านทานข้าวเป็นการประเมินแบบใหม่ สามารถนำไปใช้เป็นต้นแบบการคัดเลือกพันธุ์พืชต้านทานต่อแมลงได้

สาขาวิชาเทคโนโลยีการผลิตพืช | ลายมือชื่อนักศึกษา นภัสสร จันทร์สว่าง

ปีการศึกษา 2566 | ลายมือชื่ออาจารย์ที่ปรึกษา ....

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม ....

NAPATSON CHANSAWANG : DEVELOPMENT OF METHODS FOR RESISTANT RICE VARIETIES SELECTION TO FEEDING BEHAVIOR OF BROWN PLANTHOPPER (*Nilaparvata lugens* (Stål)). THESIS ADVISOR : ASST. PROF. DR. JARIYA RODDEE, 89 PP.

Keyword : BPH/rice resistance/AC-DC EPG/feeding behavior

The brown planthopper*, Nilaparvata lugens* (Stål) is one of the key insect pests that causes serious damage and yield loss in Asia. Removing plant sap from the phloem tissue causes hopper burn damage. Moreover, they can transmit rice ragged stunt virus (RRSV), a pathogen to plants. These effects decrease rice growth and yield. There are several methods, both chemical and non-chemical, used for prevention. However, each method has limitations in practice. The reduction of BPH, *N. lugens* infestation is breeding resistance varieties to BPH*, N. lugens*. Various methods are employed to assess plant resistance to insects, each having its distinct set of advantages and disadvantages. Therefore, this research was to study 1) the feeding behavior of BPH, *N. lugens* using AC-DC electropenetrography (The EPG test BPH, *N. lugens* appeared in several research in DC-EPG but did not appear study by AC-DC EPG) and 2) the method development for screen resistant rice varieties to the feeding behavior of BPH, *N. lugens*. The first experiment showed waveforms for BPH, *N. lugens* which were classified as seven waveform families 1) waveform Nl1 and 2) waveform Nl2 the BPH, *N. lugens* feeding on pathway phase (epidermis cells), 3) waveform Nl3; BPH, *N. lugens*'s saliva activity on phloem cell, 4) waveform Nl4, the activities of BPH, *N. lugens* was ingestion phase on phloem, 5) waveform Nl5 the character of waveform was unknown activities, 6) waveform pd; a potential drop between feeding (feeding activity in living tissues), and 7) waveform NP; a non-penetration. The second experiment consisted of six experiments as follows 1) honeydew, 2) salivary sheath, 3) life cycle development and survival, 4) EPG, 5) host preference rice variety, and 6) evaluation plant trichome. Then the correlation between the experiment's parameters and the grouping of varieties was analyzed by the program Origin 2023b. The result significantly differed in resistance method selection across 28 of 32 parameters. The result was a separate group of 8 varieties of five main groups. Group 1 included resistant varieties KDML105 and PTB33. Group 2 – Rathu Heenati, and Babawee were moderately resistant varieties, Group 3 – TN1 was moderately susceptible varieties, Group 4 included Mudgo and UBN03078-101-342-4-148 which were susceptible, the last group- ASD7 was highly susceptible. Thus, in the first report,

this research showed waveform characteristics of BPH, *N. lugens* feeding behavior with AC-DC EPG. These abilities can be used to study associated BPH, *N. lugens* feeding behavior in different rice varieties and transmit pathogen of BPH, *N. lugens* in future research. The updated evaluation of the selection method for screening rice resistance will provide valuable insights for choosing rice varieties resistant to Brown Planthopper (BPH) in the future.

School of Crop Production Technology
Academic Year 2023

Student's Signature Napatson Chansawang
Advisor's Signature ....
Co-advisor's Signature ....

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NAPATSON CHANSAWANG

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

°C	=	Degree Celsius
RH	=	Relative humidity
ml	=	Millimeter (s)
cm	=	Centimeter (s)
g	=	Gram (s)
DMRT	=	Duncan's multiple range test
PCA	=	Principal component analysis
EPG	=	Electropenetrography
BPH	=	Brown planthopper, *Nilaparvata lugens* (Stål)
SEM	=	Scanning Electron Microscope
DI	=	Deionized Water

CHAPTER 1
INTRODUCTION

Rice (*Oryza sativa* L.), mainly produced in Asia, is important for over 50% of the world's population (UN Department of Economic and Social Affairs, 2019). According to a new United Nations report, the world population of 7.6 billion is expected to reach 8.6 billion in 2030, 9.8 billion in 2050, and 11.2 billion in 2100 (UN Department of Economic and Social Affairs, 2019). This rapid increase in the global population has been the key driver of increasing rice production and productivity across the globe. However, this cereal crop (rice), solely cultivated for human consumption, is the host plant to many insect pests such as planthoppers (brown planthopper [BPH: *Nilaparvata lugens* Stål], white-backed planthopper [WBPH *Sogatella furcifera* Horvath]. BPH, *N. lugens* is one of the most dangerous insect pests that cause serious damage to rice production and yield loss in East Asia. They are sucking insects that remove plant sap from the xylem and phloem tissues of the plant. Severely damaged plants dry and take on the brownish appearance of plants that have been burnt by fire. Planthopper damage is called "hopper burn". These insects are harmful pests in Asia where they cause not only direct damage by removing plant sap but also constitute vectors of serious rice viral diseases such as grassy stunt virus transmitted (The International Rice Research Institute [IRRI], 2010).

Prevention of insect pests includes cultural, mechanical, physical, biological, and chemical methods, insect natural enemies, and microbiology control (Beck et al, 2021, Matsumura et al., 2008, Anik, and Das, 2020, Matsumura et al., 2008, Wang et al., 2008a; 2008b, Huang et al., 2000). Host-plant resistance helps decrease damage. In a previous study, Hu et al. (2016) studied about 29 dominant resistance genes of rice varieties for planthoppers that were recognized from wild rice species. The transgenic rice plants with snowdrop Lectin (*Galanthus nivalisagglutinin*) have reduced the fecundity and survival rate by postponing the development of planthoppers because of their antifeedant activity.

Several techniques are used to measure plant resistance to insects, such as direct insect feeding ingestion, the correlation of plant factors with resistance, and the measurements of insect feeding, development, and behavior. There is also the use of biotechnological methods to develop plant resistance to insects, for example, electrophoretic techniques, plant tissue and cell culture, and resistance to insects in genetically transformed plants (Smith 1989). Breeding programs that help to develop rice-resistant varieties to insect pests should therefore complement or replace conventional control strategies. Although many resistance loci have been discovered, not all can be used to protect the rice plant from BPH, *N. lugens* attack (Ishii et al., 1994). At the heart of the problem is the ability of sap-feeding insects to overcome the many adaptations that plants have evolved as protection. The complex interaction between sap-feeding pests and their host plants is only recently beginning to be understood. The pathway from the host location to sustained ingestion of phloem sap can be interrupted at several points, potentially allowing many different types of resistance. Detailed differences in the feeding behavior of BPH, *N. lugens* on different rice genotypes varying in resistance will allow underlying mechanisms to be identified, thereby providing new targets for control. the gene has not yet been cloned (Kumari et al., 2010). Wang et al. (2012) studied the microarray analysis of broad-spectrum resistance derived from RHT. However, its detailed resistance mechanism of action against BPH, *N. lugens* has not been thoroughly studied.

There are several methods of this selection, one of which is electronic penetration graph (EPG) or electropenetrography. This is an important technology in the selection of plants resistant to hemipteran. The EPG monitoring of insect feeding allows highly rigorous detection, quantification, and statistical analysis of stylet penetration behaviors of any pierce-sucking arthropod on an electrically conductive feeding substrate. A pierce-sucking insect feeds on plants by piercing cells or vascular tissues with specialized mouthparts and sucking the content. EPG has been used as an effective tool to study the feeding behaviors of sucking insects (Mclean and Kinsey, 1964; Tjallingii, 1978; Golawska et al., 2012; Trebicki et al., 2012,). The EPG monitoring study was useful in explaining the relationship between the stylet penetration behavior and the meaning of EPG waveforms (Seo et al., 2009). The feeding of planthopper is excreted with honeydew during feeding sucking sap (phloem or xylem of each planthopper species) was measured weight or area of honeydew (Cao et al., 2013). A choice test is one method used to select plant varieties that are resistant to insect pests. That useful method to observe the non-preference behavior of insect pests to host plants, which have many factors to select such as signals and information content-volatile compounds that specific individual host plant, sensory aspects-insect can receive signals from the host (Carrasco et al., 2015). From the above review, it is found that various methods were used for selecting the plant varieties resistant to pierce-sucking insects. However, the standard method used to select rice varieties resistance has not been reported. Thus, this research aims to study the feeding behavior of

BPH *N. lugens* using AC-DC electropenetrography and method development to screen resistance rice varieties to the feeding behavior of BPH *N. lugens* feeding.

CHAPTER 2
LITERATURE REVIEWS

2.1 Importance of rice

Rice (*Oryza sativa*) is an important crop in the world wild and the main economic crop in Thailand. In 2021, in-season rice area of 63.01 million rais. Harvested area 60.26 million rais. There are 26.81 million tons of paddy. And off-season rice was area of 8.34 million rais. The harvested area of 8.31 million rais. There are 5.31 million tons of paddy. The total production volume is around 31 million tons. In 2022, the production volume of rice in Thailand was around 32 million tons, indicating a slight increase from the previous year. The value of exports in 2021, was 6.30 million tons, valued at 109.77 billion baht. In 2022 at 7.69 million tons, the value of 138.45 billion baht (Figure 2.1, 2.2; Office of Agricultural Economics, 2023.).

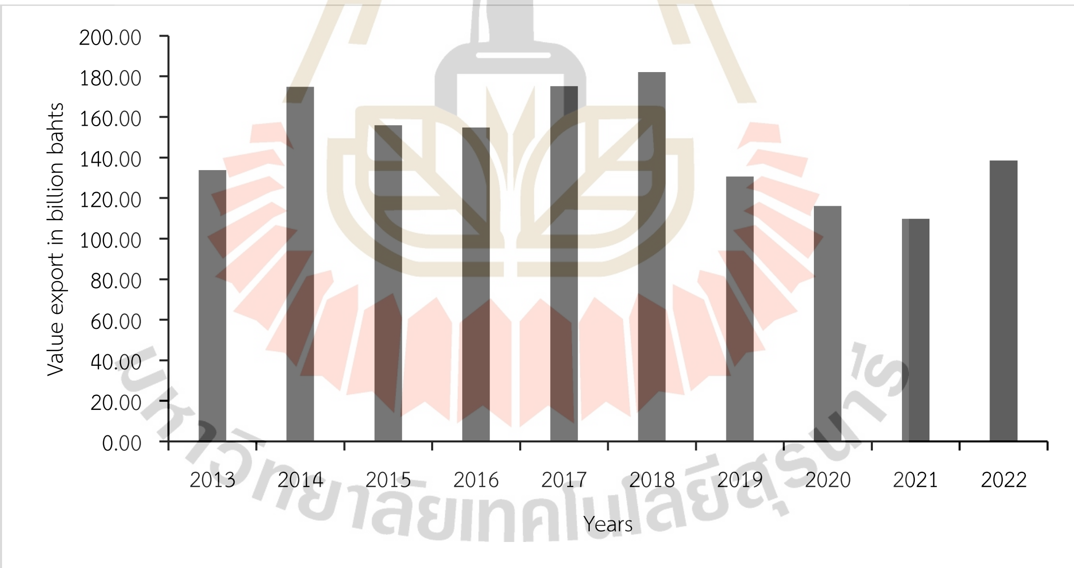

Figure 2.1 Export value of rice and products, Thailand 2013-2022
Source: Office of Agricultural Economics, 2023

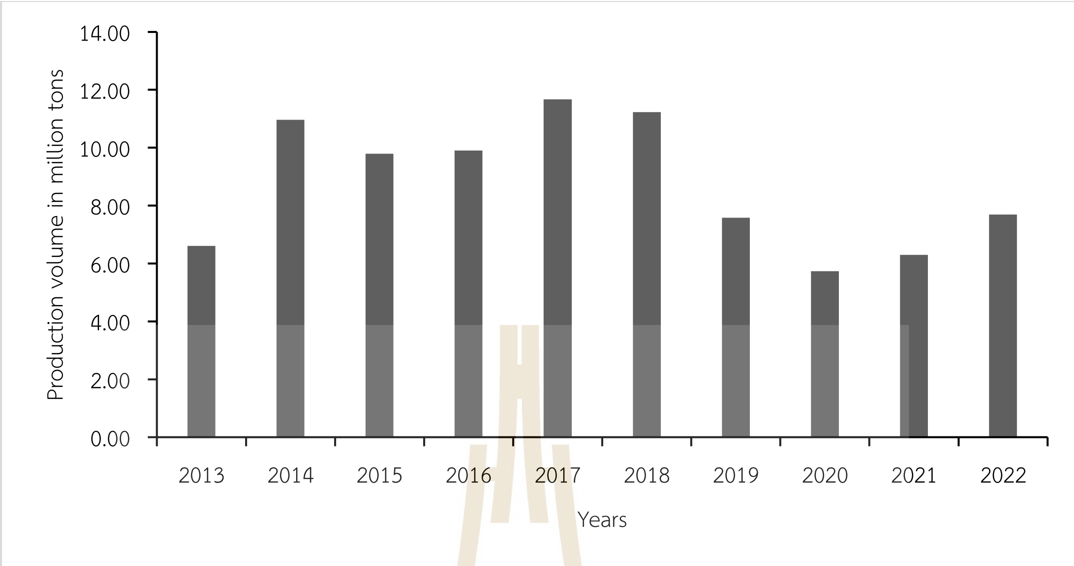

Figure 2.2 Production volume of rice and products, Thailand 2013-2022
Source: Office of Agricultural Economics, 2023

Rice classification in Thailand identified many types such as the character of varieties to grow up in different locations; upland rice plants in highlands, and mountain slopes quite to use low water. Lowland rice grows up in normal land, mostly planted in the middle region of Thailand. The floating rice tolerance drought in seedling plants and ability to adapt to high levels of water. The photoperiod-sensitive variety-producing grain depends on the period of light because rice is a short-day plant. Thus, these were quite producing grain same as a period every year. Photoperiod-insensitive variety-producing grain when the age to the maturation of life cycle, that average 90-140 days. In-season rice-planting 1 crop per year growing depends on rainwater. Off-season rice-planting 2-3 crops per year (Department of Agriculture, 1998, 1999; Boonbongkarn, n.d.)

2.2 History of rice variety

Reduced genetic variability, improved cultural practices and continuous cropping with rice have increased the genetic vulnerability of the crop. Chemical control of insects for prolonged periods in tropical climates is very expensive and impractical. The use of host-resistance for insect control is the logical approach to overcome these production constraints. Therefore, IRRI'S rice improvement program has placed major emphasis on developing germplasm with multiple resistance to major insects. Many national programs have similarly given priority to developing varieties with multiple disease and insect resistance. Fortunately, IRRI and national rice improvement programs have assembled excellent collections of rice germplasm. Pathologists and entomologists have developed screening techniques and many donors for resistance have been identified (Khush, 1977). Utilizing these donors, we have developed improved varieties with resistance to as many as four diseases and four insects. The IRRI varieties IR5, IR8, IR22, and IR24 were susceptible to most of the diseases and insects. The first variety with multiple resistance was IR26, released in i973. Since then, many varieties with multiple resistance have been developed at IRRI and by the national programs. Table 2.1 shows the disease and insect ratings of the IR varieties.

PTB33 is original variety from traditional Indian landrace (Sidhu and Khush, 1978; Jairin et al. 2007; Yadavalli et al., 2012). This variety was used for standard resistant by research of Ferrater et al. (2015), and Jairin et al. (2007).

TN1 or Taichung Native 1 is a variety released in 1960 in Taiwan that susceptible (Datta, 1981). That is a susceptible to planthopper and was used for standard susceptible variety in severely research such as Angeles, et al. (1986) study associate rice resistant to leafhopper and planthopper; Ferrater et al. (2015) study relationship on plant, symbiosis, and phloem-feeder insect; etc.

Mudgo is a local rice variety from India. This variety is used as parents for breeding programs (IRRI et al., 1996; Chen et al., 1999) because there is *bph1* gene that resistant to planthopper. Sogawa and Pathak (1970) reported this variety reduce size of planthoppers, increase period of insect growth rate and non-preference to insect.

Babawee is the Sri Lankan rice cultivar (Laksminarayana and Khush, 1977). There is *bph4* gene to resistantant planthpooer (Jairin et al., 2005).

KDML105 is local varieties in Thailand. That is main economic rice variety in Thailand. That variety is susceptible to planthopper (Jairin et al., 2005).

Rathu Heenati is the Sri Lankan rice cultivar, there is *bph3* gene to resistant to planthopper (Lakshiminarayana and Khush, 1977; Sun et al., 2005; Jairin et al., 2007).

Table 2.1 The rice resistance varieties to green leafhopper brown planthopper stemborer and gall midge named by IRRI (IR5 to IR34) and of IRRI lines named as varieties by the Philippine Government (IR36-IR66).

Varieties	Reaction [a]					
	Green leafhopper	Brown planthopper			Stem borer	Gall Midge
		Biotype 1	Biotype 2	Biotype 3		
IR5	R	S	S	S	MS	S
IR8	R	S	S	S	S	S
IR20	R	S	S	S	MR	S
IR22	S	S	S	S	S	S
IR24	R	S	S	S	S	S
IR26	R	R	S	R	MR	S
IR28	R	R	S	R	MR	S
IR29	R	R	S	R	MR	S
IR30	R	R	S	R	MR	S
IR32	R	R	S	R	MR	S
IR34	R	R	S	R	MR	S
IR36	R	S	R	S	MR	R
IR38	R	S	R	S	MR	R
IR40	R	S	R	S	MR	R
IR42	R	S	R	S	MR	R
IR44	R	S	R	S	MR	S
IR46	MR	R	S	R	MR	S
IR48	R	R	R	S	MR	S
IR50	R	R	R	S	MR	S
IR52	R	R	R	S	MR	-
IR54	R	R	R	S	MR	-
IR56	R	R	R	R	MR	-
IR58	R	R	R	S	MR	-
IR60	R	R	R	R	MR	-
IR62	R	R	R	R	MR	-

[a] S = susceptible, MS = moderately susceptible, MR = moderately resistant, R = resistant.

Source: Khush (1987)

Table 2.1 The rice resistance varieties to green leafhopper brown planthopper stemborer and gall midge named by IRRI (IR5 to IR34) and of IRRI lines named as varieties by the Philippine Government (IR36-IR66) **(continued)**.

Varieties	Reaction [a]					
	Green leafhopper	Brown planthopper			Stem borer	Gall Midge
		Biotype 1	Biotype 2	Biotype 3		
IR64	R	R	MR	S	MS	-
IR66	R	R	R	R	MR	-

[a] S = susceptible, MS = moderately susceptible, MR = moderately resistant, R = resistant.

Source: Khush (1987)

2.3 Problems in rice production

Rice paddy in Thailand a lot of problem since process planting such as, unsuitable factors of planting, grow, and produce grain, effect losses yield qualitative and quantitative (Extension and Training Office, n.d.). Which mainly unsuitable factors were 2 types; biotic factors, such as weed, disease, insect, and abiotic factors such as flood, drought, etc. Weeds are competitive factors for the growth of rice cause decrease qualitative and quantitative of yield. In Thailand, there are many weeds such as weedy rice is noxious weed. Seed of weed there is contamination in paddy that decrease qualitative of grain (Artid, 2004; Promnard et al., 2018).

Diseases attacked and damaged rice plants in every phase. They are caused by bacteria, fungi, phytoplasma, virus and nematode, some insects as vectors of pathogen. In Thailand have many important rice diseases such as, rice blast disease is caused by *Pyricularia grisea* Sacc. quite appear in-season rice. The symptoms are typically elliptical spot with gray centers and brown to margins in leaf that were first detected in the plots containing infested grain 35 days after planting (Long et al., 2001). Brown Spot Disease (BSD) is caused by *Helminthosporium oryzae* Breda de Haan, the disease damage to rice leaf occurs in a brown circle spot on the leaf in tillering phase (Extension and Training Office, n.d.).

Insect's rice pests have important around 20 species in Thailand. That is infestation because losses yield such as rice thrips-*Stenchaetothrips biformis* (Bagnall) (Figure 2.3A) infestation in drought. That ingestion of xylem sap on young leaf and symptom occur wilting and roll on margins to center, rice stem borers-or rice yellow stem borer *Scirpophaga incertulas* (Walker) in order Lepidoptera: Pyralidae (Figure 2.3B), was attacked all stages of the crop by feeding rice stem borers (Sarwar, 2012), Planthopper are sucking insect-brown planthopper, *N. lugens* (Figure 2.3C) and white-backed planthopper *S.furcifera* . (Figure 2.3D)

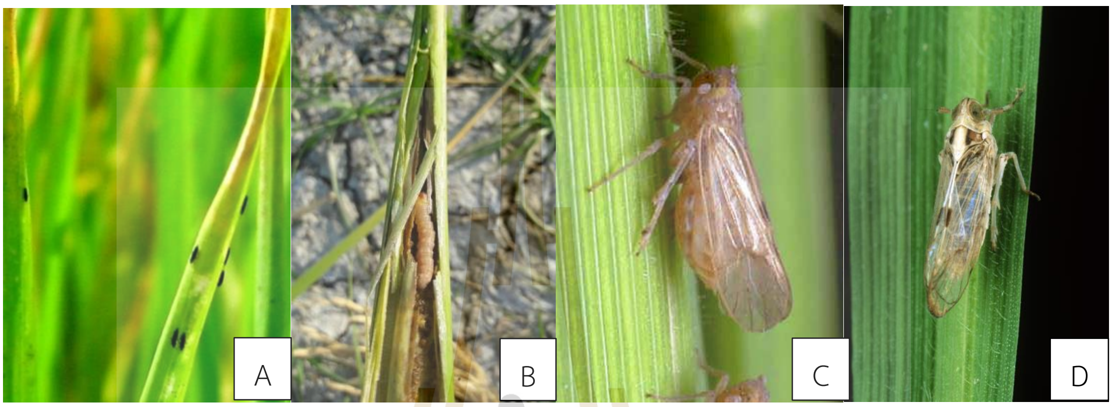

Figure 2.3 Insect pests of rice; (A) rice thrips-*Stenchaetothrips biformis* (Bagnall) (B) rice yellow stem borer, *Scirpophaga incertulas* (Walker), (C) brown planthopper, *Nilaparvata lugens* Stål, and (D) white-backed planthopper *Sogatella furcifera* Horvath

Source: Figure 2.3A: Jairin (2010), Figure 2.3B: Plantix (n.d.), Figure 2.3C: Culliney (2012), and Figure 2.3D: Cattlin (2005).

2.4 Insect pricing –sucking pest of rice and biological of planthopper

Many insects pricing– sucking pests including planthoppers brown planthopper (BPH): *Nilaparvata lugens* (Figure 2.4A) small brown planthopper (SBPH): *Laodelphax striatellus* Fallen (Figure 2.4B), green leafhopper (GLH): *Nephotettix virescens* Distant, green rice leafhopper (GRH): *N, cincticeps* Uhler, white-backed planthopper (WBPH): *S. furcifera* (Figure 2.4C) are infestation cause losses yield.

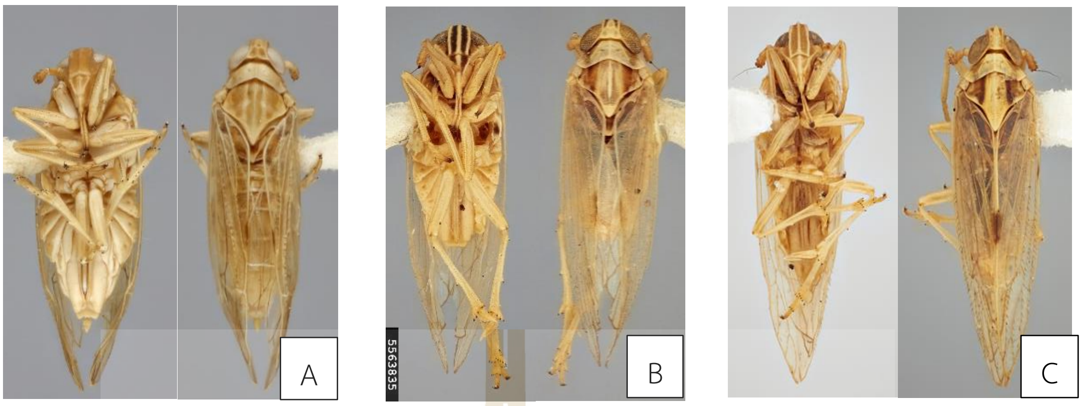

Figure 2.4 Planthoppers; (A) brown planthopper (BPH): *Nilaparvata lugens* Stål, (B) small brown planthopper (SBPH): *Laodelphax striatellus* Fallen and (C) white-backed planthopper (WBPH): *Sogatella furcifera* Horvath

Source: Langlois (n.d.)

2.4.1 Biological of planthoppers

Planthoppers are pricing – sucking insects. The taxonomy is tree as domain: Eukaryota;

Kingdom: Metazoa;

Phylum: Arthropoda

Subphylum: Uniramia

Class: Insecta

Order: Hemiptera

2.4.2 Brown planthopper, *Nilaparvata lugens* (Stål)

Brown planthopper, *Nilaparvata lugens* (Stål), (Hemiptera: Delphacidae) have 2 forms macropterous and brachypterous form, which macropterous form able to remove location far from than brachypterous form. BPH ingestion of xylem sap and phloem sap on leaf sheath Then the symptom is yellow leaf become dry brown. This was called " hopper burn" besides that is vector of rice ragged stunt cause stunt rice plant. *N. lugens* has two wing-forms body length of male size is less than female, male 2.8-3.5 mm, female 3.8-4 mm there are yellow to yellowish brown and white in back. The eggs are white color, elongated and look like a curved club. They hatch in about 8-10 days. The nymph undergoes five instars within a period of 15-20 days. The total life cycle is completed in about 20-25 days, which varies between the two sexes. The life span of male is 15-20 days and that of female is 15-30 days (Figure 2.5). The optimum temperature and humidity for the development and survival of nymph is around 30°C and 70-90%, respectively. However, each stage duration depends on temperature (Brady, 1979). Moreover, brown planthopper (BPH): *N. lugens*, is insect vector of rice grassy stunt (RGSV) and rice ragged stunt (RRSV) viruses that transmitted in a persistent manner without transovarial passage.

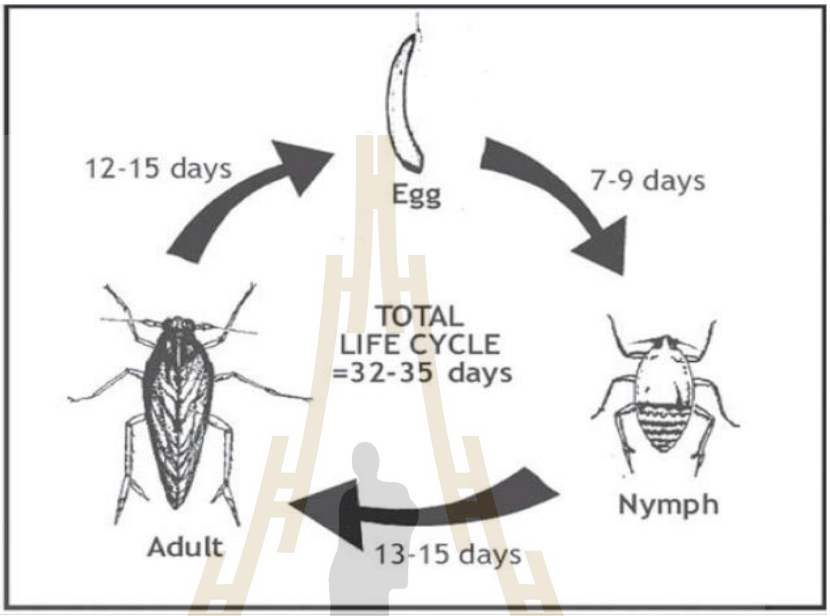

Figure 2.5 Life cycle of brown planthopper, *Nilaparvata lugens* (Stål)
Source: Albania and Mateo (2019)

Figure 2.6 Hopper burn symptom damage of brown planthopper, *Nilaparvata lugens* (Stål)
Source: International Rice Research Institute (IRRI), (2009)

2.4.3 White-backed planthopper, *Sogatella furcifera* (Horvath)

White-backed planthopper, *Sogatella furcifera* (Horvath), (Hemiptera: Delphacidae) are ingestion on xylem and phloem sap on leaf sheath and leaf occur orange yellow. White-backed planthopper is infestation broader cover location than BPH (Sriratanasuk et al., 2011) . Male size is less than female, male body length including forewing is 3.5-3.8 mm and female is 3.5-4 mm. They are yellow to yellowish brown and white on the back. The life cycle of white-backed planthoppers is 32-35 days with three stages including eggs nymph and adult stage. Eggs stage 7 days, the nymphs 1-5 stage are 12-18 days and adult 14.5-15.9 days (Figure 2.7) (Kumar et al., 2015). Both male and female are short-wings or long. It mostly occurs in Asia. (Jackson, 2018; The Invasive Species Compendium, 2021).

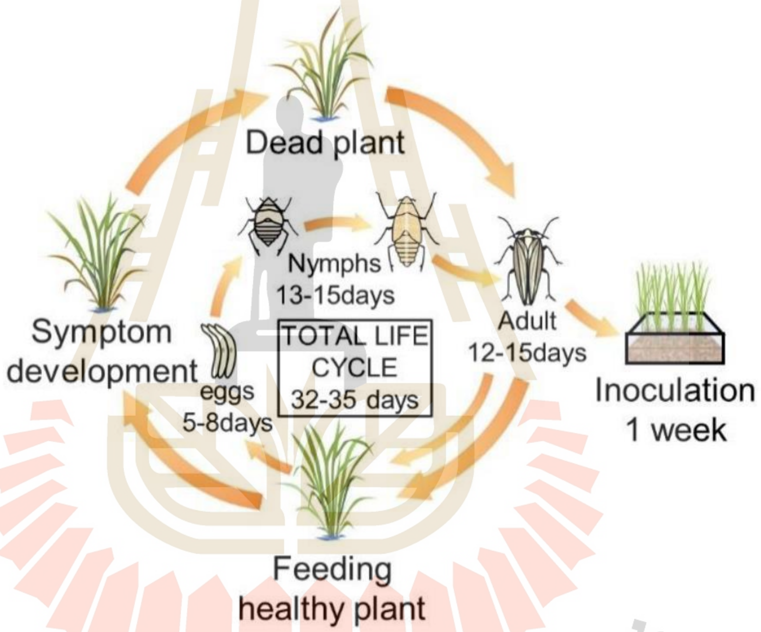

Figure 2.7 Life cycle of white-backed planthopper *Sogatella furcifera* (Horvath)
Source: Jang et al. (2020)

On 26 August 2021, Rice Department, Thailand, report detected white-backed planthopper and BPH in Chiang Rai province that probably cause to losses yield rice. Besides, in China and Vietnam that is vector of southern rice black-streaked dwarf virus (SRBSDV) cause rice black-streaked dwarf-2 (SRBSDV-2) (Zhou et al., 2008; 2010). Transmission of SRBSDV in rice by WBPH in a persistent manner, the access periods of virus were 5 min acquisition and inoculation 30 min. (Pu et al., 2012). However, SRBSDV was undetected spreading in Thailand.

2.5 Planthoppers controlling methods.

The planthopper problem is solved with several methods, the suggestion is to control the level of water in the land which generates an unsuitable environment for propagation by draining water in flooded rice paddy in the short term, selecting planting methods of indirect seeding instead of sowing, and chemical control methods, there are many types differ each period infestation.

2.5.1 Cultural method

Cultural methods are important strategy for control and management planting cultivate area for planting, use resistant varieties, crop rotation, choose time or period to planting, etc. for induce resistant of plant. During growth early-stage infestation was drain the paddies for 3-4 days and apply split applications (three times) of nitrogen fertilizer (Pacific Pests and Pathogens - Full Size Fact Sheets, n.d.). A nitrogen (N) application in three split doses is effective in minimizing the population rate of *S. furcifera* and *N. lugens* (Li et al., 2021). Silicon application is positively connected to reduce the planthopper population in rice fields (Yang et al., 2017). Water favors the growth of planthoppers and leafhoppers so 3- or 4-days fields draining during invasion have been endorsed for reducing *S. furcifera* and *N. lugens* populations. For *N. lugens*, closer plant spacing is considered as an important factor favoring hopper build-up. The lower plant portion is slightly cooler due to low sunshine and humidity and provides a favorable microclimate for pest population build-up.

2.5.2 Field sanitation

Field sanitation for the control of planthoppers is used in many rice growing areas. Volunteer rice and ratoons may act as a source for the spread of a virus. Rice crop rotation is an economical control measure. For this purpose, legumes are usually grown in most areas of Asia to reduce planthopper infestations.

2.5.3 Mechanical method

Mechanical method used Sweep a net to remove some BPH dry seedbed, but this method is not efficient if the insect lays an egg in the plant (International Rice Research Institute, n.d.).

2.5.4 Physical method

Physical methods use physiology to prevent or control number of insects until animal pest such as heat wave, light trap, temperature, etc. Use of trap crop to control *N. virescens* and *N. lugens* is suggested in a study conducted at IRRI. Trap crop transplanted 20 days before crop transplanting, and it reduced the attack of *N. lugens* on the main crop. Similarly, the banker plant system is also used in some countries, and it can boost the population of *Anagrus nilaparvatae* which helps to control rice planthoppers. In the Jiaxing city of China, the incidence of planthopper transmitted viral diseases is considerably decreased by delayed sowing and non-significant variation in the grain yield is observed.

2.5.5 Biological method

Biological method used new technology to change genetic plant to resistant insect such as genetic engineering technology, etc. and use insect natural enemies to kill them such as predatory and parasitic insects, and microbiology control, that specifically for types and species of insects. Planting flower in order to attract natural enemies (Pacific Pests and Pathogens - Full Size Fact Sheets, n.d.). *Beauveria bassiana* strain 202 (Bb-202) against multiple targeted sucking insect species showed 100% pathogenicity to *Myzus persicae* Sulzer (Hemiptera: Aphididae) (Bugti et al., 2018).

2.5.6 Chemical method

Chemical method used pesticide to control insect pests, they are indirectly controlling method, fast and high performance in short term. When detected the 1st-2nd instar WBPH on rice 0-40 days old (more than 5 insects per plant) recommend applying the buprofezin, buprofezin, isoprocarb, and etofenprox used etofenprox, dinofuran, and pymetrozine when detected more than one nymph or adult on rice 41-60 days old. And rice 61-80 days old used etofenprox, dinofuran, and carbosulfan when detected nymphs or adult (more than one insect per plant), All insecticide in above, able to use according to the label. However, some types of chemical and wrong practice can affect the environment and user, user need knowledge to use this method. In china over the period 2012–2016 reported on the status of eight insecticides resistance in *N. lugens* (Wu et al., 2018).

2.5.7 Host-plant resistance

Host-plant resistance is helpful in decreasing the damage. In a study in China, about 29 dominant resistance genes for planthoppers have been recognized from wild rice species and indicate rice varieties. The transgenic rice plants with snow drop Lectin (*Galanthus nivalisagglutinin*) have reduced the fecundity and survival rate by postponing the development of planthoppers because of there by an antifeedant activity. Breeding programs to develop rice varieties resistant to insect pests should therefore complement or replace conventional control strategies. Rathu Heenati (RHT) is a Sri Lankan rice varieties that carries a BPH-resistance gene (*bph3*) and shows a broad-spectrum resistance to all four biotypes of BPH. The BPH-resistance loci in RHT have been studied extensively and assigned to four rice chromosomes (3, 4, 6, and 10) by various research groups (Lakshiminarayana and Khush, 1977; Sun et al., 2005; Jairin et al., 2007). However, the gene has not yet been cloned (Kumari et al., 2010). Wang et al. (2012) studied the microarray analysis of broad-spectrum resistance derived from RHT. However, its detailed resistance mechanism of action against BPH has not been thoroughly studied. Moreover, the selected planting rice resistant which switch least than 2 varieties for decelerate planthopper resistance (Sriratanasuk et al., 2011) because insect can evaluation to resistant.

2.6 Resistant mechanism of plants

The plant has mechanism of resistance 3 types, antixinosis, antibiosis and tolerance.

2.6.1 Antixenosis

Insects appear non-preference for a resistant plant. Antixinosis is a mechanism of plant deterrence. That is plant defense by morphology such as wax, trichomes (Figure 2.8), etc. Experiments observing of the behavioral response of white-backed planthopper to select rice varieties that selection on resistant was lower than on susceptible variety (Huang et al., 2018).

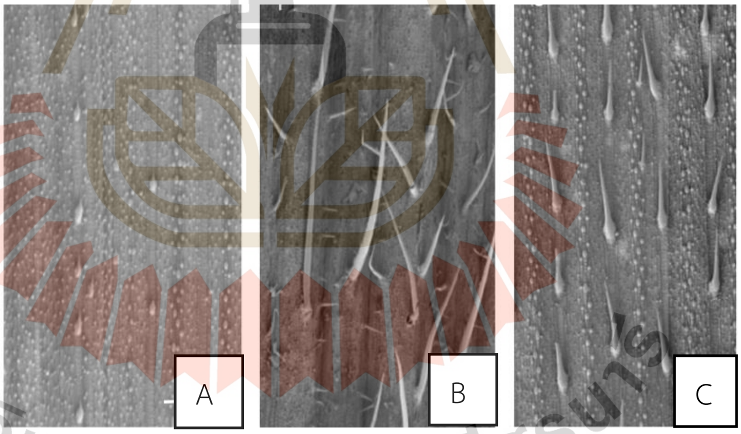

Figure 2.8 Trichomes of rice stem on susceptible variety (A) and resistance variety (B and C)

Source: Fei, et al. (2020)

2.6.2 Antibiosis

Antibiosis is a mechanism plant resistance inside, or chemical mechanism stimulate toxic to insect after feeding. That reduce the number of insect In related research were used methods as indicators this mechanism as honeydew excretion, survival rate, and emergence rate to detect the antibiotic level on rice plant

(Qiu et al., 2011). For example, the BPH is affected by both the physical barrier of silica cells and by the induced resistance mediated by Herbivore induced plant volatiles in rice as a model high Si accumulator (Table 2.2).

2.6.3 Tolerance

Tolerance is a plant response to an insect pest, plant reduce detrimental effects, they can produce grain and product even if access with insect (Kant et al., 2015). Previous studied was evaluation of resistance capability in rice to *S. furcifera*, the researcher observed leaf yellowing days after infestation, they found resistant varieties used time to leaf yellowing longer than susceptible varieties (Huang et al., 2018).

Table 2.2 Si-mediated plant resistance mechanisms and defensive responses reported in the literature.

Crop	Insect Species	Resistance Mechanism	References
Rice	Rice leaf folder *Cnaphalocrocis medinalis*	Reduced insect food quality and food conversion efficiencies; priming defense-related enzymes	Han et al. (2015), Han et al. (2016)
Rice	Rice leaf folder *Cnaphalocrocis medinalis*	Induced defense based on HIPV production	Liu et al. (2017)
Rice	Asiatic rice borer *Chilo suppressalis* Walker	Impeded Stalk penetration and prolonged. penetration duration by early instar larvae	Hou and Han (2010)
Rice	Brown planthopper *Nilaparvata lugens* Stål.	Modulation of callose deposition	Hou, et al. (2017)
Rice	Brown planthopper *Nilaparvata lugens* Stål.	Antibiotic and xenobiotic effects targeting insect. physiological functions	He, et al., (2015)
Rice	Brown planthopper *Nilaparvata lugens* Stål.	Physical barrier and induced chemical defenses	Yang, et al., (2017).

Source: Alhousari and Greger (2018)

2.7 Technique to selection plant resistance to sucking-insects

Plant resistant varieties selection is one important method to manage insect pests. In research as tomato breeding for resistance varieties to whitefly were compared genotypes in each varieties by evaluated oviposition and number of nymphs that show higher in susceptible varieties than resistance varieties (Neiva et al., 2019); To evaluate resistance against jassids and thrips of cotton were used along with their economic yield and fiber traits (Shahid et al., 2015). Selection plant resistance in BPH has many methods, such as monitoring with electronic penetration graph (EPG) or Electropenetrography. Used choice test method, testing by insect prefer selected plant each variety. The honeydew test is measurement area of insect excretion honeydew, and salivary sheath is measuring number secrete saliva of ingestion.

Technique used to measure plant resistance to sucking-insects including.

1) Plant effect measurement such as direct insect feeding injury and correlation of plant factors with resistance. Insect feeding measurements such as measurements of insect feeding and development, measurements of insect feeding behavior.

2) Use of biotechnological methods to develop plant resistance to insect for example electrophoretic techniques, plant tissue culture and cell culture and resistance to insect in genetically transformed plants (Smith 1989). Rice resistance selection method to sucking insect pests related researches; confers antibiosis resistance to planthopper use feeding behavior recorded by EPG, nymph survival rate, honeydew area, nymph population growth rate., BPH fecundity, phylogenetic analysis, and the bulked seedling test. (Dua et al., 2009). Evaluated rice resistant varieties by seed-box screening test, feeding rate test by honeydew excretion, host plant preference, tiller mass screening, and bulked sergeant simple sequence repeats analysis (Jairin, 2005). The observation of developmental stage and survival, observing of the behavioral response of WBPH to select rice varieties, Determination of rice physiological indices, Tolerance of rice were determined Evaluation of leaf yellowing days (Huang et al., 2018). However, biotechnological methods are expensive, take a long time to success and need specific skill of professional to use this method.

3) Electronic penetration graph (EPG) or electropenetrography monitoring of insect feeding, allows highly rigorous detection, quantification, and statistical analysis of stylet penetration behaviors of any piercing-sucking arthropod on an electrically conductive feeding substrate. They are important technology in selection plant resistance to homopteran feeding. There is a measure electrical in plant and insect. In the 1960s, EPG was used in aphids for monitoring probing behavior. That was developed by McLean and Kinsey. Over the 60+ years since its development, EPG instrument designs have advanced with the revolutions in electronics through three major generations (McLean and Kinsey 1964, Tjallingii 1978, Backus and Bennett 2009). That is the alternating current (AC) circuitry, and next, there was developed to direct current (DC) circuitry. The latest version, the AC-DC electropenetrograph (Backus and Bennett

2009), used in the present work, enables large insects like sharpshooters to be studied to the same depth as aphids.

In currently, EPG is used to study many piercing-sucking insects, such as (Jia et al., 2021); used EPG in monitoring the transmission SRBSDV by WBPH rice plants treated with silicon, (Cao et al., 2013); study feeding-induced interactions Between BPH and WBPH, (Backus et al., 2005); study waveform characterization of *Homalodisca coagulata* on grape and transmission of *Xylella fastidiosa*, (Zhu et al., 2020); used EPG to study effect of three triflumezopyrim concentrations in BPH and WBPH, (Seo et al., 2010); study survival rate and stylet penetration behavior of BPH on resistant rice varieties, etc. Used EPG was observed feeding behavior as interaction between the salivary sheath and rice (Huang et al., 2015). the feeding behavior of BPHs on rice plants wide types and transgenic plant by EPG (Dua et al., 2009). However, the EPG method needs professionals to use equipment and analyze results.

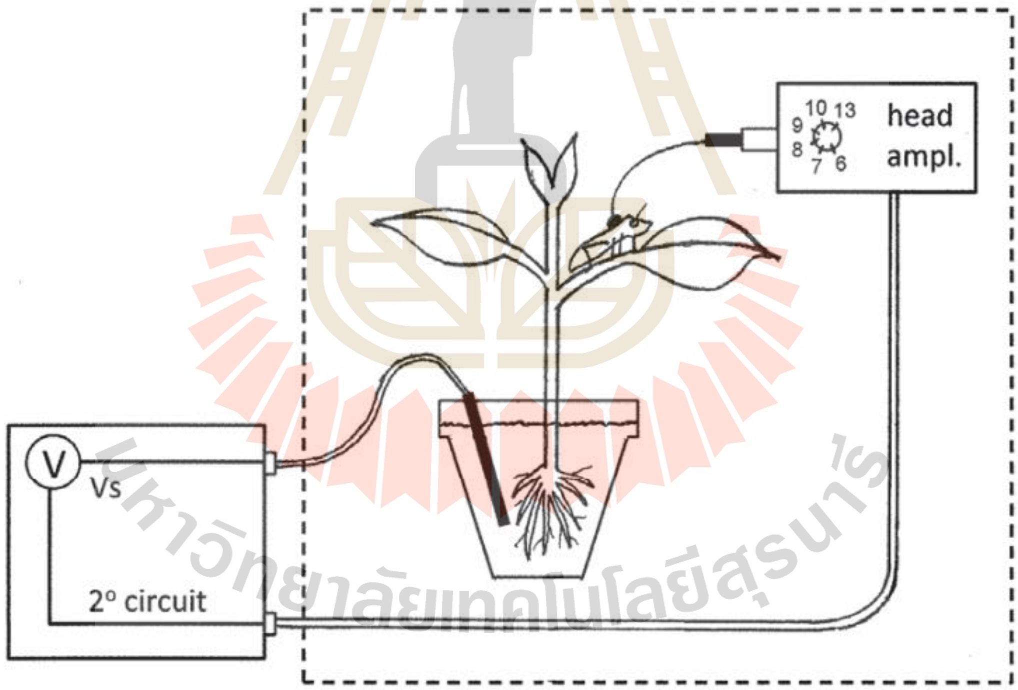

Figure 2.9 Diagram of Electropenetrography (EPG) connects with sucking insect.
Source: Backus et al. (2020)

4) Honeydew and salivary sheath

Rice planthopper is excreted honeydew during feeding sucking sap (phloem or xylem each planthopper species) using parafilm sachet method and measure weight

(Figure 2.10, Jairin et al., 2017) or area of honeydew and secrete gelling saliva were cut feeding area and immersed into 1% eosin and count the number of salivary sheaths. That is selected method for plant or rice? This method also used for selection the host preference resistant varieties (Cao et al., 2013).

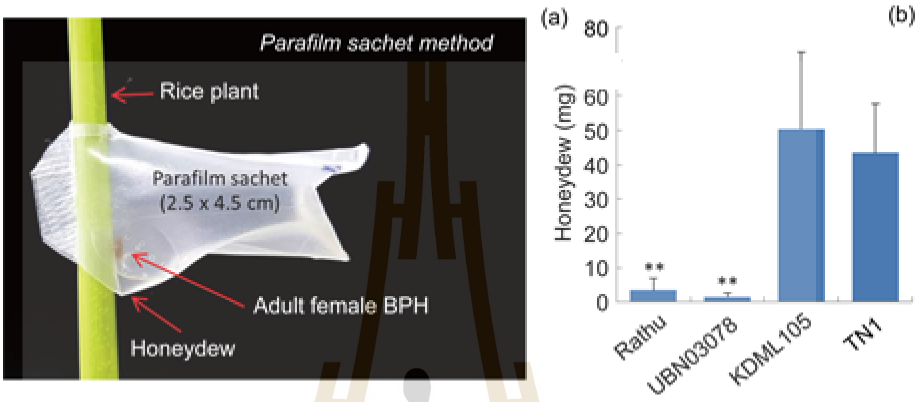

Figure 2.10 Evaluation of rice resistant to BPH *Nilaparvata lugens* (Stål) by parafilm sachet method

Source: Jairin et al. (2017)

5) The host selection by choice test is used to selected plant varieties resistance from insect pests. This method sets plant varieties for insect select host, depending on preference of insect behavior and specific character of plant (Figure 2.11). They need compatibility between them, which have many factors to select such as signals and information content-volatile compounds that specific individual host plant. Sensory aspects-insect can receive signals from host (Carrasco et al., 2015). However, this method needs the optimum time to observe insect selection (each insect has a different behavior to select host and feed on plant).

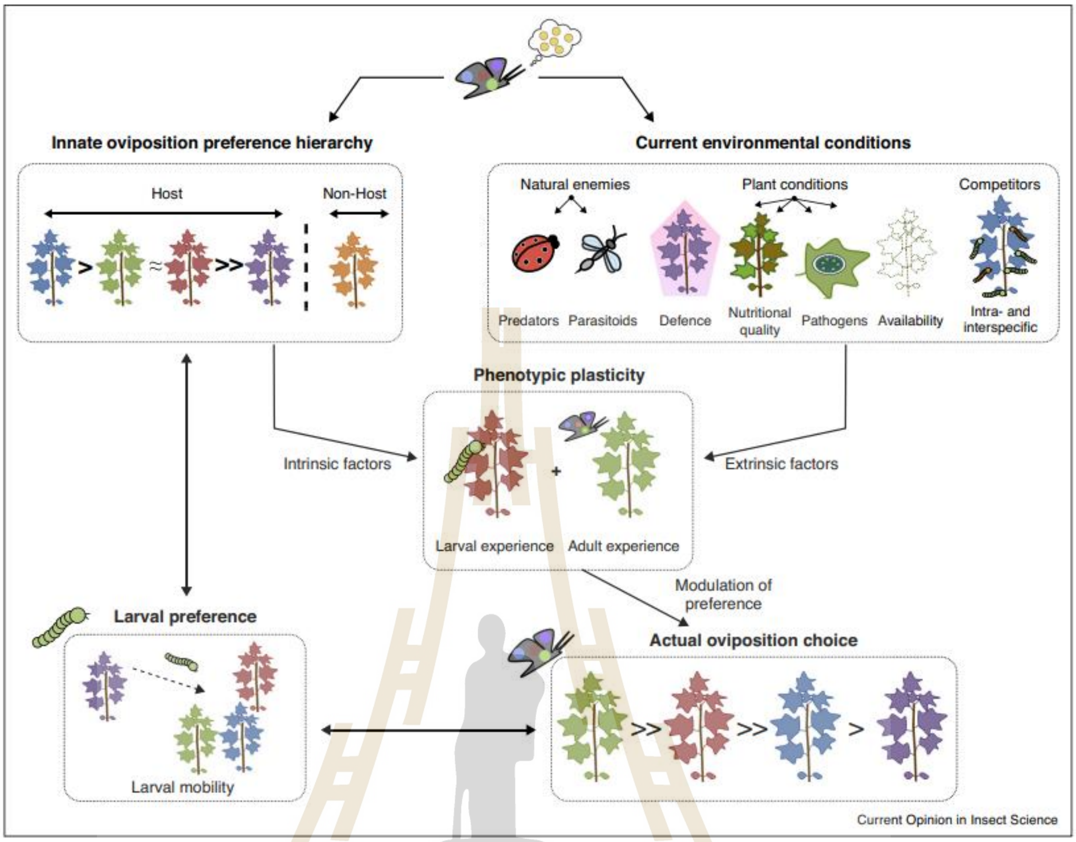

Figure 2.11 The factors influencing host plant choice in herbivorous insects.

Source: Carrasco et al. (2015)

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Study the feeding behavior of brown planthopper *Nilaparvata lugens* using the electropenetrography (EPG)

3.1.1 Insect rearing

Brown planthopper, *N. lugens* from Ubon Ratchathani Rice Research Center (year 2019). The insect was maintained for 7-14 days Khao Dawk Mali 105, (KDML105) plant in a plastic box (16x11cm) in the laboratory with 28 ± 2 °C, 60 ± 5% RH, light: dark 12:12 hours in the Entomology laboratory, Suranaree University of Technology. The next generation of planthoppers became 4-5 nymph stage and adults 5-7-day-olds, were transferred into a glass tube by an aspirator to test the feeding behavior and screening the rice resistance experimental.

3.1.2 Plant materials

Rice KDML105 variety plants were sow rice seed in a plastic box (16x11cm) with soil. The rice plants were watered every day. And then 25-30 days after transplanting in a pot (1 plant per plastic glass). The rice was maintained with flooding, in the greenhouse to control the insect pest infection with 28 ± 2 °C, 60 ± 5% RH, light: dark 12:12 hours.

3.1.3 Insect wiring.

Nymph and adult brown planthopper were removed from the rice KDML105 colony and held in small plastic vials on ice for 3-4 minutes, then wiring. A 12.5-µm diameter gold wire (sold as 0.0015 in diameter by Sigmund Cohn Corp., Mt. Vernon, NY) with a length of 3 cm was glued to the pronotum of an insect using silver glue described in more detail in Cervantes and Backus (2018). Insects were then held (without food or water) while dangling from the wire in an enclosed, humid box for 0.5 hours before EPG recording, to complete wiring for all insects.

Figure 3.1 The leafhoppers were wired with gold wire.

3.1.4 EPG recording

A 4-channel AC-DC electropenetrograph (Backus et al. 2020); manufactured by EPG Technologies, Inc.., Gainesville, FL) was used to record the probing behaviors of brown planthopper, *N. Lugens*, with 20-25 mV AC, 100-Hz substrate voltage applied to the soil of four intact, asymptomatic grapevines, and input resistor (Ri) set at 10^{10} Ohms. Each rice was placed in a plastic pot tray to electrically isolate it from the cage. The plants, insects, and amplifiers were all set up inside a Faraday cage to block external noise sources. Recordings were made under conditions of 27 ± 2 °C and 60 ± 5% RH. to monitor the stylet probing activities of brown planthopper, *N. lugens* on the KDML105 varieties. Recordings were made for 10 hours. 20 replications.

Brown planthopper stylet penetration was recorded with WINDAQ Pro+data acquisition software using a DI-720 analog to digital board (both from Dataq Industries, Akron, OH) at a sample rate of 100 Hz. Recordings are manually measured using the Notes feature in Windaq Waveform Browser. The recordings are artificially terminated (Ebert et al. 2015) for 10 hours after the start of recording (Krugner et al. 2019, Backus et al. 2021)

Figure 3.2 AC-DC electropenetrograph recording; rice in plastic pot tray inside the Faraday cage.

3.2 Screening for rice resistant varieties

Screening for rice resistant varieties 6 experiments were tests in entomology laboratory, Suranaree University of Technology.

3.2.1 Insect rearing

Brown Planthopper, *N. lugens* from Ubon Ratchathani rice research center. The insects were maintained for 7-14 days on KDML105 variety plant in a plastic box in the laboratory with 28 ± 2 °C, 60 ± 5% RH, light: dark 12:12 hours. When the next generation of plantoppers become the 4th-5th instar nymphs are transferred into a glass tube by an aspirator to test the feeding behavior and screen the rice resistance.

Figure 3.3 Insect colony was maintained using caged rice seedlings.

3.2.2 Plant materials

The 8 rice seed varieties from Ubon Ratchathani Rice Research Center (Table 3.1) were sowed rice seeds in plastic boxes with soil. The rice plants will be watered every day. And then 25-30 days after transplanting in a pot (1 plant per plastic glass). The rice was maintained with flooding, in the greenhouse to control the insect pest infection with 28 ± 2 $^{\circ}$C, 60 ± 5% RH, light: dark 12:12 hours. At 40-45 days after transplanting, the 8 rice varieties are used in the experiments.

Table 3.1 The status and sources of rice 8 varieties to use in an experiment.

No.	Varieties	Status	References	Sources
1	KDML105	Susceptible	Jairin et al., (2005)	
2	TN1	Susceptible	Angeles, et al., (1986) Ferrater et al., (2015)	
3	Rathu Heenati	Resistant	Jairin et al., (2005)	
4	Babawee	Resistant	Jairin et al., (2005)	
5	PTB33	Resistant	Ferrater et al., (2015), Jairin et al. (2007).	Ubon Ratchathani Research Center
6	Mudgo	Resistant	Sogawa, and Pathak, (1970)	
7	ASD7	Resistant	Jairin et al., (2005), Sun et al., (2006)	
8	UBN03078-101-342-4-148	Unknown	-	

To study and test the appropriate method for screening for resistant rice varieties through 6 experiments including.

3.2.3 Honeydew test

To compare honeydew production from the brown planthopper which fed on the KDML105, TN1, Rathu Heenati, Babawee, PTB33, Mudgo, ASD7, and UBN03078-101-342-4-148. Used a Completely Randomized Design (CRD), with 20 replications. The 4^{th}-5^{th} instars nymph was released on the leaf cage two insects per 40–45-day-old rice plant. The filter paper was placed on the bottom of the leaf cage (Whatman No. 1). The planthoppers were allowed to feed for 24 hours (1 insect per 1 cage). After that, the insect was removed from the stem and frozen in the -40 ˚C freezer. The filter paper was treated with ninhydrin 0.1%, for indicates the honeydew was blue-rimmed. The blue spots represent phloem-derived honeydew the filter paper was brought into a hot air oven at 90 °C for 10 minutes, after which the filter papers were collected and photographed with a Nikon digital camera (Nikon Corp Tokyo, Japan) on an illuminated bench. The area of all spots was measured from the images using Image-J version 1.48 (National Institute of Health, Bethesda, MD, USA).

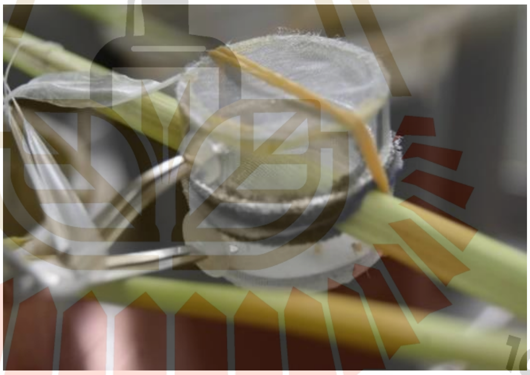

Figure 3.4 Honeydew and Salivary sheath test

3.2.4 Salivary sheath test

To compare salivary sheath, produced by brown planthopper (KDML105, TN1, Rathu Heenati, Babawee, PTB33, Mudgo, ASD7, and UBN03078-101-342-4-148). The 4^{th}-5^{th} instar nymph was released on the leaf cage two insects per 40-45 days old rice plants. The planthoppers were allowed to feed for 24 hours (1 insect per 1 plant). After that, the insect was removed from the stem. The rice stem was cut the 2.5 cm (area in the leaf cage) washed with 70% alcohol, then soaked in 1% eosin for 10 minutes and washed in Deionized water 5-6 times. The stem tissue was dry and excessively absorbed water from the stem surface with tissue paper. The salivary sheaths were counted within a stereo microscope. The experiment used a Completely Randomized Design (CRD), with 20 replications per rice variety.

3.2.5 Development, survivorship, and leafhopper life cycle test

The first instar nymph was released on the 8 rice varieties plants (40-45 days old) 1 plant was put in a plastic pot and covered by a clear plastic cage (10 cm diameter, 40 cm height) with 1 insect per plant. After 24 hours, the insects were observed daily to record the developmental and survival rate from the nymph to the adult stage. The experiment used a completely Randomized Design, with 20 replications. The development, survival rate in each stage of the planthopper and gender were recorded and analyzed.

Figure 3.5 Survivorship test and life cycle observation on 8 rice varieties.

3.2.6 Study the feeding behavior of brown planthopper *Nilaparvata lugens* using electropenetrography (EPG) on 8 rice varieties.

The feeding behavior of brown plantoppers was studied on 8 rice varieties. The details of Insect wiring follow 3.1.3

3.2.6.1 EPG recording

A direct current electrical penetration graph (Wageningen Agricultural University, Wageningen, The Netherlands; Giga-8 EPG amplifier with a 10^9-Ω input resistance) was used to record probing behaviors of brown planthopper, *N. lugens*. Each rice was placed in a plastic pot tray to electrically isolate it from the cage. The plants, insects, and amplifiers were set up inside a Faraday cage to block external noise sources. Recordings were made under conditions of 27 ± 2°C and 60±5% RH.

Recordings were performed to monitor the stylet probing activities of brown planthopper, *N. lugens* on the 8 rice varieties. A total of 20 individual brown planthoppers were recorded for each of the 8 varieties. Recordings were made for 10 hours, using a Completely Randomized Design.

Brown planthopper stylet penetration was recorded with EPG stylet +d software and the waveforms were classified using the Stylet +a software for Windows. Then the waveforms were transformed by EPG_ParProc_JKI_mult for statistical analysis via Statistical Analysis System (SAS).

3.2.7 Preference rice variety by choice test

The 8 rice varieties' preferences were tested for brown planthopper in each rice variety by a Completely Randomized Design, with 4 replications. The 8 varieties of rice were planted with 10 plants per variety on one tray (size 30x50 cm) and the tray was put in a net cage (size 50x50x50 cm). When 40-45 days rice plants aged used in an experiment. The 4^{th}-5^{th} instars of brown planthopper were prepared in a Petri dish (100 insects per plate) and released on the tray center. After 24 and 48 hours, count the number of brown planthoppers per rice variety plant. And observed the symptoms of rice plants after 3, 5, 7, and 10 days.

3.2.8 Evaluation of plant trichome by scanning electron microscope (SEM)

The scanning electron microscope (SEM) was used to observe the trichome on the rice stem. Trichome types were examined to compare their shapes on the leaf sheath materials of each rice variety. The rice stem was cut into 5x5-mm pieces, fixed overnight with 6% paraformaldehyde, and washed thrice in phosphate-buffered saline. The scanning electron microscopy (SEM) samples were allowed to dry in a desiccator under a vacuum and were observed under SEM 1000X (SEM, FEI, Quanta450, and The Netherlands). The trichome character were identified two types: prickle and macro trichomes.

3.2.9 Evaluation of the selection method to screen the rice resistance.

Evaluation of the selection method to screen the rice resistance, data from six experiments were used for evaluation of the selection method to screen the rice resistance following Table 3.3 and analyze the correlation of parameters, principal component analysis, and cluster analysis of the rice resistance by Origin 2023b.

3.3 Data analysis

The EPG waveforms were analyzed with Windaq software (company). EPG waveforms were described in terms of the standard characteristics, including maximum peak voltage per waveform (mV), voltage level (extracellular, positive; or intracellular, negative), frequency (Hz), identified NP: non-penetration, Nl1 and Nl2 waveform: pathway phase, Nl3 waveform: salivation, Nl4 waveform: ingestion phase (Backus, and Shih 2020)

Experimental has used a Completely Randomized Design (CRD), from 3.2.3 to 3.2.6 repeat 20 replications. And 3.2.7 repeats 4 replications. The average honeydew areas, the average number of salivary sheaths, and the development, and survival rate in each stage of the planthopper were analyzed with SAS® OnDemand for Academics and mean comparison by Duncan’s New Multiple Range Test method.

The AC-DC EPG waveforms were quantified and subjected to statistical analysis using SAS software (SAS Institute, Cary, NC, USA). The Backus v2.0 SAS program (Backus et al. 2007; Ebert et al. 2015) was employed for conducting a mixed model analysis of variance and mean comparison using Fisher’s least significant difference with a significance threshold of $P < 0.05$. The variables analyzed included the number of waveform events per insect, waveform duration per insect (WDI), and waveform duration per event per insect (WDEI). The analyze waveform with, identified Np: non-penetration, Nl1 = penetration initiation, Nl2 = salivation and stylet movement, Nl3 = extracellular stylet activity near the phloem region, Nl4-a = intracellular stylet activity in the phloem region without ingestion, Nl4-b = phloem sap ingestion. pd = potential drop and Nl5 = unknown waveform.

The symptoms of rice plants were evaluated by a score of the standard evaluation system (SES) for rice (2013) following IRRI (2013); Rungrat et al. (2021) Table 3.2

Table. 3.2 The score of the standard evaluation system (SES) for rice.

Score	Description of symptoms	Level of resistance
0	Absent symptom or no damage, normal leaf color	Highly resistant
1	Tip of leaves slightly dried and light green	Resistant
3	First and 2nd leaves of most plants partially yellowing.	Moderately Resistant
5	Pronounced yellowing and stunting or about 10-25% of the plants wilting or dead and the remaining plants severely stunted or dying.	Moderately susceptible

Source: IRRI (2013); Rungrat et al. (2021)

Table. 3.2 The score of the standard evaluation system (SES) for rice. **(continued)**

Score	Description of symptoms	Level of resistance
7	More than half (≥50%) of the plants yellowing, wilting, or dying.	Susceptible
9	All plants dead	Highly susceptible

Source: IRRI (2013); Rungrat et al. (2021)

Table 3.3 The expectation of parameter use evaluation of the selection method to screen the rice resistance.

Methods	Characteristic	Resistance	Moderately Resistance	Moderately Susceptible	Susceptible	Moderately Susceptible
Honeydew	Honeydew drops production (mm^2)	.....	.....	.....	.....	.....
Salivary sheath	Number of salivary sheath (spot)	.....	.....	.....	.....	.....
Electropenetrography	Number of np waveform per insect (times)	.....	.....	.....	.....	.....
	The mean duration of np waveform per insect (minutes)	.....	.....	.....	.....	.....
	The total duration of np waveform per insect (minutes)	.....	.....	.....	.....	.....
	Number of Nl1-Nl2-Nl3 waveforms per insect (times)	.....	.....	.....	.....	.....
	The mean duration of Nl1-Nl2-Nl3 waveform per insect (minutes)	.....	.....	.....	.....	.....
	The total duration of Nl1-Nl2-Nl3 waveform per insect (minutes)	.....	.....	.....	.....	.....
	Number of nl4-a waveforms per insect (times)	.....	.....	.....	.....	.....
	The mean duration of Nl4-a waveform per insect (minutes)	.....	.....	.....	.....	.....
	The total duration of Nl4-a waveform per insect (minutes)	.....	.....	.....	.....	.....
	Number of Nl4-b waveform per insect (times)	.....	.....	.....	.....	.....
	Mean duration of Nl4-b waveform per insect (minutes)	.....	.....	.....	.....	.....
	Total duration of Nl4-b waveform per insect (minutes)	.....	.....	.....	.....	.....
Life cycle	Total of nymph's stage Life cycle duration (day)	.....	.....	.....	.....	.....
	Total of adult stage Life cycle duration (day)	.....	.....	.....	.....	.....
Survivor rate	Percentage of survivor rate (%)	.....	.....	.....	.....	.....
Choice test	A score of symptoms on rice plant after insects' infestation	.....	.....	.....	.....	.....
	Insects' preference number (insects)	.....	.....	.....	.....	.....
Plant trichome by SEM	Dentistry of macro trichome	.....	.....	.....	.....	.....
	Dentistry of prickle trichome	.....	.....	.....	.....	.....

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Study the feeding behavior of brown planthopper *Nilaparvata lugens* (Stål) using the electropenetrography (EPG)

Electropenetrography (EPG) waveforms for *N. lugens* were hierarchically divided into waveforms and types, based on biological correlations and characteristic frequencies of other AC-DC EPG waveforms (Almeida and Backus, 2004). *N. lugens* waveforms were alphabetically ordered and considered a waveform family (i.e., Nl1, Nl2, Nl3, Nl4, Nl5, pd, and NP). When the waveforms were further expanded to examine details of their fine structure, they were categorized by waveform type numerically within the family designations (i.e., Nl2a, Nl2b, etc.). In this study, we explored the probing behavior of the BPH *N. lugens* on rice plants using AC-DC EPG, a valuable technology for studying hemipteran behavior. Using EPG, ten distinct waveforms were identified, each characterizing different aspects of the animals' feeding behavior. Although some waveforms showed similarities in appearance and biological significance to those observed in other sucking insects such as sharpshooters, pea aphids, stink bugs, and citrus psyllids (Almeida and Backus, 2004; Backus et al., 2005, 2016; Lucini and Panizzi, 2017; Shugart et al., 2019), we noted qualitative differences in the feeding behavior of the BPH *N. lugens*

4.1.1 NP waveform

The NP waveform started as a line at 0 Volt; every graph's initial phase included insects not currently ingesting anything (Figure 4.1A, Table 4.1). The NP waveform corresponded to the baseline and was associated with walking resting oviposition, and labial dabbing. The NP waveform represented the insect standing still on the plant's surface, whereas the NP waveform indicated the insect walking on the plant's surface. Both waveforms are typical of sucking insects and denote activities like landing, adhesion to the plant surface, oviposition, and walking (Almeida and Backus, 2004). Interestingly, the NP waveform was also observed in the BPH *N. lugens* using DC-EPG (Ghaffar et al., 2011)

4.1.2 Nl1 waveform

The Nl1 waveform was characterized by moderately regular, very slow peaks at the beginning of the probe (Figure 4.1A and 4.1B). These peaks were usually seen at the highest amplitudes. The average voltage of this waveform was 4.73 ± 0.91 Volt (Table 4.1) and started after the NP waveform. Sometimes Nl1 waveforms were interrupted by Nl2 waveforms (Figure 4.1A). The Nl1 waveform was associated with the pathway phase, which showed similar characteristics to the A and B waveforms of sharpshooters (Backus and Shih, 2020; Backus et al. 2016). Backus and Shih (2020) showed that the A waveform represented the first insertion of a stylet, and the B waveform represented the extension of sheath branches. Further, the A and B (B1s) waveforms represented probing activities in the epidermis and mesophyll cells.

4.1.3 Nl2 waveform

The Nl2 waveform was always observed after the Nl1 waveform and was followed by either Nl3 or Nl1 (Figure 4.1A). The Nl2 waveform occurred at all voltages. It's typical chevron-shaped phase, amplitudes, and frequency (Table 4.1) made it easily recognizable. In addition, more than one Nl2 waveform sometimes occurred in a single waveform event, and more than one event sometimes occurred in a single probe. This led to dividing this waveform into two subtypes: Nl2a and Nl2b.

The Nl2a waveform appeared after the Nl1 waveform. Nl2a was a pathway phase like the Nl1 waveform, but Nl2a waveforms often had a pattern of low-voltage peaks that looked like spikes (Figure 4.1A, 4.1C, 4.2B, and 4.2A). This waveform had a minimum absolute amplitude of 4.46 ± 1.01 Volt (Table 4.1).

The Nl2b waveform appeared after the Nl2a waveform. Nl2b waveforms showed deep troughs with spikes on top (Figure 4.1A, 4.1D, 4.2B, and 4.3A). The waveform had a maximum absolute amplitude of 6.18 ± 0.38 Volt (Table 4.1).

The Nl2 waveform was associated with the pathway phase, and these waveforms showed similar characteristics to the A and B waveforms of sharpshooters (Backus and Shih, 2020; Backus et al., 2016), which was previously discussed in the Nl1 waveform. Moreover, the Nl2 waveforms seen in the present study were similar to C waveforms of the Asian citrus psyllid representing insects probing on epidermis, parenchyma, and cambium tissue into vascular tissue (Shugart et al., 2019).

The Nl1 and Nl2 waveforms were associated with the pathway phase, and these waveforms showed similar characteristics to the A and B waveforms of sharpshooters (Backus and Shih, 2020; Backus et al. 2016). Backus and Shih (2020) showed that the A waveform represented the first insertion of a stylet, and the B waveform represented the extension of sheath branches. Further, the A and B (B1s) waveforms represented probing activities in the epidermis and mesophyll cells. The Nl2 waveforms in the present study were similar to the C waveforms of the Asian citrus psyllid, representing insects probing the epidermis, parenchyma, and cambium tissue into vascular tissue (Shugart et al., 2019).

4.1.4 Nl3 waveform

Nl3 waveform, the spikelet waveform high peak, continues in the short term (2–3 spike), and the low peak continues in the short term (2–3 spike) that appears before Nl4 and sometimes appears between or after Nl4. Waveform Nl3 was a short event with a high absolute amplitude of 6.35 ± 1.0 Volt (Table 1). This waveform appeared between the NL4 waveform (Figure 4.1A, 4.2A, and 4.2B).

The Nl3 waveform was associated with phloem tissue. This waveform showed similar characteristics to other sucking insects, such as the E1 waveform of the Asian citrus psyllid (Shugart et al., 2019), representing insect probing and salivation on phloem tissue. The Nl3 waveform had a similar appearance to other phloem (E1 and E2 waveforms) and xylem (XC1 waveform) ingestion waveforms in heteropterans (Backus et al., 2005; Backus and Shih, 2020). The XC1 waveform represents the mixing and uptake of saliva and injection into cells by sharpshooters; this waveform represents activity in the xylem cells (Backus and Shih, 2020).

4.1.5 Nl4 waveform

The Nl4 waveform began only after the Nl3 waveform and appeared as repeating spike waveforms that continued over long events. The Nl4 waveform had a high absolute amplitude of 6.43 ± 1.20 Volt (Table 4.1). The Nl4 waveform was often interrupted by the short waveform Nl3 (Figures 4.1A, 4.2A, 4.2B, 4.2C). The Nl4 waveform showed the highest mean WDI (Table 2).

The Nl4 waveform was associated with phloem tissue. The appearance of the Nl4 waveform showed similar characteristics to other xylem and phloem ingestion waveforms in some heteropterans (Backus et al., 2005; Cervantes et al., 2016; Lucini and Panizzi, 2018; Backus et al., 2019; Shugart et al., 2019; Backus and Shih, 2020). The Nl4 waveform was similar to the XC2 waveform of sharpshooters, representing activity in xylem cells and insects testing the viscosity of the saliva mixture

(Backus and Shih, 2020). Moreover, the Nl4 waveform is similar to the Df2 waveform, showing ingestion on xylem sap, and the Df4b waveform, showing seed endosperm ingestion.

The Nl3 and Nl4 waveforms were associated with phloem tissue; the Nl3 waveform showed similar characteristics to other sucking insects, such as the E1 waveform of the Asian citrus psyllid (Shugart et al., 2019), representing insect probing and salivation on phloem tissue. The Nl3 waveform had a similar appearance to other phloem (E1 and E2 waveforms) and xylem (XC1 waveform) ingestion waveforms in heteropterans (Backus et al., 2005; Backus and Shih, 2020). The XC1 waveform represents the mixing and uptake of saliva and injection into cells by sharpshooters; this waveform represents activity in the xylem cells (Backus and Shih 2020). The appearance of the Nl4 waveform showed similar characteristics to other xylem and phloem ingestion waveforms in some heteropterans (Backus et al., 2005; Cervantes et al. 2016, Lucini and Panizzi 2018; Backus et al., 2019; Shugart et al., 2019; Backus and Shih, 2020). The Nl4 waveform was similar to the XC2 waveform of sharpshooters, representing activity in xylem cells and insects testing the viscosity of the saliva mixture (Backus and Shih 2020). Moreover, the Nl4 waveform is similar to the Df2 waveform, showing ingestion on xylem sap, and the Df4b waveform, showing seed endosperm ingestion.

4.1.6 pd waveform

Pd waveforms occurred between Nl4 waveforms for a short duration with a low absolute amplitude of 2.46 ± 1.11 Volt (Table 1).

4.1.7 Nl5 waveform

The Nl5 waveform almost always showed after Nl4 and sometimes appeared during waveform Nl2b. Thus, this waveform was divided into Nl5a, Nl5b, and Nl5c (Figures 4.3 A, B). The Nl5a waveform took the form of spikes arranged in an up-and-down pattern like a bell curve. Waveform Nl5a was seen in short events with a low absolute amplitude of 3.84 ± 0.43 Volt (Table 1). The Nl5b waveform was similar to Nl5a, with spikes arranged pointing up and down like a mountain range. It had an average absolute amplitude of 2.94 ± 0.21 Volt (Table 1). The Nl5c waveform appeared following Nl5a. Nl5c showed a similar spike pattern to Nl5a with spikes arranged like a mountain range (low peak spike arranged almost a line). It had a peak absolute amplitude of 3.79 ± 0.42 Volt (Figure 4.3B, Table 4.1)

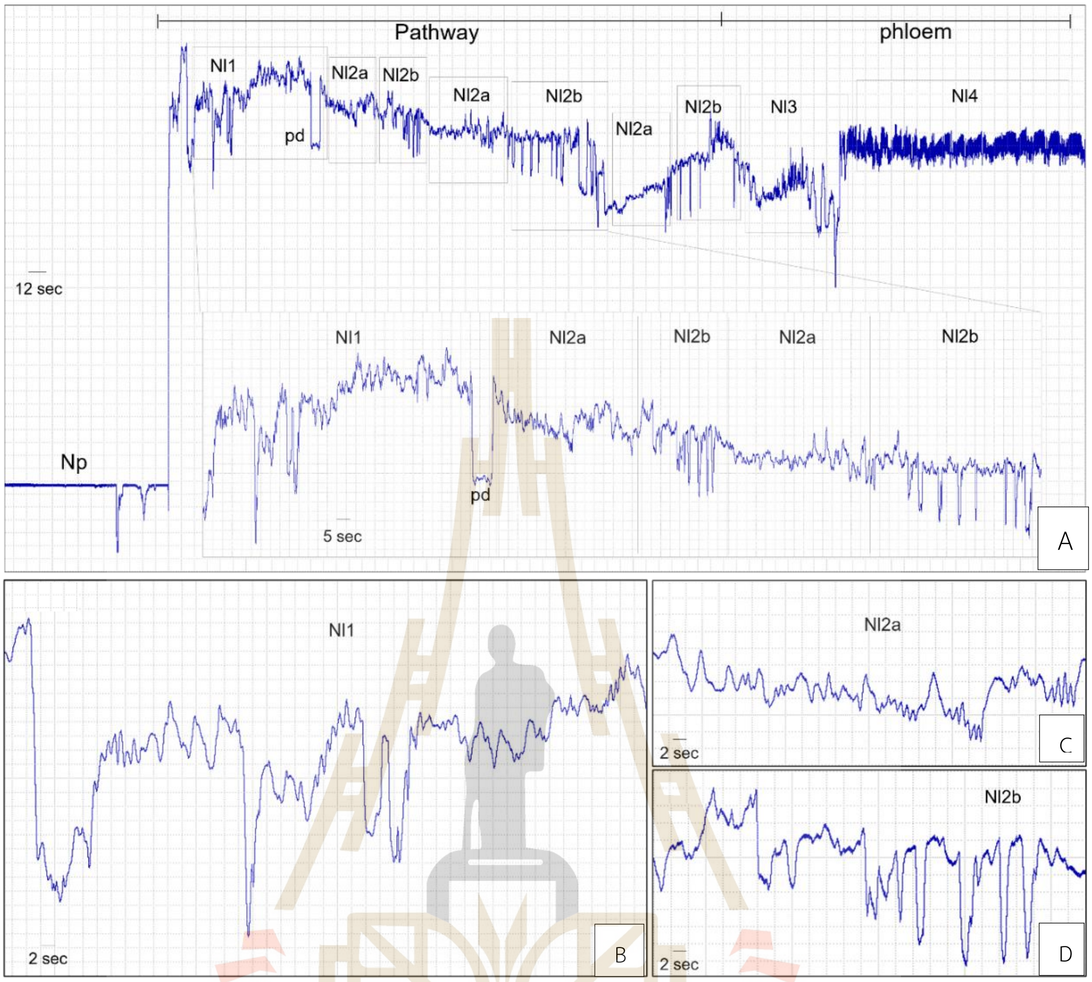

Figure 4.1 The AC -DC waveforms generated by the brown planthopper *Nilaparvata lugens* (Stål) probing on rice plants.

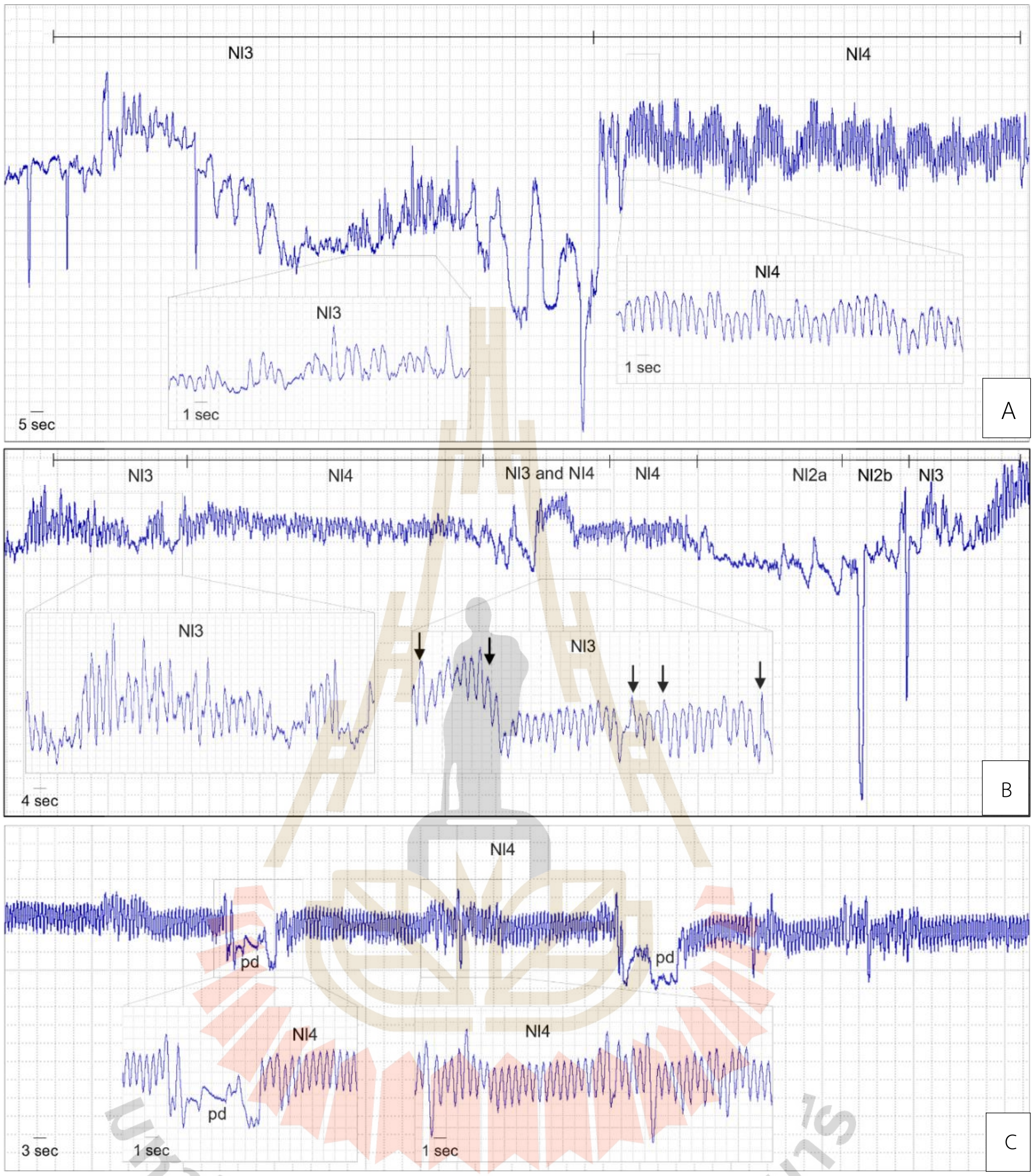

Figure 4.2 The AC-DC waveforms generated by brown planthopper *Nilaparvata lugens* (Stål) probing on rice plants.

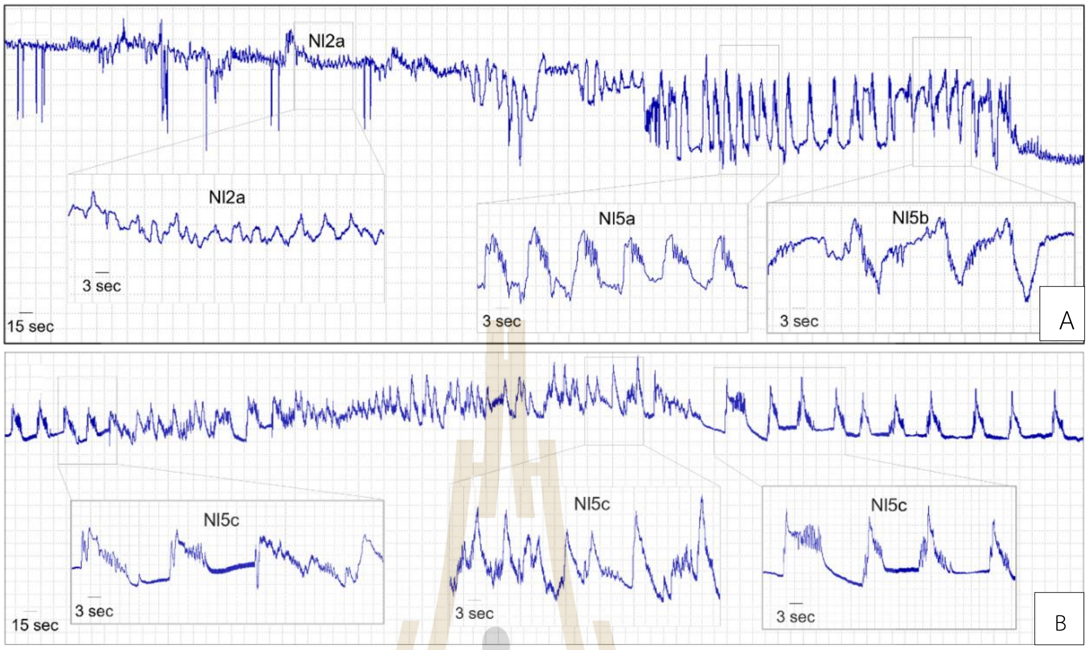

Figure. 4.3 The AC-DC waveforms generated by brown planthopper *Nilaparvata lugens* (Stål) probing on rice plants.

Table 4. 1 Summary of AC- DC electropenetrography waveforms, their main characteristics, and proposed biological meaning for each waveform recorded during the feeding behavior of the brown planthopper *Nilaparvata lugens* (Stål) on rice stems.

EPG Waveforms	Type	Frequency (Hz)	Voltages (mean ± SD)	Electrical origin	Suggested biological meaning, stylet activities
NP		0	0	-	No movement on the stem surface landing, adhesion on stem surface, oviposition, walking on the stem surface
Nl1		mixed	4.73 ± 0.91	-	Stylet penetration and salivary sheath secretion
Nl2	a	mixed	4.46 ± 1.01	R/emf	Stylet penetration, on epidermis
	b	mixed	6.18 ± 0.38	R	Mesophyll, parenchyma
Nl3		5.46	6.35 ± 1.83	R/emf	Salivation activities, Near phloem,
Nl4		8.12	6.43 ± 1.20	R/emf	Phloem sap ingestion
Pd			2.46 ± 1.11	emf	Rapid stylet withdrawal and re-insert / Resting with stylets inserted into any cell
Nl5	a		3.84 ± 0.43	R/emf	Unknown
	b		2.94 ± 0.21	R/emf	Unknown
	c		3.79 ± 0.42	R/emf	Unknown

4.1.8 Feeding process of adult brown planthopper *N. lugens*

The results of these recordings help to understand style movement during the feeding process and how this correlates with EPG waveforms (Figure 4.4). In the beginning, the tip of the stylet touches the plant surface from above. Here the EPG showed non-probing waveforms and then penetration waveforms. The stylet then contracts repeatedly to penetrate deeper into the plant tissue (penetration waveform). The two mandibular stylets penetrate the plant tissue alternating with the maxillary stylets, and all the stylets reach the phloem tissue (salivation and ingestion waveforms). Finally, the head is lowered, and the first labial segment of the beak is partly retracted into the head capsule. The stylets contract to pull up the pouches to generate space for the first labial segment packed into the head capsule.

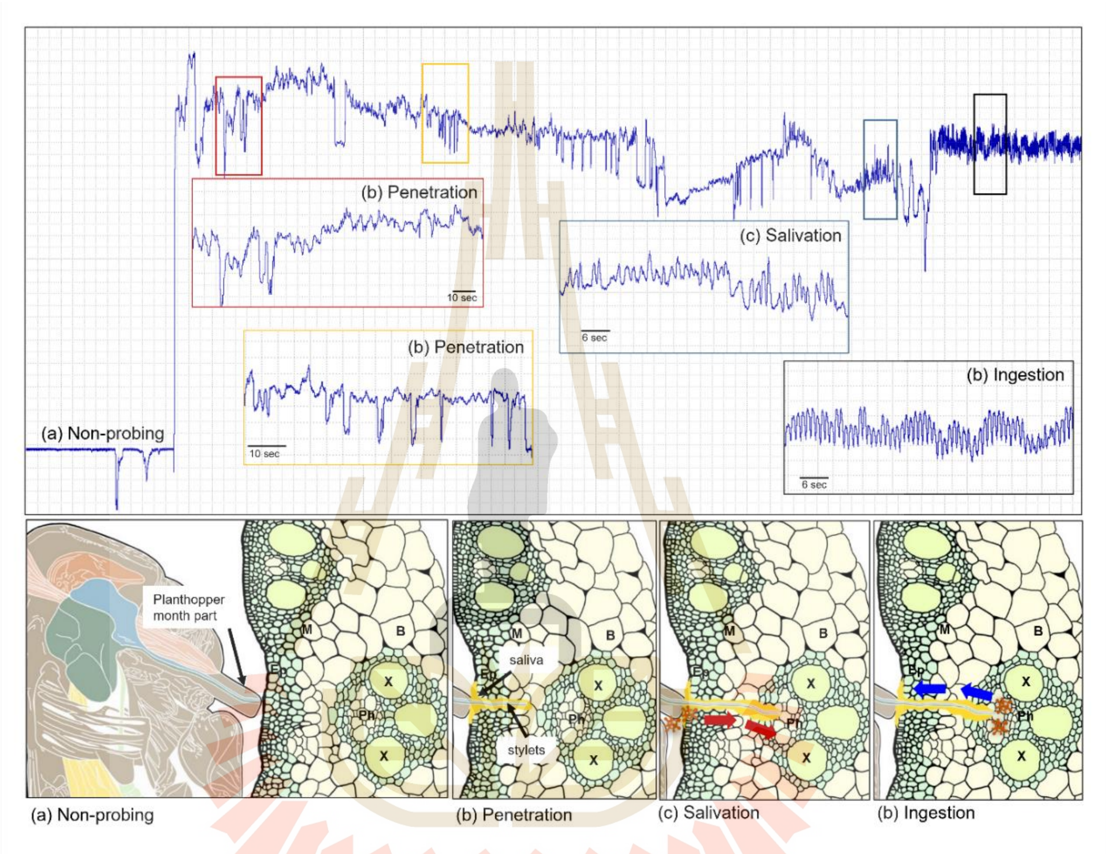

Figure 4.4 The feeding process of adult brown planthoppers feeding on plant tissue shown with the corresponding EPG waveforms.

4.1.9 Analysis of waveform measurements

1) Number of waveform events per insect; NWEI (counts)

The numbers of waveform events per insect for the Nl2b Nl3, Nl4, Nl5a, Nl5b, Nl5c, pd, and NP waveforms were not significantly different among individuals. However, there was a significantly higher frequency of Nl1 and Nl2a waveform events per insect than all other events ($F_{9, 1364} = 5.509$, $P < 0.0001$; Table 4.2).

2) Waveform duration per insect; WDI (seconds)

The WDI for the Nl1, Nl2a, Nl4, and NP waveforms did not significantly differ from each other, but they were significantly different from the other waveforms ($F_{9,59}$ = 7.953, P < 0.0001 (Table 4.2). The BPH showed longer mean WDI for Nl1, Nl2a, Nl4, and NP waveforms than other waveforms (Table 2) and showed the shortest mean WDIs for pd waveforms.

3) Waveform duration per event per insect; WDEI (seconds)

The WDEI for Nl4 waveforms did not show any significant differences with the WDEI of NP waveforms. In contrast, the WDEIs for Nl1, Nl2a, Nl5a, Nl5b, Nl5c, pd, and NP waveforms were significantly different from each other ($F_{9,1364}$ = 8.909, P < 0.0062; Table 4.2). BPH individuals showed the longest WDEI for NP waveforms (1466.78 ± 1239.24 s), followed by Nl4 and Nl2a waveforms (Table 4.2). In addition, the Nl5b and Nl5c waveforms did not show any significant differences in WDEI; the Nl2b waveform did not show any significant differences in WDEI with Nl3 waveforms. The shortest WDEI was seen for pd waveforms.

Table 4.2 AC-DC electropenetrography waveforms of the brown planthopper *Nilaparvata lugens* (Stål) feeding on rice stems.

EPG waveforms	NWEI (mean ± SEM)	WDI (mean ± SEM)	WDEI (mean ± SEM)	Percentage of recording time
Nl1	57.50 ± 4.54 a[/1]	6932.26 ± 864.53 a	144.22 ± 16.81 cd	19.25 a
Nl2a	52.17 ± 3.34 a	11487.87 ± 997.78 a	286.63 ± 27.43 bc	31.9 a
Nl2b	15.17 ± 3.11 b	204.65 ± 47.54 de	11.03 ± 0.70 f	0.568 de
Nl3	22.00 ± 1.00 b	245.03 ± 13.58 bcd	11.26 ± 0.41 f	0.681 bcd
Nl4	13.50 ± 0.98 b	4865.13 ± 385.61 a	438.83 ± 50.99 ab	13.51 a
Nl5a	6.50 ± 0.91 b	198.87 ± 34.32 cd	22.89 ± 2.25 ef	0.552 cd
Nl5b	8.67 ± 0.99 b	386.10 ± 51.46 bc	35.48 ± 2.79 de	1.072 bc
Nl5c	14.67 ± 1.48 b	849.27 ± 110.03 b	79.69 ± 10.29 de	2.359 b
Pd	22.00 ± 2.32 b	23.43 ± 2.12 e	1.07 ± 0.04 g	0.065 e
NP	15.17 ± 1.22 b	10814.32 ± 946.35 a	1466.78 ± 189.36 a	30.03 a
F-value	5.509	7.953	8.909	7.653
***P*-value**	<0.0001	<0.0001	<0.0001	<0.0001

NWEI; number of waveforms per events per insect (time ± SEM), WDI; waveform duration per insect (sec ± SEM), WDEI; waveform duration per events per insect (sec ± SEM),[1] Mean followed by the same letters in each column do not significantly differ at $P > 0.05$ by Fisher's least significant difference test.

4.1.10 Transitional probability and kinetograms of EPG waveforms

Transitional probabilities are the frequency of occurrence of a waveform following in sequence from the previous waveform. The transitional probabilities of the BPH *N. lugens* on rice plants recorded as part of this study are summarized in kinetograms in Figure 4.5. Not all waveforms followed each waveform; the non-probe NP waveform was only followed by waveform Nl1. The probing sequence for all insects in this study always began with the Nl1 waveform, representing the intercellular pathway phase. Once the Nl1 waveform began, BPH returned to the NP phase rather than initiated to the Nl2 (pathway phase), Nl5, Nl3, or pd waveforms (Figure 4.5). There was approximately a 50% chance of changing to Nl2, and 18% to Nl5 waveforms (Figure 8). Waveform Nl2 transited to the Nl1, pd, Nl5, or Nl4 waveforms at 36%, 22%, 20%, 14%, and 3%, respectively (Figure 8). The Nl3 waveform transitioned to Nl1 or pd waveforms but not to the NP waveform. The Nl3 waveform transitioned to Nl4 at a rate of approximately 50%. Nl4 waveforms almost always transitioned to Nl3 but not to NP or Nl3 waveforms. Waveform Nl5 transitioned to all other waveforms, and Nl5 waveforms almost always transitioned to Nl1 or Nl2 waveforms. The pd waveform transitioned to Nl1 or Nl5 but not transited NP waveforms.

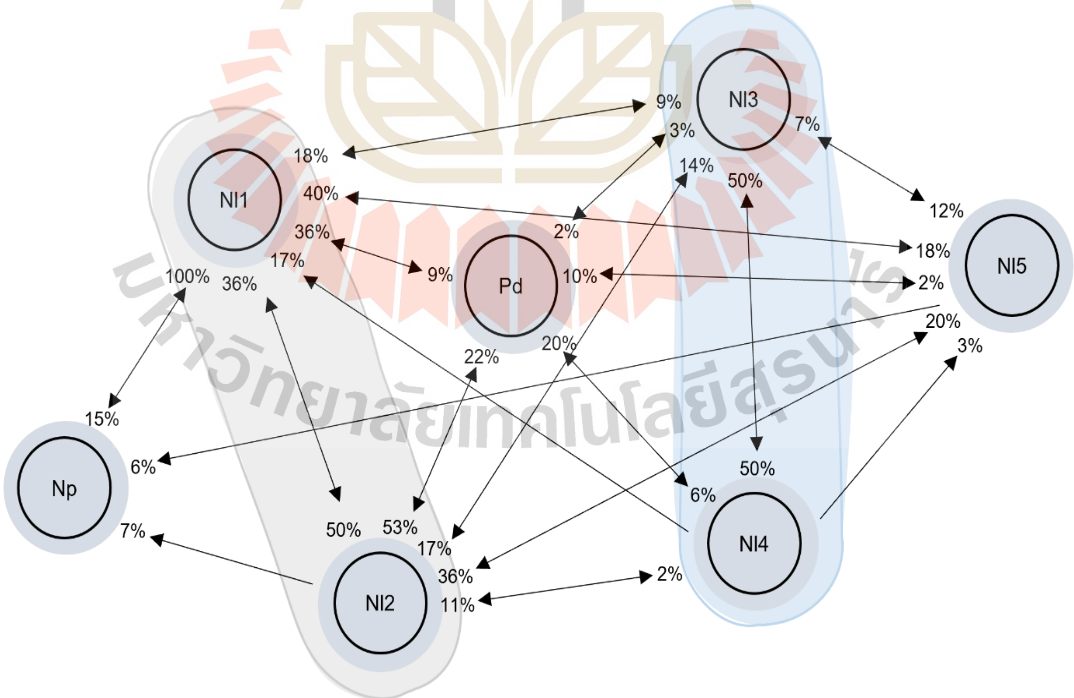

Figure 4.5 Kinetogram of waveform transitions in the brown planthopper *Nilaparvata lugens (Stål)*

4.2 Screening for rice resistant varieties

Feeding behavior of brown planthopper *N. lugens* determined by honeydew area, salivary sheath, electropenetrography, and host choice test experiment.

4.2.1 Honeydew test

The honeydew area of the brown planthopper *N. lugens* fed on different rice varieties showed a significant difference ($P < 0.0001$), the highest on the KDML105. However, the honeydew area of planthopper fed on KDML105 was not significantly different from the Babawee and TN1 varieties. Moreover, that showed the lowest honeydew area on Rathu Heenati, Mudgo, and PTB33 (Figure 4.6). The resistance varieties/cultivar/germplasms were less honeydew on these than on the susceptible ones. The feeding behavior of brown planthopper *N. lugens* determined by honeydew area was excreted during brown planthopper *N. lugens* feeding on phloem sap that showed susceptibility higher than resistance varieties (Ghaffar et al., 2011; Seo et al., 2010; Yorozuya, 2017). The experiment found that KDML105, Babawee, and TN1 varieties showed a high area of honeydew, meaning the brown planthopper *N. lugens* fed preference on these varieties (susceptible). Which honeydew test (48 hours) on TN1 shows the highest honeydew area contrast Mudgo, Rathu Heenati, and ASD7 (Qiu et al., (2011). However, Ghaffar et al., (2011) reported that the Babawee variety was resistant and showed a low area of honeydew studied by a honeydew clock. However, this result showed a high area probably because Babawee varieties had high transpiration during the experiment that appeared on filter paper (probably similar to the honeydew area). That showed a different result probably because they used a different technique: the honeydew clocks technique which filters paper Petri dish circle plate rotate for 12 hours between feeding on EPG. The honeydew clocks technique showed droplets on each hour and compared them with EPG feeding behavior. Moreover, a honeydew measurement technique uses the parafilm sachet method (Jairin et al., 2017). They measure the weights of honeydew excretion by using a 0.01-mg sensitivity balance (ME204, Mettler Toledo). This probably used the filter paper in the leaf cage method was unsuitable for rice or insects that should apply other techniques or should not be experimental when high transpiration. Furthermore, each rice stage is probably associated with showing resistance or susceptibility to rice-resistant genes.

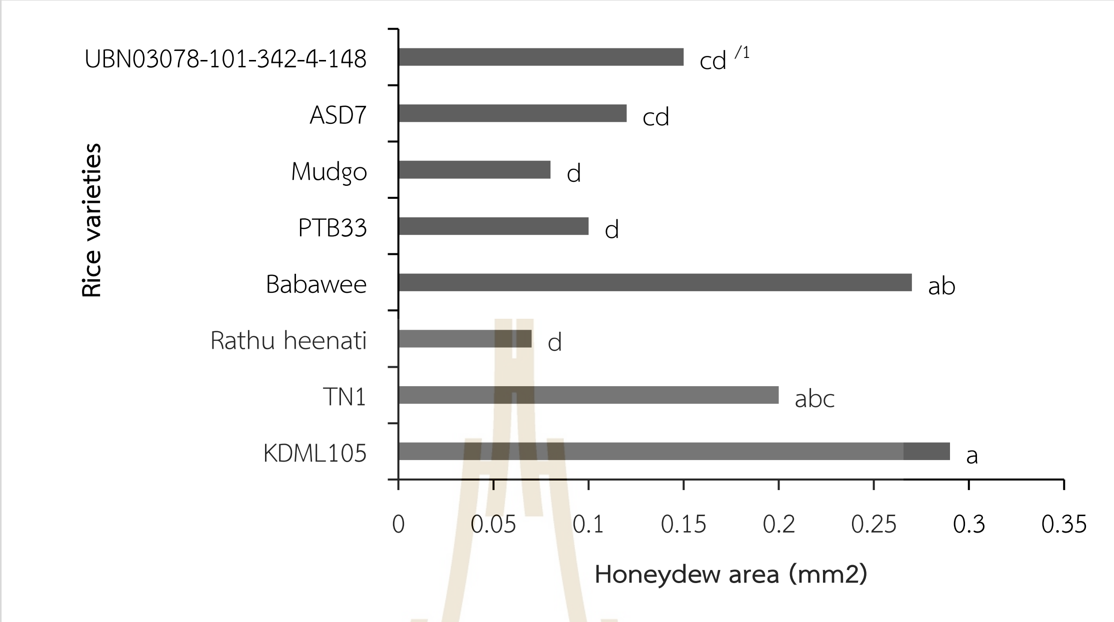

Figure 4.6 The honeydew area (mm^2) of brown planthopper *Nilaparvata lugens* (Stål) fed on different rice includes 8 varieties.

[/1] Data in the same letters were not significantly different at P > 0.05 by DMRT

4.2.2 Salivary sheath test

The number of salivary sheaths from the brown planthopper *N. lugens* showed the highest significant difference (P < 0.0001) on the PTB33 variety from other varieties. In contrast, that showed the low on TN1, KDML105, Babawee, and Rathu Heenati varieties (Figure 4.7).

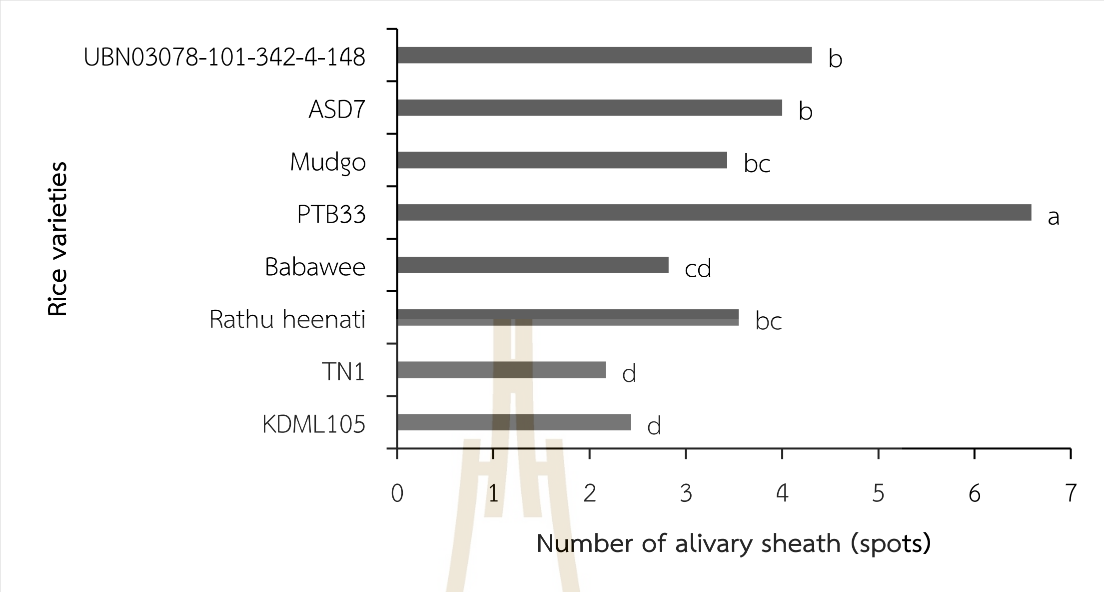

Figure 4.7 The salivary sheath (spots) of brown planthopper *Nilaparvata lugens* (Stål) fed on different rice includes 8 varieties.

Data in the same letters were not significantly different at P > 0.05 by DMRT.

From the salivary sheath experiment, in some insects, the mean number of salivary sheaths showed the highest on the resistant variety, which means the insect spent more frequency sucking on the resistant variety, such as the planthopper (Sogawa and Pathak, 1970). In contrast to the report by Zeilinger et al. (2008), the number of flanges and relative growth rate positively correlated with the stink bug. There was a high frequency of salivary sheath, which meant more feeding of stink bugs. According to brown planthoppers, *N. lugens* showed more frequent salivary sheaths in the susceptible rice variety than in the resistant variety (Wang et al., 2008). However, this result was shown differently because of a different period for the release of insects to feed on plants. Wang et al., (2008) released insects feeding for 4 days showed more sheath than on susceptible varieties. In this result, there was less time for insect feeding (24 hours probably showed movement BPH and tried to feed on resistant, but on susceptible 24 hours they can feed on phloem sap and then probably move to another position). Moreover, the result of the salivary sheath found in the PTB33 reported the resistance variety move frequency of the spot. This is probably the insect trying to feed food and get the nutrients. In contrast, the TN1 and KDML105 varieties found low salivary sheath spots. That means the insect accepted these varieties and localized nutrients on phloem enough for the insect development.

4.2.3 Development, survivorship, and life cycle test

The development of insects depended on the lack of nutrients, if plants had an abundant nutrient the insect still long life and reproduction. The brown planthopper *N. lugens* development showed significant differences as first-instar nymphs were development stage longest on the Babawee was (2.29 ± 0.47 days) not significantly different with KDML105, TN1, PTB33, Mudgo, and ASD7 varieties. However, the Babawee, KDML105, and Mudgo significantly differed from the Rathu Heenati and UBN03078-101-342-4-148 (Table 4.3).

The second-instar nymphs exhibited significantly different developmental stages, with the longest duration observed in KDML105 (3.12 ± 1.07 days), significantly different from the other varieties. However, the seven varieties did not significantly differ (Table 4.3).

The third-instar nymphs were longest development stage on PTB33 (3.50 ± 1.45 days) that not significantly different with KDML105, TN1, Babawee, Rathu Heenati, ASD7, and UBN03078-101-342-4-148 varieties (Table 4.3).

The fourth and fifth-instar nymphs were not significantly different (Table 4.3). On pre-adult was the longest development stage on PTB33 (15.46±6.09 days) that was not significantly different with KDML105, Babawee, Rathu Heenati, and Mudgo varieties (Table 4.3). Moreover, adults showed a highly significant difference ($P < 0.0001$), on KDML105 and TN1 longer than other varieties (Table 4.3). That relatively susceptible variety 28 - 30 °C was developing time complete 44 days (Manikandan et al., 2015). These results show nymphs' period of development was long for the adult stage on resistance varieties. In contrast, insects have faster development in the nymph stage on susceptible varieties which means they have a long life for oviposition.

Table 4.3 Development stage (day) (the mean ± standard error) and sex ratio of adult brown planthopper *Nilaparvata lugens* (Stål) fed on different rice varieties.

Rice varieties	1st instar	2nd instar	3rd instar	4th instar	5th instar	Pre-adult	Adult	Sex ratio Male:Female
KDML105	2.18 ± 0.94$^{a/1}$	3.12 ± 1.07^{a}	2.93 ± 0.94^{a}	2.54 ± 0.79	3.39 ± 2.41	14.14 ±1.80^{abc}	23.93 ± 9.02^{a}	1 : 2
TN1	1.83 ± 0.58^{ab}	2.33 ± 0.89^{b}	2.50 ± 0.52^{ab}	2.00 ± 0.60	3.25 ± 1.91	11.92 ±1.31^{c}	26.75 ± 12.92^{a}	1 : 1.4
Rathu Heenati	1.60 ± 1.01^{b}	2.29 ± 1.41^{b}	3.37 ± 2.52^{a}	2.60 ± 1.99	3.57 ± 3.11	13.43 ± 5.05^{abc}	9.37 ± 10.18^{c}	1 : 1.25
Babawee	2.29 ± 0.47^{a}	1.95 ± 1.30^{b}	2.52 ± 0.83^{ab}	2.19 ± 2.46	6.24 ± 6.66	15.19 ± 6.25^{ab}	9.24 ± 10.99^{c}	1 : 1.4
PTB33	1.93 ± 0.60^{ab}	2.04 ± 1.00^{b}	3.50 ± 1.45^{a}	3.43 ± 2.82	4.57 ± 5.96	15.46 ± 6.09^{a}	7.07 ± 9.22^{c}	1 : 1.8
Mudgo	2.18 ± 0.38^{a}	2.29 ± 1.90^{b}	1.89 ± 0.87^{b}	2.83 ± 0.92	3.80 ± 2.48	12.97 ± 3.14^{abc}	16.77 ± 10.17^{b}	1.2 : 1
ASD7	1.97 ± 0.93^{ab}	1.83 ± 0.91^{b}	2.53 ± 1.17^{ab}	2.33 ± 1.36	3.67 ± 3.98	12.23 ± 4.75^{c}	9.40 ± 10.87^{c}	1 : 1.4
UBN03078-101-342-4-148	1.61 ± 0.86^{b}	1.85 ± 1.03^{b}	3.21 ± 2.13^{ab}	2.48 ± 1.86	3.48 ± 3.22	12.64 ± 3.86^{bc}	9.85 ± 12.82^{c}	1 : 4
P-value	0.0026	0.0063	0.0007	0.1654	0.2145	0.0416	<0.0001	

[/1] Data in the same column with the same letters were not significantly different at P > 0.05 by DMRT

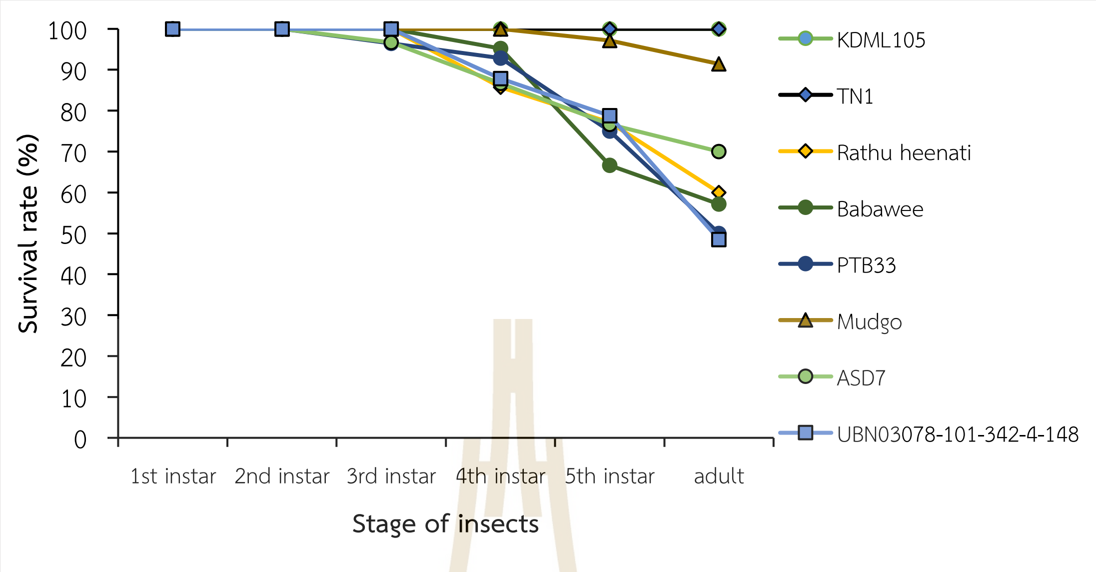

Figure 4.8 The survival rate (%) of brown planthopper *Nilaparvata lugens* (Stål) fed on different rice includes 8 varieties.

Besides, the survival rate of insects was high on preference and abundant of nutrient of plant for insect life (Qiu et al., 2011). This result showed the highest (100 %) on the KDML105 and TN1 varieties and 90% on the Mudgo variety, respectively (Figure 4.8). In contrast, in other varieties, the survival rate of nymphs was decreased (Figure 4.8). Qiu et al., (2011) reported a survival rate of more than sixty-five percentage was high survival including ASD7, Rathu Heenati, and TN1 and a low rate on the Mudgo variety.

4.2.4 Study the feeding behavior of brown planthopper *Nilaparvata lugens* using electropenetrography (EPG) on different 8 rice varieties.

1) Number of waveform events per insect; NWEI, (time)

The frequency of feeding behavior of the brown planthopper *N. lugens* on 8 rice varieties appears significant on the NP waveform ($P < 0.0002$). That same to Nl1-Nl2-Nl3 and Nl4-a waveforms ($P < 0.0001$), the Nl4-b ($P = 0.0019$), and Probe ($P = 0.0002$).

The brown planthopper *N. lugens* shows the highest NP waveform on TN1 varieties but not significantly with PTB33 and UBN03078-101-342-4-148, respectively (Figure 4.9). In contrast, KDML105 shows the lowest and no significant difference with Babawee, Rathu Heenati, Mudgo, and ASD7, respectively (Figure 4.9).

The number of Nl1-Nl2-Nl3 shows the same direction as the NP waveform. That shows the highest number of NP waveforms on TN1 but not significantly with PTB33. Besides, PTB33 was not significantly with UBN03078-101-342-4-148 (Figure 4.9). While the respective of low the number of Nl1-Nl2-Nl3 waveforms similar to NP waveform.

The Nl4-a shows the highest frequency on UBN03078-101-342-4-148 but not significantly with PTB33. While PTB33 did not significantly with TN1, Mudgo, and ASD7, respectively. In contrast to that, the lowest Babawee, was not significantly with KDML105 and UBN03078-101-342-4-148 (Figure 4.9). The brown planthopper *N. lugens* made more frequency on probe and phloem sap ingestion (Nl4-b) waveform for TN1 variety but not significantly with PTB33 and UBN03078-101-342-4-148 (Figure 4.9).

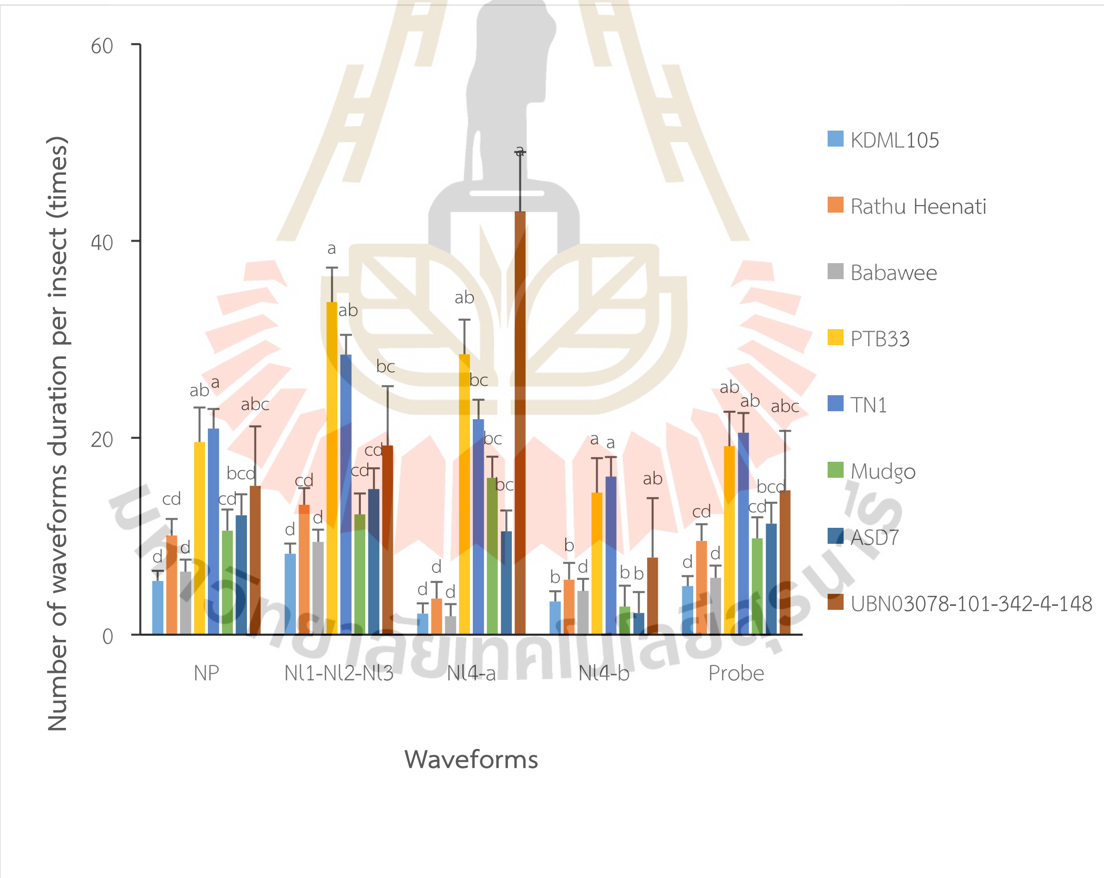

Figure 4.9 Number of waveforms duration per insect (times) of brown planthopper *Nilaparvata lugens* (Stål) fed on rice includes 8 varieties. Data in the same letters were not significantly different at P > 0.05 by DMRT.

2) Waveform duration per insect (WDI)

The result showed mean of behavior the brown planthopper *N. lugens* on 8 rice varieties appear significance on NP and Nl1-Nl2-Nl3 waveform ($P < 0.0001$). That same to Nl4-b waveform ($P < 0.0038$) and probe ($P = 0.0028$). In contrast the Nl4-a waveform that not significant ($P = 0.0835$).

The waveform duration of NP was the highest on Babawee significant difference with UBN03078-101-342-4-148, and PTB33. The waveform duration of Nl1-Nl12-Nl3 was high on Babawee, KDML105 and Rathu Heenati varieties. The waveform of ingestion activities (phloem sap ingestion), the brown planthopper *N. lugens* spent a long duration on the Nl4-b waveform when fed on Mudgo and TN1 varieties were significantly different from other varieties ($P < 0.05$; Figure 4.10). The total duration of probe the brown planthopper *N. lugens* spent long duration on KDML105, Babawee and PTB33 Figure 4.10).

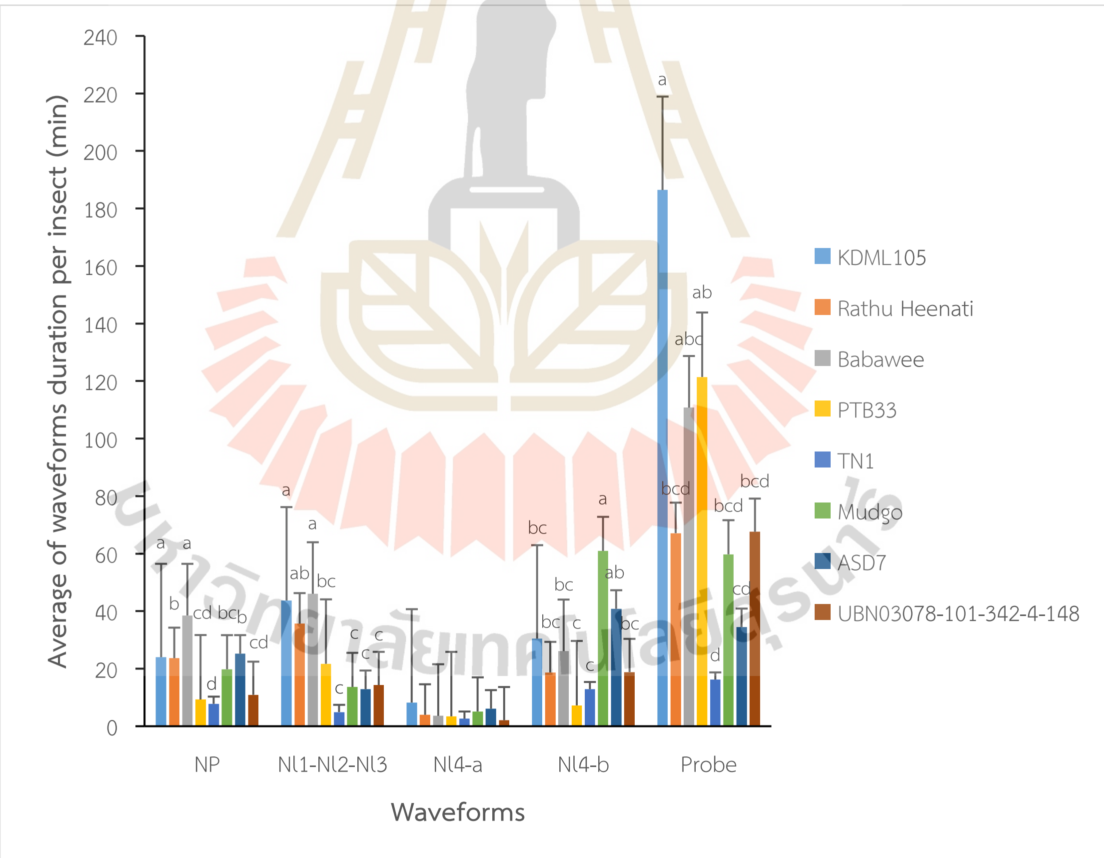

Figure 4.10 Mean of waveforms duration per insect (min) of brown planthopper *Nilaparvata lugens* (Stål) fed on rice includes 8 varieties. Data in the same letters were not significantly different at $P > 0.05$ by DMRT.

3) Waveform duration per event per insect (WDEI)

The waveform duration of NP was significantly (P = 0.0296), same to the Nl1-Nl2-Nl3 waveform (P < 0.0001), Nl4-a waveform (P < 0.0075) and Probe (P = 0.0002). In contrast the waveform duration of Nl4-b that not significant with other waveforms (P = 0.0835). The waveform duration of NP was the highest on ASD7, not significantly with Babawee, and Rathu Heenati, these results were lowest on PTB33, UBN03078-101-342-4-148, TN1, KDML105, and Mudgo, respectively.

The Nl1-Nl2-Nl3 waveform duration per event per insect shows the highest on Rathu Heenati not significantly with PTB33, Babawee, and KDML105. This result was lowest on TN1, Mudgo, ASD7, and UBN03078-101-342-4-148, respectively. The waveform duration of Nl4-a waveform shows the highest on Mudgo followed by PTB33, UBN03078-101-342-4-148, ASD7, KDML105, and TN1. However, the duration of Nl4-a waveform was lowest on Babawee, that not significant with Rathu Heenati (Figure 4.11).

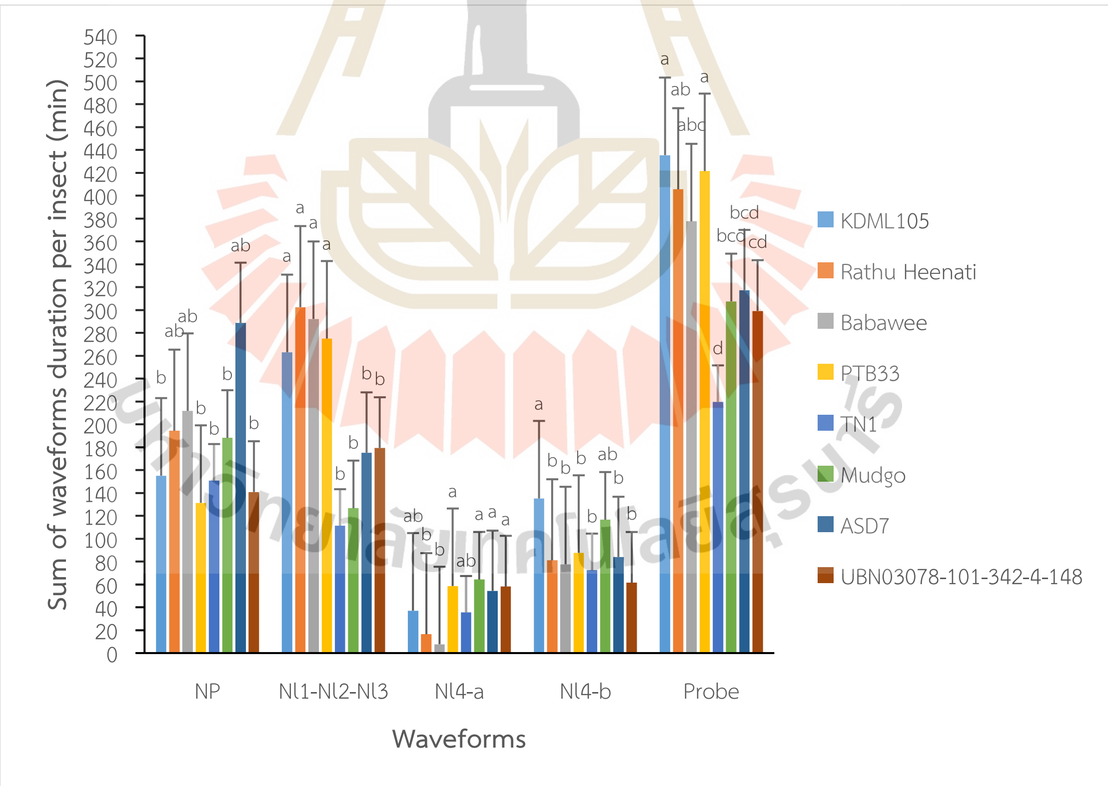

Figure 4.11 Sum of waveforms duration per insect (min) of brown planthopper *Nilaparvata lugens* (Stål) fed on rice includes 8 varieties. Data in the same letters were not significantly different at P > 0.05 by DMRT.

From this research, KDML105 shows the highest N4-b waveform duration (Figure 4.11) followed by Mudgo, PTB33, ASD7, Rathu Heenati, Babawee, TN1, and UBN03078-101-342-4-148 varieties, respectively. The KDML105 shows the highest probe followed by Rathu Heenati, Babawee, and PTB33 where high feeding behavior was probably those susceptible varieties. In contrast, TN1, Mudgo, UBN03078-101-342-4-148, and ASD7 were lower.

Waveform NP, Nl1-Nl2-Nl3 (pathway phase), Nl4-a, and Nl4-b were used to classify the waveform from the experiment (EPG) that was reported by Seo et al. (2009); Ghaffar et al. (2011); He et al. (2011). The result showed the highest on N4-b as high ingestion of that variety (Seo et al. 2009; Ghaffar et al. 2011). Furthermore, Mudgo and TN1 varieties show the highest mean of N4-b waveform duration. KDML105 and Mudgo show the highest sum of N4-b waveform duration. TN1 PTB33 and UBN03078-101-342-4-148 show the longer probe and phloem sap ingestion. While Ghaffar et al. (2011) found TN1 was higher on the N4-b waveform that means TN1 was classified in the susceptible varieties.

4.2.5 Preference rice variety by choice test

The number of insects host preference and symptom scored after infestation showed susceptibility or resistance on plants. The result of rice varieties preference found that the ASD7, KDML105 and Mudgo were shown the highest density of brown planthoppers per plant at 24, and 48 hours (28 and 33 insects per 10 plants), (33 and 29 insects per 10 plants), (31, and 28 insects per 10 plants), respectively (Figure 4.12). The symptoms score showed that the PTB33 scored lower in 3, 5, 7, and 10 days. The UBN03078-101-342-4-148 scores highest in 3, 5, 7, and 10 days of observation (Table 4.4). The previous report that showed host preference for Rathu Heenati higher than KDML105 and TN1 (Khetnon et al., 2022). However, the host prefers brown planthoppers different from the previous report that show host preference for TN1 higher than Rathu Heenati (Wang et al., 2012) and PTB33 (Sandhu and Sarao, 2021).

In addition, TN1 showed score symptoms after infestation higher than another variety. Wang et al., (2012) show the result after 7 days of infestations of brown planthoppers on the TN1 variety appear brownish and die. According to Khetnon et al., (2022), hopper burns symptoms on 7 days showed an average score the highest. Moreover, the TN1 variety spent less time to chance yellow leaf contrast with Mudgo, Rathu Heenati, and ASD7 (Qiu et al., 2011). This method shows tolerance varieties with many infestations but low damage.

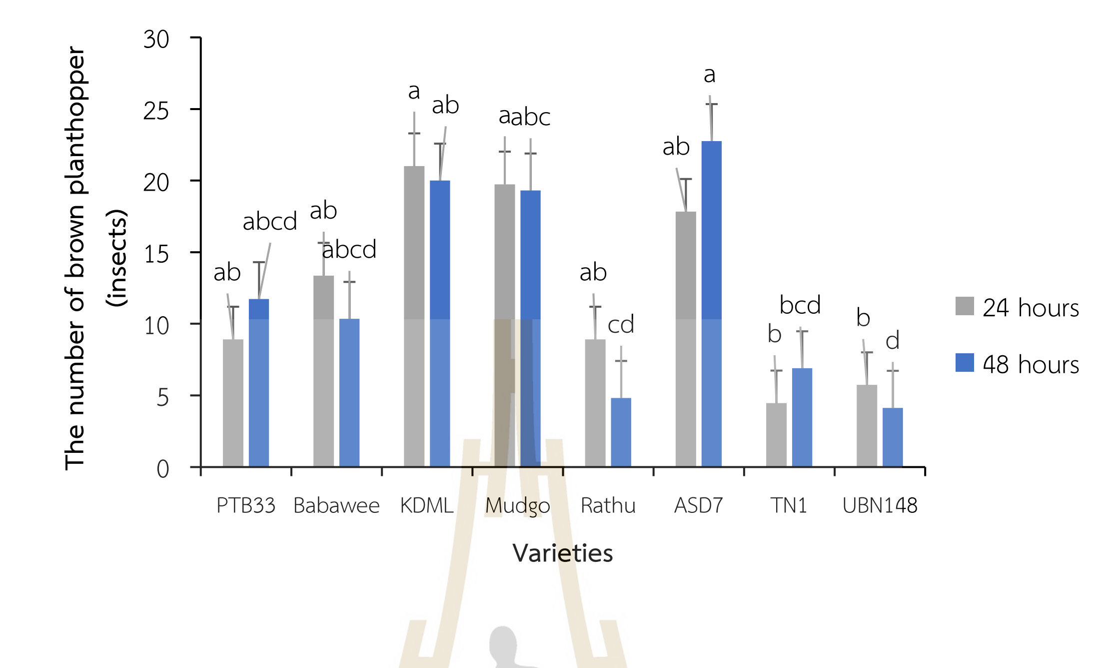

Figure 4.12 The total number of brown planthoppers, *Nilaparvata lugens* (Stål) per varieties at 24, and 48 hours. Data in the same letters were not significantly different at $P > 0.05$ by DMRT.

Table 4.4 The damage symptoms (hopper burn) score (mean ± SD) of difference 8 varieties infection by brown planthopper, *Nilaparvata lugens* (Stål) on 3, 5, 7, and 10 days

Rice Varieties	Day 3		Day 5		Day 7		Day 10	
PTB33	1.28 ± 3.25	d[1]	2.54 ± 3.10	d	2.62 ± 3.41	c	6.67 ± 3.44	b
Babawee	1.67 ± 3.77	cd	3.13 ± 3.49	cd	3.90 ± 4.08	bc	7.97 ± 0.00	a
KDML	2.51 ± 3.69	bcd	4.67 ± 2.28	ab	5.13 ± 2.91	ab	8.21 ± 0.00	a
Mudgo	3.10 ± 4.48	abcd	3.62 ± 4.29	cd	4.87 ± 3.66	ab	7.15 ± 0.00	ab
Rathu	1.95 ± 0.00	c	3.51 ± 4.11	cd	4.74 ± 4.11	ab	7.77 ± 0.00	ab
ASD7	3.72 ± 4.05	ab	5.54 ± 3.32	a	5.56 ± 4.16	ab	8.03 ± 0.00	a
TN1	3.79 ± 4.40	ab	4.64 ± 4.40	ab	5.28 ± 4.40	ab	8.08 ± 0.00	a
UBN03078-101-342-4-148	4.92 ± 3.83	a	6.41 ± 2.18	a	6.59 ± 3.70	a	7.69 ± 0.00	ab
P-Value	<0.0000		<0.0000		<0.0000		<0.0000	

[1] Data in the same column with the same letters were not significantly different at P > 0.05 by DMRT

4.2.6 Evaluation plant trichome by scanning electron microscope (SEM)

From results found arrowhead two types of trichome macro trichome was long thin of arrow (Figure 4.13A) and prickle trichome is short of arrow (Figure 4.13B) followed by trichome character reported by Khetnon et al (2022). The PTB33 and TN1 show sum of trichome density (two characters). The PTB33 showed the highest density of the prickle trichome. According to Sandhu and Sarao, (2021) showed the highest density of the trichome on PTB33 variety. In addition, the result show TN1 found the highest density of the macro trichome (Figure 4.15). Considering preference of insects on PTB33 showed insect densities higher than TN1 that similar to those reported by Khetnon et al (2022).

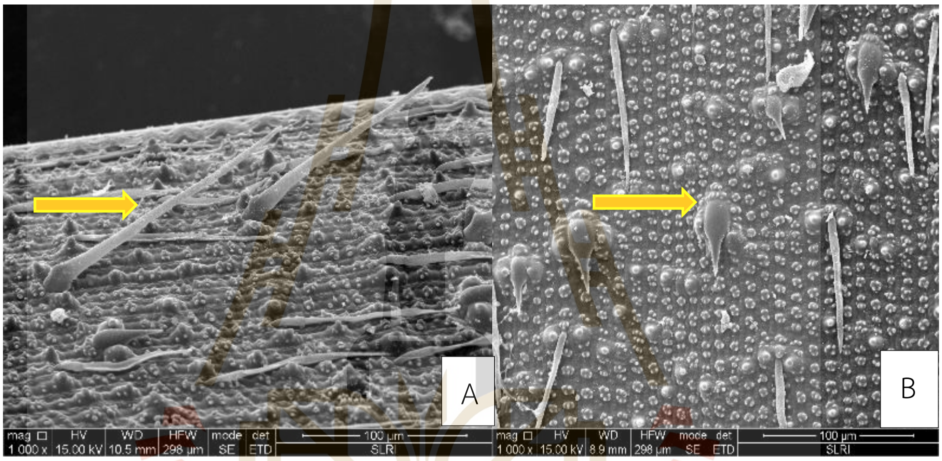

Figure 4.13 The trichome types of rice varieties by the scanning electron microscopy (SEM) 1000X, Arrowhead: macro trichome (A), prickle trichome (B).

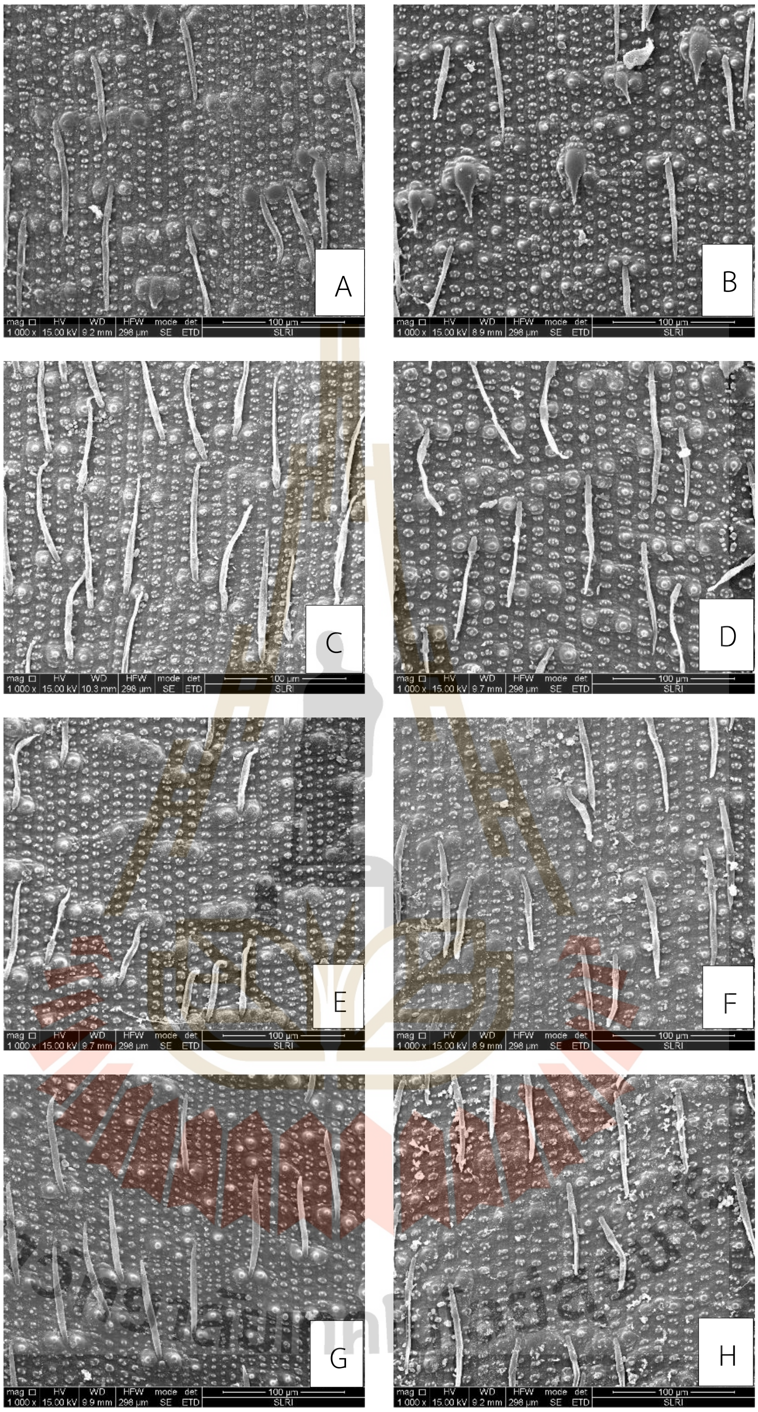

Figure 4.14 The trichome on rice sheath 8 varieties; KDML105 (A), PTB33 (B), TN1 (C), Babawee (D), Mudgo (E), Rathu heenati (F), ASD7 (G), UBN03078-101-342-4-148 (H) by the scanning electron microscopy (SEM) 1000X

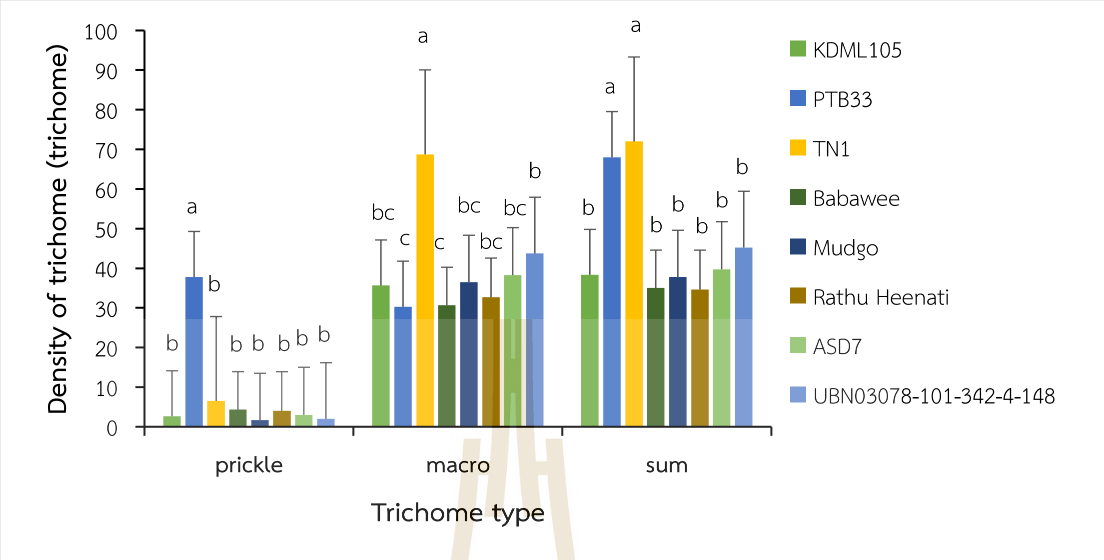

Figure 4.15 The Density of trichome rice includes 8 varieties. Data in the same letters were not significantly different at P > 0.05 by DMRT.

4.2.7 Evaluation of the selection method to screen the rice resistance.

To evaluate the selection method to screen the rice resistance variety, a total of 32 characters were used for analysis of the statistics including honeydew production, salivary sheath, *N. lugens* feeding behavior EPG, feeding preference the average development, survival ship, damage symptoms, and trichome density (Table 4.5). In univariate analysis (Table 4.5), 28 of 32 activities showed highly significant differences between varieties. That were analyzed principal component analysis and correlation between the group as honeydew and salivary sheath experiments (Attached, Figures 1 and 2), life cycle and EPG (phloem phase) experiments (Attached, Figures 3 and 4), survival and EPG (phloem phase) experiments (Attached, Figures 5 and 6), preference rice variety by choice test (Attached, Figures 7 and 8), preference rice variety by choice test and trichome density experiments (Attached Figures 9 and 10) and correlation of all parameters analyze (Attached, Figure 11). The correlation of the 28 characters was used for analysis correlation.

Table 4.5 The list of 32 characters used for the analysis of the statistics.

No.	Parameters (Full name)	Parameters (Short name)	Significance level P>F
1	Honeydew	Honeydew	<0.0001
2	Salivary sheath	Salivary sheath	<0.0001
3	1st instar (life cycle)	1st life	0.0026
4	2nd instar (life cycle)	2nd life	0.0063
5	3rd instar (life cycle)	3rd life	0.0007
6	4th instar (life cycle)	4th life	0.1654
7	5th instar (life cycle)	5th life	0.2145
8	Pre-adult (life cycle)	Pre-adult	0.0416
9	Adult (life cycle)	Adult	<0.0001
10	Number of waveform events per insect (time) NP	num NP	0.0002
11	Number of waveform events per insect (time) Nl1-Nl2-Nl3	num Nl1-Nl2-Nl3	<0.0001
12	Number of waveform events per insect (time) Nl4-a	num Nl4-a	<0.0001
13	Number of waveform events per insect (time) Nl4-b	num Nl4-b	0.0019
14	Number of waveform events per insect (time) Probe	num Probe	0.0002
15	Waveform duration per insect (WDI) NP	ave NP	<0.0001
16	Waveform duration per insect (WDI) Nl1-Nl2-Nl3	ave Nl1-Nl2-Nl3	<0.0001
17	Waveform duration per insect (WDI) Nl4-a	ave Nl4-a	0.0835
18	Waveform duration per insect (WDI) Nl4-b	ave Nl4-b	0.0038
19	Waveform duration per insect (WDI) Probe	ave Probe	0.0028
20	Waveform duration per event per insect (WDEI) NP	sum NP	0.0296
21	Waveform duration per event per insect (WDEI) Nl1-Nl2-Nl3	sum Nl1-Nl2-Nl3	<0.0001
22	Waveform duration per event per insect (WDEI) Nl4-a	sum Nl4-a	0.0075
23	Waveform duration per event per insect (WDEI) Nl4-b	sum Nl4-b	0.22
24	Waveform duration per event per insect (WDEI) Probe	sum Probe	0.0002
25	Preference rice variety by choice test (24 hours)	24 prefer sum	0.021

Table 4.5 The list of 32 characters used for the analysis of the statistics **(continued)**.

No.	Parameters (Full name)	Parameters (Short name)	Significance level P>F
26	Preference rice variety by choice test (48 hours)	48 prefer sum	0.021
27	The symptoms score (day 3)	sym 3	<0.0000
28	The symptoms score (day 5)	sym 5	<0.0000
29	The symptoms score (day 7)	sym 7	<0.0000
30	The symptoms score (day 10)	sym 10	<0.0000
31	Trichome density (prickle trichome)	prickle density	<0.0001
32	Trichome density (macro trichome)	macro density	<0.0001

Linear correlations between *N. lugens* feeding behavior, strong positive correlation between the number of probes, Nl4-b waveform, and number of NP (non-probe) waveform (r=1 and r=0.88, respectively). A high positive correlation was also found between the number of probes, the Nl4-b waveform, and the number of Nl1-Nl2-Nl3 waveforms (r=0.96 and r=0.92, respectively). A high positive correlation was found between damage symptoms scores at 5 and 7 days, and damage symptoms score at 3 days (r=0.92 and r=0.91, respectively). Also, a high positive correlation was found between waveform Nl4-a duration per insect (sum Nl4-a), the total number of insect preferences per plant at 24 hours, and the total number of insect preferences per plant at 48 hours (r=0.91 and r=0.84 respectively). Moreover, the total number of insect preference honeydew areas showed a positive correlation with the waveform Nl4-b duration per insect (ave Nl4-b) waveform Nl4-a duration event per insect (sum Nl4-a) and the total number of insect preferences per plant at 48 hours (r=0.83, r=0.90, and r=0.89, respectively, (Figure 4.16). In contrast, waveform NP duration event per insect (sum NP) showed a highly negative correlation with the number of NP waveform events per insect, the number of Nl1-Nl2-Nl3 waveforms, the number of waveforms Nl4-a, and the number of the probe (r=-0.87, r=-0.81, and r=-0.80, r=-0.87, respectively, (Figure 4.16). A negative correlation was found between pre-adult development and damage symptoms score at 3 and 7 days (r=-0.85 and r=-0.82 respectively). Moreover, a negative correlation was also found between first-instar development, the number of NP waveform events per insect, and the number of probes (r=-0.82).

The 32 characters (Table 4.5) was separated for antixenosis resistance including 10, 11, 15, 16, 20, 21, 25, 26, 31, and 32 characters. Antixenosis is also referred to as a nonpreference, in which a plant characteristic poses a chemical or biophysical barrier that deters or repels the arthropod without causing it harm. It may be affected by morphological factors (such as trichomes, waxiness, or pigmentation) or chemical factors (such as phenolic compounds, volatile compounds, enzymes, or other secondary metabolites) that adversely affect the actions of insects, prompting them to choose an alternative host plant (Sandhu and Sarao, 2021). In antibiosis resistant mechanism is affecting the biology of the insect so pest abundance and subsequent damage is reduced compared to that which would have occurred if the insect was on a susceptible crop variety. Antibiosis resistance often results in increased mortality or reduced longevity and reproduction of the insect (Ghaffar et al, 2011, Qiu et al, 2011). Antibiosis resistant including the number 1-9, 12-14, 17-19, and 22-24 characters. Tolerance is resistance in which a plant can withstand or recover from damage caused by insect pest abundance equal to that damaging a plant without resistance characters (susceptible). Tolerance is a plant response to an insect pest. Thus, tolerance resistance differs from antibiosis and antixenosis resistance in how it affects the insect-plant relationship. Antibiosis and antixenosis resistance cause an insect response when the insect attempts to use the resistant plant for food, oviposition, or shelter. Tolerance resistant including the number 27-30 (Qiu et al, 2011).

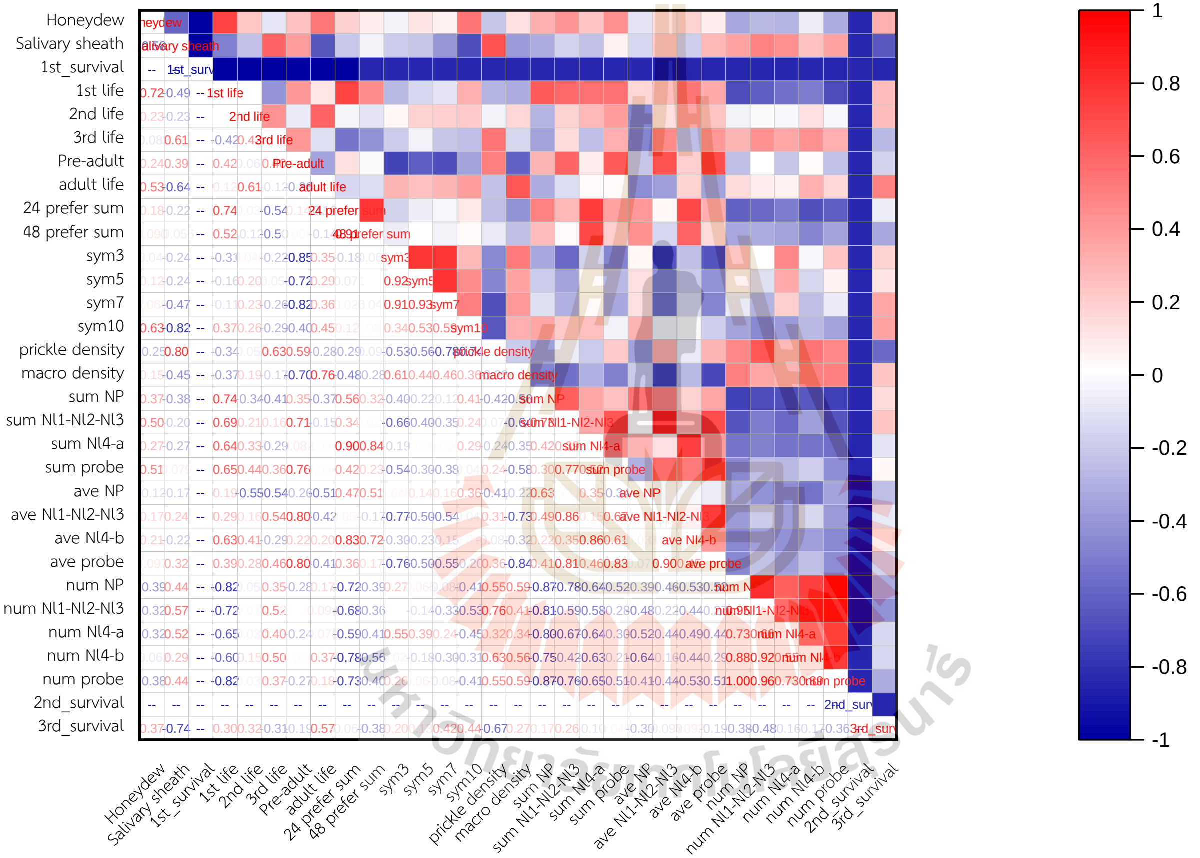

Figure 4.16 Correlations of parameters analyze (28 out of 32 characters).

Cluster analysis using Origin 2023b method based on the Euclidean Distance was performed using 28 activities derived from honeydew production, salivary sheath, *N. lugens* feeding behavior EPG, feeding preference the average development, survival ship, damage symptom, and trichome density. The resulting dendrogram (Figure 4.17) divided the 8 rice varieties into five main groups. Group 1 includes KDML105 and PTB33. Group 2 – Rathu Heenati, and Babawee, Groups one and two were distant from the other three groups. Group 3 – TN1, Group 4 includes Mudgo and UBN03078-101-342-4-148, the last group- ASD7. While Ghaffar et al. (2011) reported the resulting dendrogram divided the 12 rice varieties into only three groups. They use EPG and honeydew drop (38 of 56-character activities).

When considering a group of cluster analysis and all results from experiments appears Group 1 (KDML105 and PTB33) was resistant. Group 2 (Rathu Heenati, and Babawee) were moderately resistant. Group 3 (TN1) was moderately susceptible, Group 4 (Mudgo and UBN03078-101-342-4-148) was susceptible, and Group 5 (ASD7) was highly susceptible. The classified differences previous report as KDML105 and TN1 were susceptible varieties (Ghaffar et al. 2011; Khetnon et al., 2022). However, Ghaffar et al. (2011) reported Rathu Heenati, and Babawee was resistant that according to the result. In the same way, Khetnon et al. (2022) reported resistance varieties as PTB33, PTB33 was reported as resistance varieties by several genes BPH2-NIL, BPH17-ptb-NIL, and BPH32-NIL. BPH2 and BPH17-ptb (Nguyen et al., 2021).

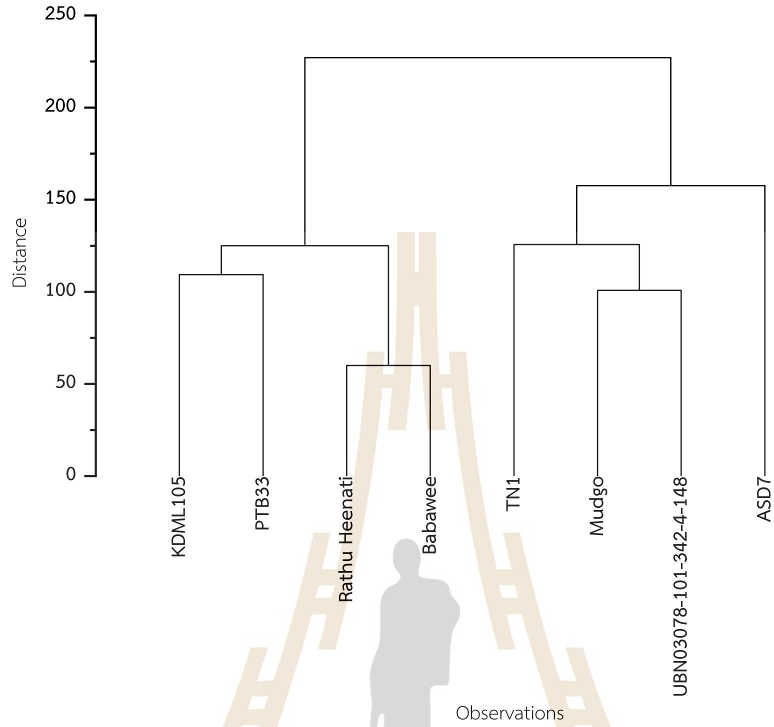

Figure 4.17 Dendrogram derived used cluster analysis on 28 characters. 8 rice varieties have been divided into 5 groups.

Table 4.6 The expectation of parameter use evaluation the selection method to screen the rice resistance.

Methods	Characteristic/ Parameter	Resistance	Moderately Resistance	Moderately Susceptible	Susceptible	Highly susceptible
Honeydew	Area of the honeydew drop (mm^2)	<0.1	0.1-0.12	>0.12-0.15	>0.15-0.2	>0.2
Salivary sheath	Number of salivary sheath (spot)	>6.5	>3-6.5	>2.5-3	>2-2.5	<2
Electropenetrography	Number of NP waveform per insect (times)	>20	>15-20	>10-15	6-10	<6
	Mean duration of NP waveform per insect (min)	>38	>20-38	>10-20	8-10	<8
	Total duration of NP waveform per insect (min)	>190	>160-190	>140-160	130-140	<130
	Number of Nl1-Nl2-Nl3 waveform per insect (times)	>33	>20-33	>15-20	9-15	<9
	Mean duration of Nl1-Nl2-Nl3 waveform per insect (min)	>35	>15-35	>12-15	5-12	<5
	The total duration of Nl1-Nl2-Nl3 waveform per insect (min)	>290	>250-290	>200-250	>100-200	<100
	Number of Nl4-a waveforms per insect (times)	<2	2-21	>21-28	>28-40	>40
	The mean duration of Nl4-a waveform per insect (min)	<3	3-6	6-8	8-10	>10
	Total duration of Nl4-a waveform per insect (min)	<7	7-17	>17-38	>38-65	>65
	Number of Nl4-b waveforms per insect (times)	<3	3-6	>6-7	>7-10	>10
	The mean duration of Nl4-b waveform per insect (min)	<12	7-12	>12-20	>20-40	>40
	The total duration of Nl4-b waveform per insect (min)	<60	60-80	>80-90	>90-120	>120
Life cycle	Total of nymph's stage development duration (day)	>15	14-15	<14-13	<13-11	<11
	Total of adult stage development duration (day)	<7	>7-9	>9-15	>15-26	>26
Survivor rate	Percentage of survivor rate (%)	<30	30-49	40-59	89-60	100-90
Choice test	Score of symptoms on rice plant after insects' infestation	<3	3-4	>4-5	>5-8	>8
	Insects' preference number (insects)	<4.5	4.5-6	>6-10	>10-14	>17
Plant trichome by SEM	macro trichome	>65	39-65	33-38	30-32	<30
	prickle trichome	>35	>6-35	>3-6	>1-3	<1

CHAPTER 5
CONCLUSIONS

5.1 Study the feeding behavior of brown planthopper *Nilaparvata lugens* (Stål) using the electropenetrography (EPG)

Electropenetrography (EPG) waveforms for *N. lugens* were classified as a waveform family (i.e., Nl1, Nl2, Nl3, Nl4, Nl5, pd, and NP). The NP waveform's initial phase included insects not currently ingesting anything, activities like landing, adhesion to the plant surface, oviposition, and walking. The Nl1 waveform was associated with the pathway phase, probing activities in the epidermis and mesophyll cells. In the same way in the Nl2 waveform was the pathway phase, probing activities identified two subtypes: Nl2a and Nl2b. Besides Nl3 waveform was saliva activities in the phloem phase. While The Nl4 waveform was associated with phloem tissue, ingestion activities. The pd waveform was a potential drop between Nl4 waveforms. The last waveform was the Nl5; there were three subtypes: Nl5a, Nl5b, and Nl5c, that unknown phases and activities of insect behavior. In addition, EPG waveforms help to understand the behavior of brown planthopper *N. lugens* on rice plants, and their measurement means comparing the frequency, duration, and total appearances of each waveform for guidelines and comparisons in future research.

5.2 Screening for rice resistant varieties

The honeydew area of the brown planthopper *N. lugens* fed on different rice varieties showed feeding behavior, their hypnotized high excretes (high area of honeydew) caused high feeding. The number of salivary sheaths showed high numerals and high resistance variety. The brown planthopper *N. lugens* development was influenced by nutrients from rice, which showed a long life in the pre-adult phase, it was good for rice more than a long life in the adult phase because the adult phase was able to reproduce, and that hypothesis was the long adult phase more reproductive. Moreover, the nutrients from rice influenced the survivorship of insects were survivors.

The EPG compared the feeding behavior of brown planthopper *N. lugens* on 8 rice difference varieties. The result showed that the high activity of NP, Nl1-Nl2- Nl3 waveform is not famous for insect feeding, while the high activity of Nl4-a, Nl4-b waveform is insect preferred to feeding.

The rice varieties' preference showed the famous varieties for insects, insects prefer high numerical famous varieties. However, the observed insect numerical preference on rice plan is proven by the symptoms score; this method shows tolerance varieties where there are many infestations but shows low damage. In addition, all 8 varieties found two characters of trichome as macro trichome and prickle trichome, which the prickle trichome probably cause to insect density on host preference.

To evaluate the selection method to screen the rice resistance variety, 28 of 32 activities showed highly significant differences between varieties. The correlation of the 28 characters was used for analysis correlation. And those were used cluster analysis from honeydew production, salivary sheath, *N. lugens* feeding behavior EPG, feeding preference the average development, survival ship, damage symptom, and trichome density. They were classified into five main groups. Group 1 includes KDML105 and PTB33 were resistance varieties. Group 2 – Rathu Heenati and Babawee were moderately resistant varieties; Group 3 – TN1 was moderately susceptible varieties; Group 4, including Mudgo and UBN03078-101-342-4-148, were susceptible; the last group- ASD7, was highly susceptible.

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APPENDIX

APPENDIX A

REAGENTS

I Regents for honeydew test

1. **0.1% ninhydrin**

Ninhydrin	0.1	g
Acetone	99	ml

II Regents for salivary sheath test

1. **1% eosin**

Eosin	1	g
DI water	99	ml

2. **70% ethanol**

Absolute ethanol	70	ml
DI water	30	ml

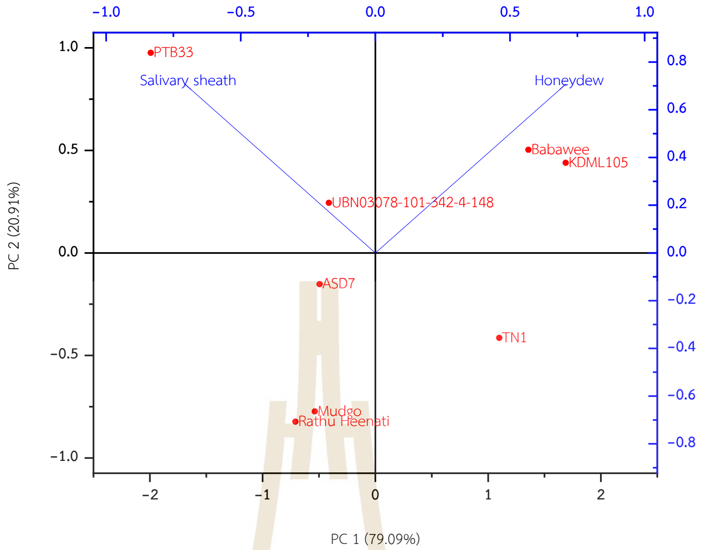
III Regents for electropenetrography (EPG)

1. **Water base silver glue**

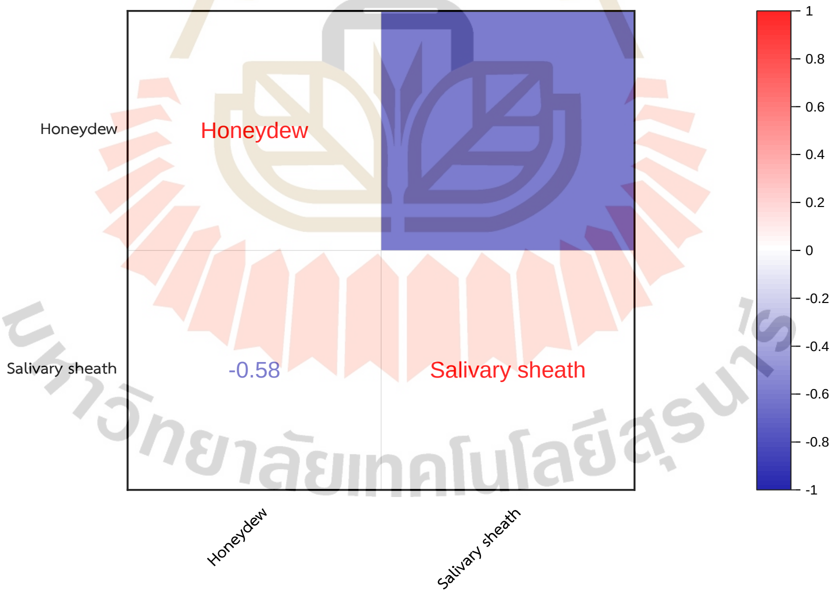
Silver flake	5	g
Liquid glue: latex glue	5	ml
DI water	5	ml

APPENDIX B

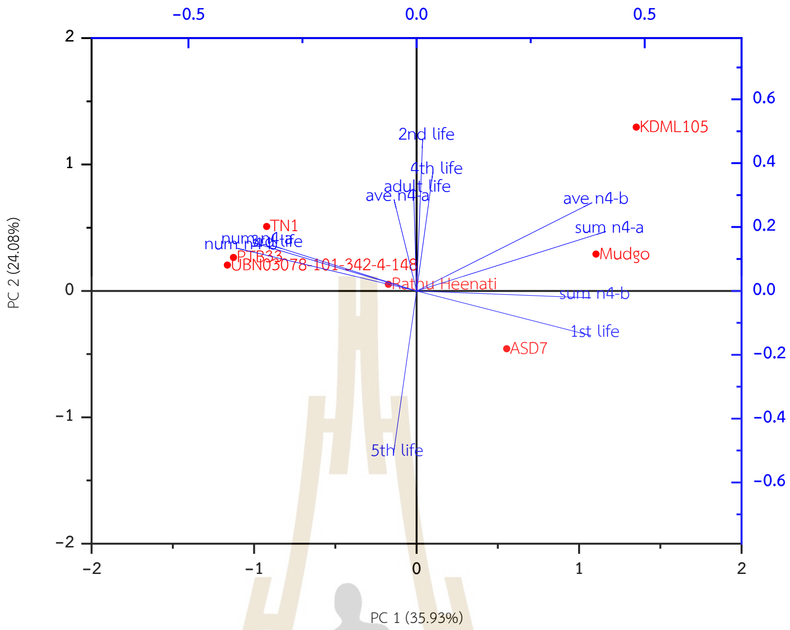
ATTACHED FIGURE



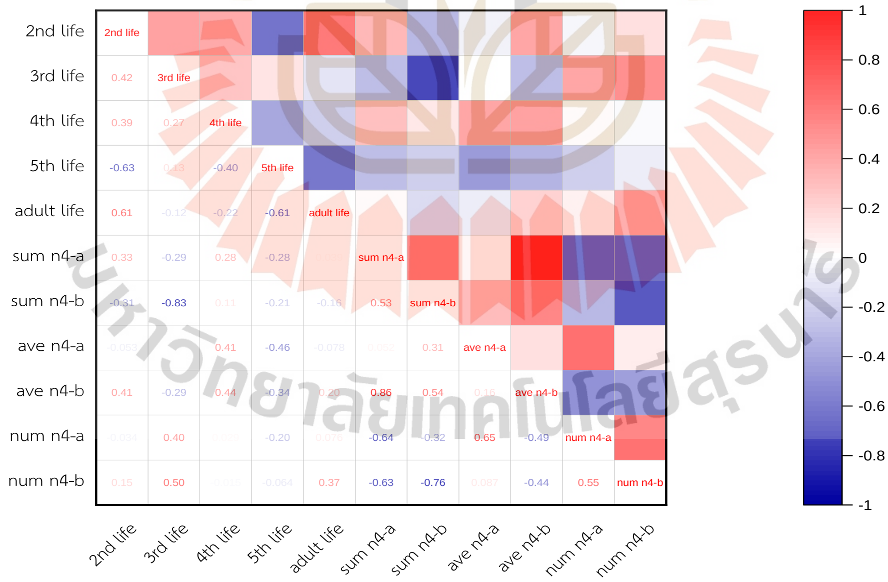
Attached Figure 1 Principal component analysis rice includes 8 varieties and parameter of honeydew and salivary sheath experiments.



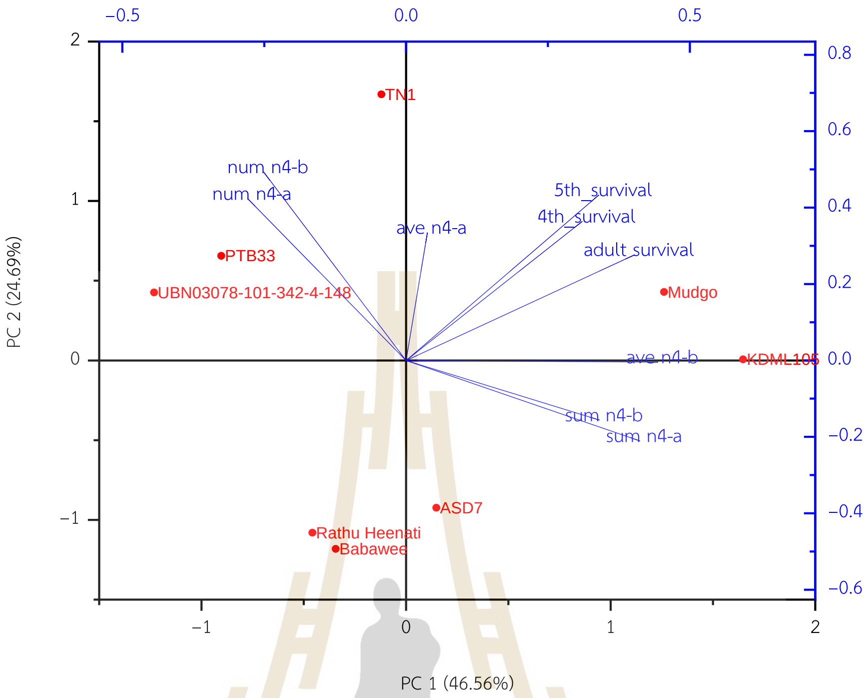
Attached Figure 2 Correlations of parameters analyze of honeydew and salivary sheath experiments.



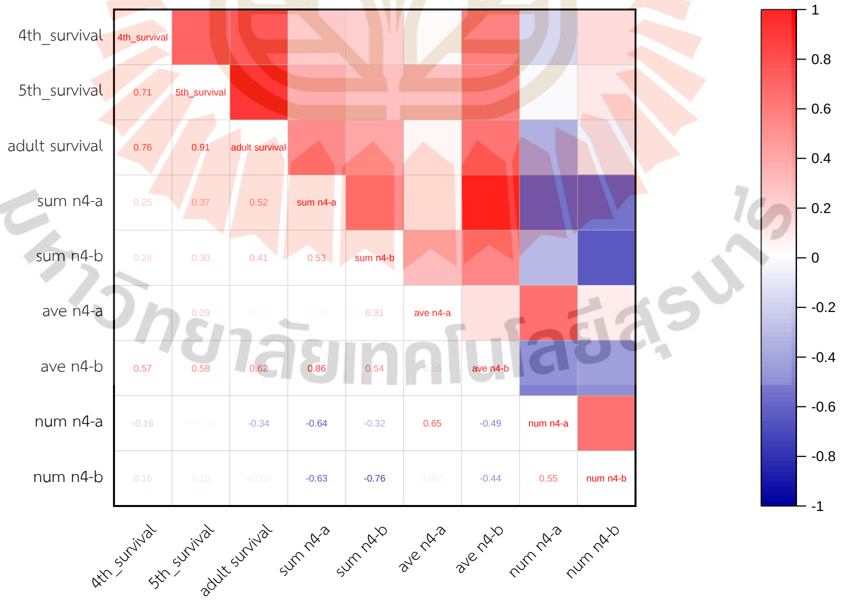
Attached Figure 3 Principal component analysis rice includes 8 varieties and parameter of life cycle and EPG (phloem phase) experiments.



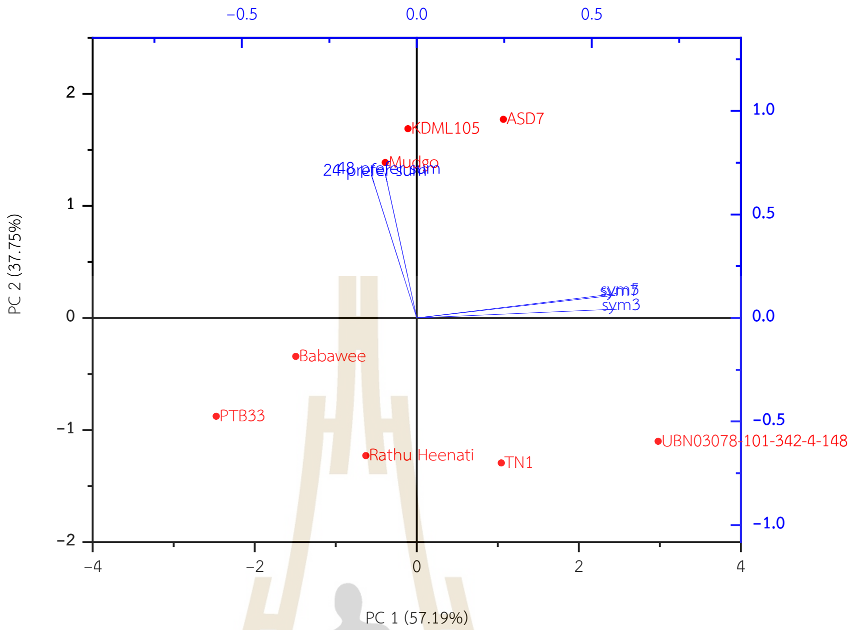
Attached Figure 4 Correlations parameters analyze of life cycle and EPG (phloem phase) experiments.



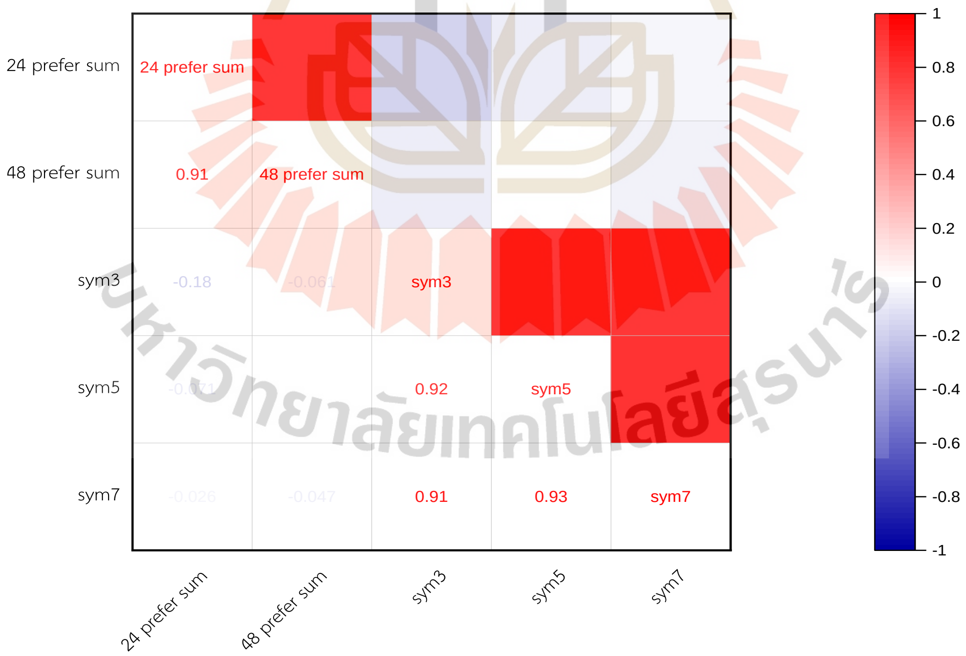
Attached Figure 5 Principal component analysis rice includes 8 varieties and parameter of survival and EPG (phloem phase) experiments.



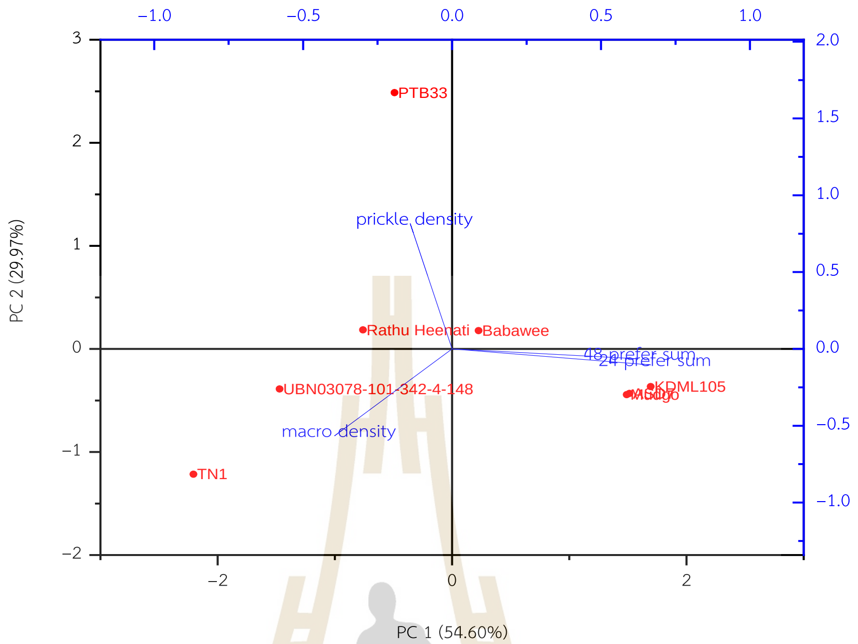
Attached Figure 6 Correlations of parameters analyze of survival and EPG (phloem phase) experiments.



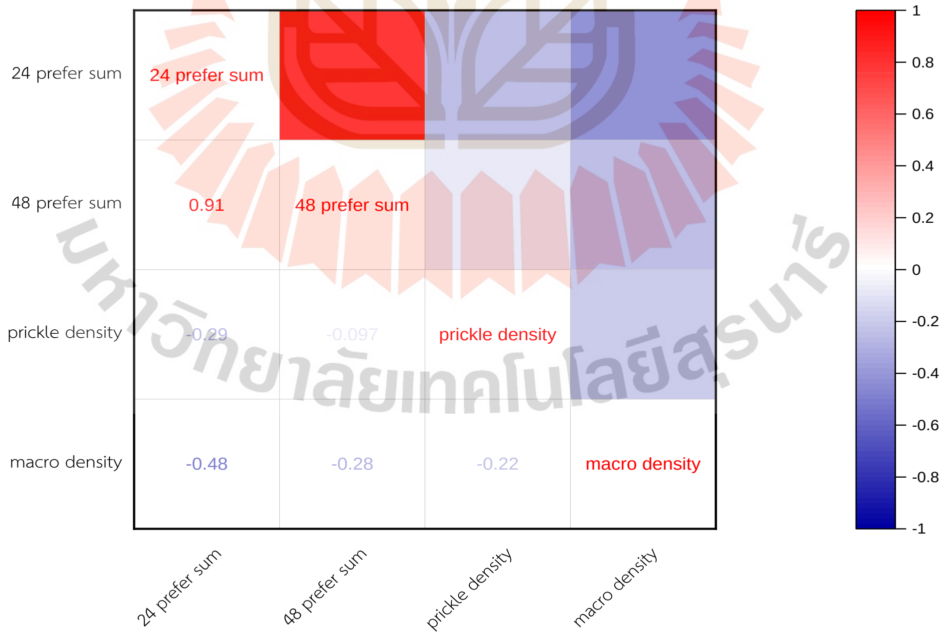
Attached Figure 7 Principal component analysis rice includes 8 varieties and parameter of preference rice variety by choice test.



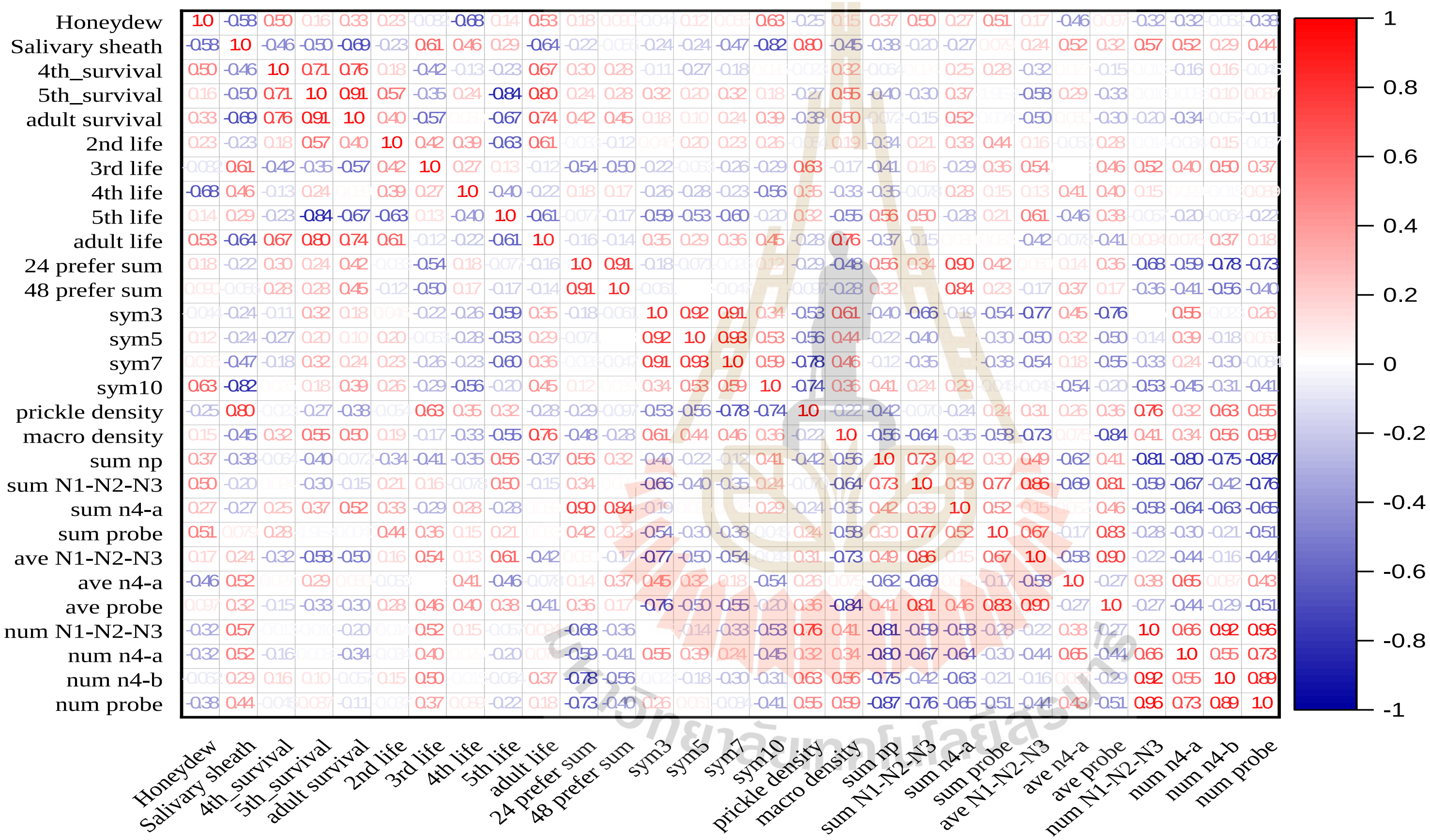
Attached Figure 8 Correlations of parameters analyze of preference rice variety by choice test.



Attached Figure 9 Principal component analysis rice includes 8 varieties and parameter of preference rice variety by choice test and trichome density experiments.



Attached Figure 10 Correlations of parameters analyze of parameter of preference rice variety by choice test and trichome density experiments.



Attached Figure 11 Correlations of all parameters analyze (32 characters)

VITAE

Miss Napatson Chansawang was born on July 11, 1998, in Pathum Thani, Thailand. She graduated from the Suranaree University of Technology, Nakhon Ratchasima 30000 Thailand. She received her Bachelor of Science in the Institute of Agricultural Technology from Suranaree University of Technology, Thailand in 2021. She received a *SUT Sakayabandit* scholarship from the Suranaree University of Technology which supported her tuition, fees, and allowance. In 2021, she pursued a Master of Science program in the School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology. She received a *Kittibandit* scholarship from Suranaree University of Technology which supported her tuition, fees, and allowance. In the meantime, she worked as a teaching assistant (TA) of the School of Crop Production Technology in 2021-2022. In 2023 she published in Khon Kaen Agriculture Journal included: The effectiveness of containers for black soldier fly larvae (*Hermetia illucens* L.) domestication in household., and the bio-produces of entomopathogenic fungus *Beauveria bassiana* (Balsamo) like pellets to control the brown planthopper (*Nilaparvata lugens* (Stål)). In addition, she received a good oral presentation award from the 24th Agricultural Conference: "Agriculture for Achieving the Sustainable Development Goals".