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THESIS

MECHANISMS OF *Bph3*, *Qbph6* AND *Qbph12* RESISTANT GENES/QTLS AGAINST *Nilaparvata lugens* (Stål) IN KAO DAWK MALI 105 INTROGRESSION LINES

WISSARUT SUKHAKET

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Wissarut Sukhaket 2011: Mechanisms of *Bph3*, *Qbph6* and *Qbph12* Resistant Genes/QTLs against *Nilaparvata lugens* (Stål) in Kao Dawk Mali 105 Introgression Lines. Master of Science (Entomology), Major Field: Entomology, Department of Entomology. Thesis Advisor: Associate Professor Intawat Burikam, Ph.D. 68 pages.

Mechanisms of resistance to brown planthopper, *Nilaparvata lugens* (Stål) of two introgression lines, UBNKD6–56 and UBN4–283, within *Bph3* resistant gene derived from BC_3F_7 progenies of KDML105 and Rathu Heenati crosses, and the other two introgression lines, KPSKD17–173 and KPSKD55–220, carrying *Qbph6,12* resistant *QTLs*, unknown genes derived from BC_4F_8 progenies of KDML105 and Abhaya crosses were determined. All of introgression lines were not found the antixenotic properties of the donors and the insects preferred settlement on them. However, these introgression lines were recorded low feeding rate, nymphal survival, growth index, functional plant loss index, tolerance index, and longer development period compared with KDML105 and the susceptible check TN1. The results suggested that the host–plant defense is the complex trait of resistant mechanisms between antibiosis and tolerance.

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Student's signature   Thesis Advisor's signature

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Wissarut Sukhaket

April 2011

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LIST OF ABBREVIATIONS

BPH	=	Brown plantopper
KDML105	=	Kao Dawk Mali 105
RH	=	Rathu Heenati
AB	=	Abhaya
TN1	=	Taichung Native 1
AX	=	Antixenosis
FR	=	Feeding rate
AHD	=	Area of honeydew excretion
NS	=	Nymphal survival
DP	=	Developmental period
GI	=	Growth index
SES	=	Standard evaluation system
FPLI	=	Functional plant loss index
PH	=	Plant height
TI	=	Tolerance index
HAI	=	Hours after infestation

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MECHANISMS OF *Bph3*, *Qbph6* AND *Qbph2* RESISTANT GENES/QTLS AGAINST *Nilaparvata lugens* (Stål) IN KAO DAWK MALI 105 INTROGRESSION LINES

INTRODUCTION

Rice (*Oryza sativa* L.) is the primary food source for nearly half of the world's population. Economically, rice is among the top three export commodities in Thailand. The indica rice cultivar Kao Dawk Mali 105, KDML105 (KD), is characterized by its good eating quality with desirable fragrance and has been accepted in markets as premium jasmine rice. Additionally, the cultivar can be widely adapted under rain fed lowland areas in Northeast of Thailand. Thus, KD has been extensively used as a favorable quality parental line to develop new cultivars. However, one limitation of this cultivar is its susceptibility to the brown planthopper (BPH), *Nilaparvata lugens* (Stål), a major insect pest in rice–growing areas. The BPH is causing enormous yield losses every year. In addition to feeding on rice plants directly, the BPH also causes indirect damage by acting as a vector for the viruses that cause ragged and grassy stunt diseases. Growing rice all year round, extensive use of insecticides and high application rates of nitrogen fertilizer often cause outbreaks of the BPH in rice fields. One strategy to reduce the losses due to the BPH is the utilization of rice varieties with BPH resistance genes.

The application of resistant rice cultivars has been recognized as the most economic and effective measure for the BPH control. The genetic basis of qualitative and quantitative BPH resistance has been well studies with major resistance genes (*Bph1* to *bph19*) identified from cultivated rices and wild relatives.

Two resistant rice cultivars, Rathu Heenati, a local variety in Sri Lanka, and Abhaya, an Indian local variety, were found to confer a strong and broad–spectrum resistance to the BPH in Thailand (Jairin *et al.*, 2007). *Bph3*, one of the major resistant genes derived from the cultivar Rathu Heenati , have been mapped to the

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short arm of chromosome 6. *Qbph6* and *Qbph12*, other of resistant unidentified–genes derived from Abhaya have been analyzed by QTL mapping on chromosome 6 and 12, respectively. These resistant unknown–genes have showed a highly resistant reaction against a broad range of the BPH populations in Thailand (Jairin *et al*., 2005). Both Rathu Heenati and Abhaya have been used as donors of the BPH resistance in various conventional breeding programs.

Although, the progenies from a crossing between KDML105 and two resistant varieties expressed a high resistance in vegetative stage to BPH (Jairin *et al*., *2007*), but the resistant mechanisms of rice plant such as antixenosis, antibiosis and tolerance in these resistant varieties have not yet been studied and resistant levels of these varieties have not yet been compared as well.

This current study analyzed and compared the mechanism of resistance of progenies resulting from a cross KDML105 and Rathu Heenati against KDML105 and Abhaya. Detailed observations resistant mechanisms would help to manage the BPH populations in Thailand.

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OBJECTIVES

To identify and compare the mechanisms of resistance in KDML105 introgression lines with the *Bph3* from Rathu Heenati and *Qbph6, 12* from Abhaya.

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LITERATURE REVIEW

The Brown planthopper (BPH) *Nilaparvata lugens* (Stål) (Homoptera : Delphacidae) has in recent years caused extensive damage to the rice crop in Asia. Large scale damages by the insect have been reported in India, Indonesia, the Philippines, and Sri Lanka, including Thailand and infestations of varying degrees are now commonly observed in the remaining Asian countries. Direct damage of rice caused by feeding behavior of both nymphs and adults of the BPH. The insects feed on phloem sap using their piercing–sucking mouthparts. If the insect density is high, the plant dies and a condition known as hopper burn results. The insect may also transmit the grassy stunt disease, which can further reduce yield. Epidemics of grassy stunt have followed major pest outbreaks in India, Indonesia, and the Philippines.

1. Life cycle of the brown planthopper

The eggs are usually laid as egg–groups in the tissue of the lower part of the rice plant, mainly in sheaths but also in leaf blades. But the sizes and sites of egg–groups depend upon the stages of the rice plants. When the adult population is high, eggs are found in the upper parts of rice plants. The egg stage is about 7 to 11 days in the tropics. The nymphal stage is 10 to 15 days (Figure 1). The preoviposition period averages 3 or 4 days for brachypterous females and 3 to 4 days for macropterous females (Figure 2). Duration of each stage depends on temperature and cultivars. In the greenhouse each female lays about 100 to 200 eggs. The adults and nymphs usually stay on the lower parts of rice plants (Figure 3). However, when the population is very high, they are observed to swarm even on flag leaves, the uppermost internodes of panicles, and panicle axes.

The adults of BPH are yellowish brown or dark brown. Length of macropterous male 2.3–2.4 mm (3.8–4.2 mm, including fore wing), female 2.8–3.2 mm (4.4–4.8 mm, including fore wing), brachyterous male 2.0–3.1 mm, female 2.7–3.5 mm, post–tibial spur with 30–36 teeth (Okada, 1977). BPH is belonging to family Delphacidae, the largest (more than 1,100 species) among 15 families of infra–order,

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Fulgoromorpha. Variations of the macropterous fore wings or teguments and the spur or calcar at the apex of the hind tibia are the genus *Nilaparvata.*

The average temperatures in tropical lowlands range from about 20 to 30°C–20 to 31°C at Calcutta, 25 to 31°C at Bangkok, 26 to 28°C at Jakarta, and 25 to 30°C at Manila. The time from appearance of the adult in one generation to that in the following generation is 28 to 32 days at 25°C constant, and 23 to 25 days at 28°C constant. The growing duration of existing rice cultivars in the tropics ranges from 78 to 230 days (Grist, 1968). Thus, *N. lugens* may be calculated to have 2 to 8 generations during one rice cropping season in tropical lowlands. In fact, *N. lugens* has five generations on a single rice crop in southern Japan (Mochida, 1964a), five or six generations in the central part of China (Lei and Wang, 1958), and four or five generations in Java (Mochida *et al.,* 1977).

The seasonal occurrence of *N. lugens* depends on the presence of rice plants in the tropics. In many rain fed paddy fields in Java, rice is absent during the dry season. *N. lugens* is not found so abundantly in such fields, but is found in some rice fields irrigated in the dry season (Mochida *et al.,*1977).

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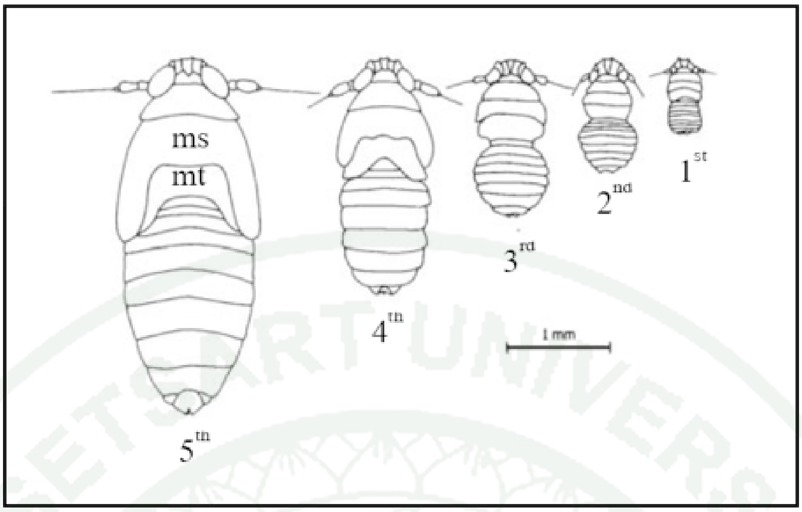

Figure 1 First to fifth instars of the brown planthopper.,
ms= mesonotum,
mt= metanotum

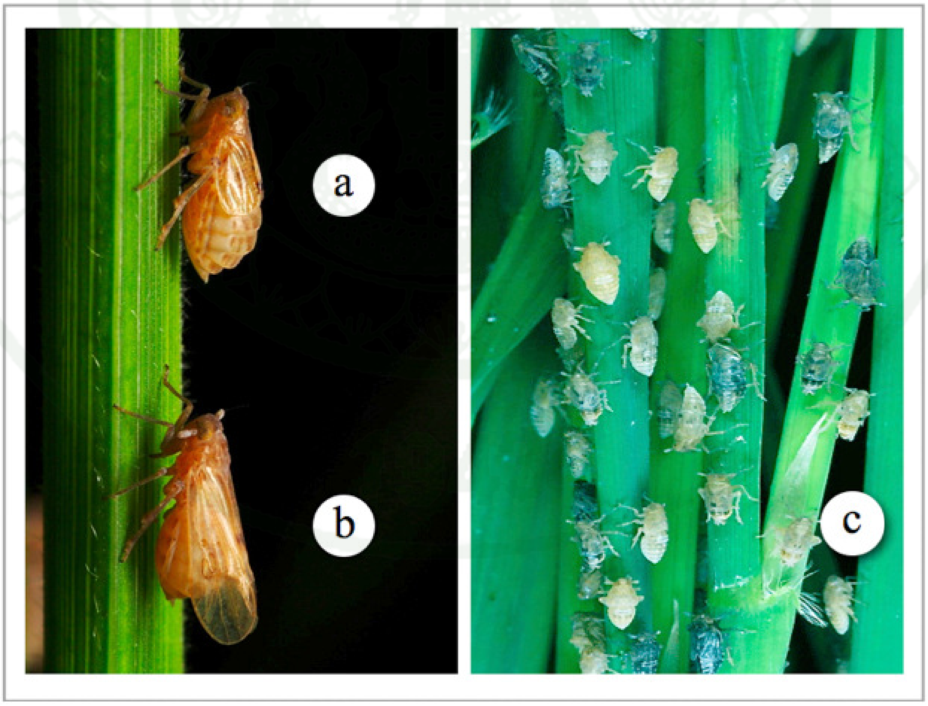

Figure 2 Two wing forms of adult female and theirs nymphs.

a. Brachypterous wing form

b. Macropterous wing form

c. Nymphs of brown planthopper

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Figure 3 The brown planthopper settled on lower part of rice plant.

2. Reproduction of the brown planthopper

Reproductive organs. Spermatogenesis and ogenesis have been studied by Suenaga (1963). Rudimentary reproductive organs are found in first–instars nymphs. The spermatogonia and the spermatocytes in early stages are present in the middle of the first–instars span. The maturation division of spermatogenesis starts between the end of the third–instars and the early fifth–instars period. The spermatozoa are seen at the bottom of the testicular tubules in the fifth–instars. Because the *N. lugens* female has acrotrophic ovarioles, ogenesis advances rapidly after emergence, as in other delphacids (Mochida, 1973a).

Copulation, egg–formation, and egg–deposition. Adult males on rice plants. before copulation, are attracted by the abdominal vibration of females even from a distance of about 80 cm (Ichikawa and Ishii, 1974; Ichikawa *et al.,* 1975; lchikawa 1976). For 24 hours after emergence males cannot copulate. Their ability to copulate increases up to 5 days after emergence, then decreases (Takeda, 1974). A male can copulate at most with nine females for 24 hours; a female can copulate two or more

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times during her life time. Egg–formation is not related to copulation, but discharge of fully grown eggs from the vitellarium of the ovariole is related to copulation, as in the delphacid *J. pellucida* (Mochida, 1970, 1973a). The number of eggs laid by females during their life spans ranges between 0 and 1,474 eggs. The number of eggs laid is correlated to life span and ovipositional period, as mentioned in the section on effect of temperature. Although there are many data from several authors, because of large individual variations it seems difficult to decide whether females of one wing–form produce more eggs than females of the other. However, brachypterous females start to oviposit earlier than macropterous females in cool environments. The preoviposition period averages 3 or 4 days for brachypterous females but 3 to 10 days for macropterous ones at constant temperatures of 20 to 33°C.

3. Dispersal of the brown planthopper

The migration and flight of macropterous adults are influenced greatly by their age, sex, and climatic conditions. Macropterous adults take off for flight around sunrise and sunset. Light intensity adequate for takeoff flight is about I to 200 lux. The frequencies of flight activity of *N. lugens* form a bimodal crepuscular curve at times in the temperate zone when low temperatures may suppress sunset flight to produce a unimodal pattern. The low temperature threshold for takeoff is about 17°C. Flight behavior or takeoff is suppressed by winds more than 11 km/hour (Ohkubo and Kisimoto, 1971). Flight activity seems to continue under conditions of low temperature, high humidity, and weak wind (Ohkubo 1973). MacQuillan (1975) observed in the tropics that the diurnal flight activity of *N. lugens* has a unimodal crepuscular pattern.

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4. Distribution of the brown planthopper

BPH is widely distributed in rice growing areas throughout South and Southeast Asia. It is also found in East Asia, The South Pacific Islands, and Australia (Figure 4) (Dyck and Thomas, 1979; Khush, 1979).

Asia: Japan, Korea, China, Taiwan, Philippines, Vietnam, Laos, Thailand, Myanmar Malaysia, Indonesia, Brunei, Cambodia, Nepal, India, Bangladesh, Bhutan, Sri Lanka, Pakistan, Papua New Guinea

Australia: Queensland

The South Pacific Island: Fiji, Solomon Islands

Figure 4 Distribution of the brown planthopper in Asia and Australia.

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5. Feeding physiology of the brown planthopper

The BPH has mouth parts specialized for intake plant sap. The most conspicuous elements are stylets, which serve as a piercing and sucking organ. Stylets that are about 650–700 μm long consist of an outer pair of mandibular and an inner pair of maxillary stylets (Sogawa, 1973). The maxillary stylets are interlocked to form two canals, the dorsal and the ventral canal. The dorsal canal functions as the sucking canal and communicates with the sucking pump via the pharyngeal duct. The ventral canal is the salivary canal. Saliva is forced out by the action of the salivary pump through this canal (Sogawa, 1982). A coagulable saliva is secreted during stylet penetration to form a stylet sheath or salivary sheath. The stylet sheaths seem to play a major role in the bundling of the stylets protruding beyond the labial tip so as to enable them to function as a piercing and sucking organ. They support for stylet penetration, by sealing them into the sucking sites of the rice plant tissues (Sogawa, 1982).

The BPH is a typical vascular feeder. It primarily sucks the phloem sap. The BPH is attracted to the fresh rice plant–by–plant volatile substances. The volatile substances are considered to play an important role in the BPH attraction to, and persistence on, the host plant. Prior to starting stylet probing, the BPH applies the labium perpendicularly to the plant epidermis and explores the surface. It seems to be that the specific sites of stylet penetration are determined in response to the surface texture of host plant (Sogawa, 1982). The BPH produces an average of 16 feeding marks in a day on seedlings of susceptible rice variety, while on those of a resistant variety is about 30–50 feeding marks (Sogawa and Pathak, 1970). The frequently of the probing sites is depending on the acceptable host plant of the insect. The BPH tends to change the probing sites more frequently on less acceptable host plant such as N–deficient rice plant or resistance variety (Sogawa, 1970a; Sogawa and Pathak, 1970).

Sucking activity at the end of the probing is immediately followed by a characteristic stylet movement consisting of the protrusion of only the maxillary

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stylets beyond the stylet sheath. During sustained sucking, the BPH excretes a relatively small amount of liquid called honeydew (Sogawa, 1982). The honeydew contains about 2–5% of carbohydrates. Most of the carbohydrates occur as soluble polysaccharides. Glucose, fructose, sucrose, a few oligosaccharides, various free amino acids and amides are contained in BPH honeydew. Aspatic and glutamic acids are the major amino acids detected in the honeydew (Noda *et al*., 1973). The rate of honeydew excretion by female adult is estimated at 1.3–2.0 µl (Sogawa, 1970b).

6. Feeding Damage of the brown planthopper

Both the nymphs and adults of BPH feed on the leaf sheaths at the basal portion of the rice plants. In most cases the BPH severely damages rice plants in the post–flowering stage. The removal of assimilates and reductions in photosynthetic rate of leaves by the BPH feeding have the greatest effect on growth and yield on rice plant (Watanabe and Kitagawa, 2000). The typical sucking damage caused by BPH is commonly referred to as hopper burn. The first symptom of hopper burn injury appears on rice plants as a yellowing of the older leaf blades. It extends progressively to all parts of the plants that are above the ground. In the paddy fields, hopperburn usually appears as a browning of plants in scattered patches. In severe cases the patches spread rapidly on a large scale (Sogawa, 1982). A probable cause of hopper burn damage is the reduction in the rate of translocation of photosynthetic to the root system because of the drain of phloem sap and the physiological disruption of active transportation in the phloem by sustained feeding and stylet probing. Disturbance of the physiological activities of the root system enhances leaf senescence causing the accumulation of free amino acids and amides in the leaf blades (Sogawa, 1982).

Wilting symptoms can occur if the amount of energy supplied is less than that required for tissue maintenance (Watanabe and Kitagawa, 2000). The wilting symptom from the infestation is differed from those of plants under drought stress, in which the leaf blades dry up with little loss of green color. The chlorophyll content of the leaf blades of the BPH–infested plant decreased with the reduction in moisture content. The total free amino acid content of chlorotic leaf blades is conversely more

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than four times that of healthy ones. The concentration of aspartic acid, glutamic acid and valine decreased in the infest plants (Sogawa, 1982).

7. Mechanism of Resistance

Antixenosis, the effect of plant resistance on insect behavior (Smith, 1989) , a term derived from a Greek word xeno (guest), describes the inability of a plant to serve as a host to an insect herbivore. As a result, a potential pest insect is forced to select an alternate host plant. The term antixenosis resistance in plant was proposed by Kogan and Ortman (1978) to describe more accurately the non–preference reaction of insects to resistant plant, which was originally defined by Painter (1951). Non–preference resistance in the group of plant characters that lead to a plant being less damaged than another plant lacking these characters and the insects' response to them.

Both antixenosis and non–prefernce denote the presence of morphological or chemical plant factors that adversely alter insect behavior, resulting in selection of an alternate host plant. Physical barriers such as thickened plant epidermal layers, waxy coatings on leaves and stems, or trichomes (plant hairs) may force to insect abandon their efforts to feed on an otherwise palatable host plant. Insect–resistant crop plants may be devoid of, or lack sufficient levels of, phytochemicals that stimulate feeding or oviposition. Antixenosis in plants may also be due to the possession of unique phytochemicals that repel or deter insect herbivores from feeding or oviposition.

Antibiosis is the category of plant resistance to insects that describes the negative effects of a resistant plant on the biology of an insect attempting to use that plant as a host. Both chemical and morphological plant defenses mediate antibiosis, and antibiotic effects of resistant plants range from mild to lethal. Lethal effects may be acute, often affecting young larvae and eggs, The chronic effects of antibiosis lead to mortality in older larvae, prepupae, pupae and adult, when larvae and pupae fail to pupate and eclose, respectively. Individuals surviving the direct effects of antibiosis may also suffer the debilitating effects of reduced body size and weight, prolonged

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periods of development in the immature stage, and reduced fecundity as surviving adults (Smith, 1989).

Antibiosis occurs because of either the presence of plant allomones or the absence of plant kairomones. Antibiosis resistant cultivars may lack the proper quantities of basic insect nutrient or contain phytochemicals that are toxic to insects. Antibiosis may also occur owing to high concentrations of structural plant substances, such as lignin and silica that reduce insect digestion (Smith, 1989).

Tolerance, the effect of plant growth characteristics on resistance to insects. Plants may also be resistant to insects by possessing the ability to withstand or recover from damage caused by insect populations equal to those on susceptible cultivars. The expression of tolerance is determined my inherent genetic ability of plant to grow an insect infestation or to recover and add new growth after the destruction or removal of damaged tissues. From an agronomic perspective, the plants of a tolerant cultivar produce a greater yield than plant of non–tolerant, susceptible cultivars. Unlike antixenosis and antibiosis, tolerance involves only plant characteristics and is not part of an insect–plant interaction. However, tolerance often occurs in combination with antibiosis and antixenosis. Because of its unique nature in plant resistance to insects, the quantitative assessment of tolerance is accomplished by using different experimental procedures from those used to study antixenosis or antibiosis (Smith, 1989).

8. Varietal Resistance of Rice

Studies on varietal resistance in rice to BPH were initiated at the International Rice Research Center (IRRI). The methodology for mass rearing of BPH and mass screening of test varieties had been established since 1967. IRRI has systematically emphasized identified of resistance germplasm, genetic analysis of resistance varieties, and incorporation of BPH resistance genes into improved lines. Most of the resistance germplasms were found among traditional *indica* varieties that originated on the Indian subcontinent, particularly southern India and Sri Lanka (Khush, 1979).

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The experimentation on biological interactions between the BPH and resistance rice varieties has demonstrated various adverse effects of resistance varieties on the BPH life cycle. If the BPH is forced to stay and feed on the resistance varieties, there is a striking deterioration in nymphal development, with high mortality and irregular prolongation of the nymphal period (Cheng and Chang, 1979; Sogawa and Pathak, 1970). Only a small proportion of nymphs developed to adulthood and the adults were small. However, some characteristic of rice varieties are allowed BPH populations to build up but the varieties can ability to withstand insect infestations and yield satisfactorily in spite of injury levels that would debilitate nonresistance varieties.

9. Genetic of Resistance to the BPH

Genetic Analysis for Major Gene Resistance Inheritance of resistance to BPH has been investigated since 1968 (Khush, 1979). Four resistance varieties, Mudgo, ASD7, CO22 and MTU15, were initially analyzed. F2 populations from the crosses of susceptible TN1 with resistance varieties, Mudgo, MTU15 and CO22, segregated into a ratio 3 resistances: 1 susceptible, indicating that three varieties have a dominant gene for resistance to BPH. The F2 population from the cross TN1 x ASD7 segregated into 1 resistance: 3 susceptible, indicating that ASD7 has a recessive gene for resistance (Athwal *et al*., 1971). The single dominant gene in Mudgo, MTU15 and CO22 was at the same locus. This locus was designated as *Bph1*. The resistance in ASD7 is controlled by a single recessive gene, designated as *bph2* (Khush, 1979). No recombination between Bph1 and *bph2* was observed. It was indicated that these two genes are closely linked (Athwal *et al*., 1971).

Later studies, Lakshminarayana and Khush (1977) analyzed 28 resistance varieties. Nine of the varieties had *Bph1* and 16 had *bph2* for resistance. Two new loci for resistance were discovered. A single dominant gene governs resistance in Rathu Heenati segregated independently of *Bph1* and was designated as *Bph3*. A single recessive gene in Babawee segregated independently of *bph2* and was designated as *bph4*. Resistance in PTB21 is controlled by one dominant, *Bph3* (Ikeda, 1985) and one recessive gene, *bph2* (Ikeda and Kaneda, 1983).

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A new resistance gene that resistance to the BPH biotype 4 but not to *Bph1*, *bph2*, *Bph3* and *Bph4* was evaluated at the Bangladesh Rice Research Institute (BRRI). This gene was designated as *bph5* (Khush *et al.*, 1985). Seventeen resistance varieties which resistance to biotype 4 but susceptible to biotype 1, 2 and 3, were genetically analyzed. Seven were found to have single dominant gene, which segregated independently of *bph5*. The single dominant gene was designated as *Bph6* (Kabir and Khush, 1988). The remaining ten cultivars were found to have recessive resistance genes and eight of them were allelic to bph5 but the recessive gene of two cultivars was non–allelic to *bph5*. The recessive gene of T12 was designated as *bph7* (Kabir and Khush, 1988).

Nemoto *et al.* (1989) studied on two Thai varieties, Col.5 Thailand and Col.11 Thailand, and Chin Saba from Myanmar. He found a single recessive gene, which was allelic to each other but was non–allelic to *bph2* and *bph4*. The recessive gene of these three cultivars was also non–allelic to *bph5* and *bph7*, which did not confer resistance to biotype 1, 2, and 3, but the new gene did. Therefore, this new recessive gene was different from all the other recessive genes and was designated as bph8. In 1988, other new gene, Bph9, has been found in Kaharamana, Pokkali, and Balamawee (Nemoto *et al.*, 1989).

An introgression line, IR65482–4–136–2–22, from a cross IR31917–45–3–2/O. australiensis was found to have a single dominant gene governing BPH resistance, which has been tentatively designated as *Bph10* (Ishii *et al.*, 1994). The other unregistered resistance genes such as *Bph*(t) (Guoqing *et al.,* 2000), bph(t) (Hirabayashi *et al.,* 2000) and *Bph*(t) (Jena *et al.*, 2000), were investigated.

The genes for resistance in rice varieties can be inferred without genetic analysis by determining their reaction to different biotypes. If a variety is resistance to biotype 1 and 3, it is likely to have *Bph1*; if a variety is resistance to biotype 1 and 2, it has *bph2*; and if it is resistance to all three biotypes, it may have any of these, *Bph3*, *bph4*, *bph8*, or *Bph9* (Panda and Khush, 1995).

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Table 1 Sources of resistant genes for the brown planthopper

Gene	Variety	Reference
Bph1	Mudgo, MTU15, CO22	Athwal *et al*., 1971
	MGL2	Athwal and Pathak ,1972
	IR747B2–6	Martinez and Khush, 1974
	Tibiriwewa, Balamawee, CO10, Heenakkulama,	Lakshminarayana and Khush, 1977
	MTU 9, Sinnakayam, SLO12, Sudhubalawee,	
	Sudurvi 305	Ikeda and Kaneda, 1983
	Asdaragahawewa	Ikeda and Kaneda, 1986
	Balamawee	
bph2	ASD7	Athwal *et al.,* 1971
	PTB18	Athwal and Pathak, 1972
	H 105, IR1154–243	Martinez and Khush, 1974
	Anbaw C7, ASD9, Dilwee 328, Hathiel, Kosatawee, Madayal, Mahadikwee, Malkora, M.I.329, Murungakayan302, Ovarkaruppan, Palasithari 601, PK–1, Seruvellai, Sinna, Karuppan, Vellailangayan	Lakshminarayana and Khush, 1977
	PTB21, PTB34, H5, IR9–60,	Chang *et al*., 1971
	Kaosen–Yu 12	Ikeda and Kaneda, 1983
	PTB33	Jeon *et al*., 1999
	Gatabyeo	
Bph3	Rathu Heenati	Lakshminarayana and Khush, 1977
	PTB19, Gangala, Horana Mawee, Kuruhondarawala, Mudu Kiriyal, Hondarawala 378	Sidhu and Khush, 1978
	PTB21	
	PTB33	Ikeda, 1985
		Lakshminarayana and Khush, 1977; Angeles *et al*., 1986

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Table 1 (Continued)

Gene	Variety	Reference
bph4	Babawee Gambada Samba, Heenhoranamawee, Hotel, Samba, Kahata Samba, Kulukuruwee, Lekam, Samba, Senawee, Sulai, Thirissa, Vellai Illankali	Lakshminarayana and Khush, 1977 Sidhu and Khush, 1978
bph5	ARC10550 Leb Mue Nahng, ARC15872, ARC13788, S61, ARC11367, ARC15694, ARC14342A, ARC15831 (a)	Khush *et al.*, 1985 Kabir and Khush, 1988
Bph6	Swarnalata	Kabir and Khush, 1988
bph7	T12	Kabir and Khush, 1988
bph8	Thai Col.5, Thai Col.11, Chin Saba	Nemoto *et al.*, 1989
Bph9	Kaharamana, Pokkali, Balamawee (70–518)	Nemoto *et al.*, 1989
Bph10	*Oryza australiensis*	Ishii *et al.*, 1994
bph(t)	*Oryza officinalis*	Hirabayashi *et al.*, 2000 Huang *et al.*, 2001
Bph(t)	Sanguuizhan	Mei *et al.*, 1996
Bph(t)	*Oryza eichingeri*	Guoqing *et al.*, 2000
Bph(t)	*Oryza latifolia*	Yang *et al.*, 2002
Unknow–gene (Qbph 6)	Abhaya	Jairin *et al.*, 2005
Unknow–gene (Qbph 12)	Abhaya	Jairin *et al.*, 2005

Source: Jairin (2008)

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MATERIALS AND METHODS

Materials

1. Plant materials

Plant materials used in this study were two introgression lines (UBNKD6–56, UBNKD4–283) of BC_3F_7 from crossing between KDML105 and Rathu Heenati and two introgression lines (KPSKD17–173, KPSKD55–220) of BC_4F_8 from crossing between KDML105 and Abhaya. The parents from two introgression line, Rathu Heenati, Abhaya and KDML105 were compared with the all of progenies. Taichung Native 1 (TN1) was used for susceptible check. Completely Randomized Design was used in all experiment.

2. Insect materials

Stock culture of *N. lugens* was originated from the insect population collected from Ubon Ratchathani province, north–eastern of Thailand in 2003. The insect were reared on young seedlings of a susceptible cultivar, Taichung Native 1 (TN1) following the method of Yushima *et al.* (1991). All insect cultures were maintained under controlled conditions (approx. 25°C and 70% relative humidity) inside a nylon mesh cage. Mass rearing of BPH population, adults BPH were reared on tillering of TN1 inside a wire screen cage measuring 1 x 1 x 1 m in a greenhouse.

Methods

1. Antixenosis agianst the BPH nymphs in the rice seedlings

Antixenosis (AX) (preference of nymphs on seedling) was assessed by comparing KDML105 background within different resistant genes (*Bph3* and *Qbph6, 12*). The three seedlings of each two different lines were opposite planted in a 12 onz. on translucent plastic cup following the list of treatment (Table 2). At 7 DAS, 30

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second instars nymphs were released on the seedling. The number of nymphs settling on each seedling was counted at first hour after releasing, and 4 hours interval till 1 days. After 24 hours, the number of nymphs settling was counted 12 hours intervals untill 1 week.

To detect the antixenosis mechanism of *Bph3* and *Qbph6, 12* on 4 KDML105 introgression lines, the different treatment were defined following: Non–different morphology with representing and non–representing of resistant genes from introgression lines and KDML105, respectively, were compared in treatment 1 – 4. Representing of resistant genes with KDML105–liked and resistant cultivar morphology from introgression lines and donors (RH and AB), respectively were compared in treatment 5 – 8. Those two above criteria of comparison could illustrate antixenosis expression from KDML105 introgression lines. Representing of resistant genes and non–different morphology of introgression lines were compared in treatment 9 and 10 to illustrate other possible antixenotic factor. The level of antixenosis of Rathu heenati and Abhaya were compared in treatment 11. Non–different combination of parent lines was used for control treatment to prove the hypothesis that the insects would settle freely without disturbance of normal olfaction or vision.

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Table 2 Comparison treatment

Treatment	Comparison	
1	UBNKD6–56	KDML105
2	UBNKD4–283	KDML105
3	KPSKD17–173	KDML105
4	KPSKD55–220	KDML105
5	UBNKD6–56	Rathu Heenati
6	UBNKD4–283	Rathu Heenati
7	KPSKD17–173	Abhaya
8	KPSKD55–220	Abhaya
9	UBNKD6–56	UBNKD4–283
10	KPSKD17–173	KPSKD55–220
11	Rathu Heenati	Abhaya
12	Rathu Heenati	Rathu Heenati
13	Abhaya	Abhaya
14	KDML105	KDML105

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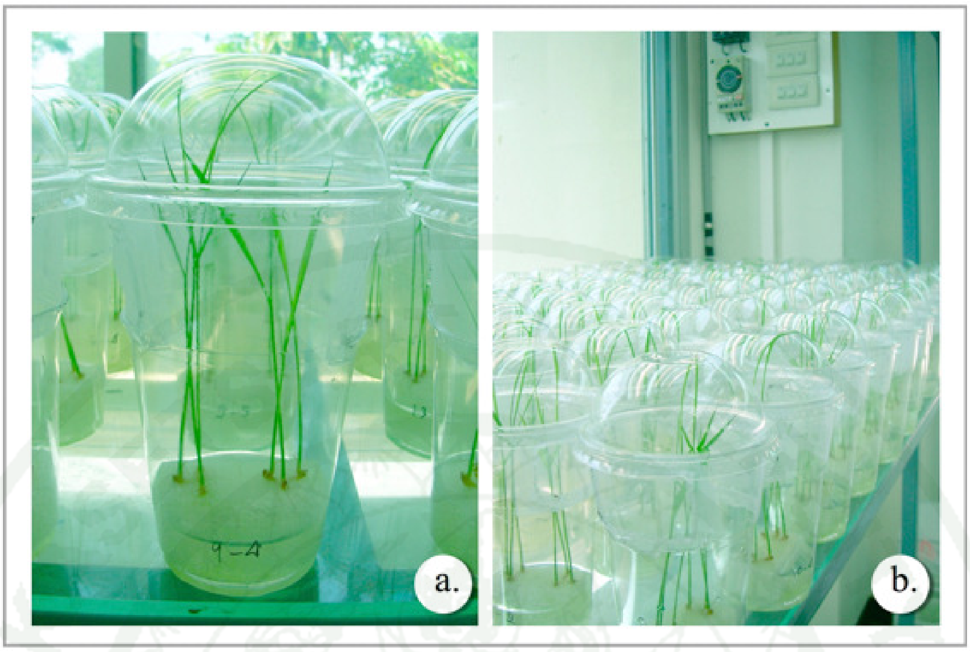

Figure 5 Antixenosis on seedling experiment

a. Two difference lines were opposite placed in a chamber

b. The experiment was placed in a conditional control room

3. Antibiosis to area of honeydew excretion on tillering

The test of honeydew excretion, as a measure of feeding rate, was conducted as described in Henrichs *et al.* (1985). Seeds were pregerminated with tissue paper on plastic tray (10 x 10 cm) then transplanted into 12 onz. of plastic glass by using clay as media. During the seedling growing, urea fertilizers were applied every 1 week until rice plant getting to tillering stage. At 20 days after sowing, rice plants were removed arthropods preventing contamination. Newly emerged adult female were starved for four hours in nylon mesh cage with a moist cotton ball then transferred into honeydew deposited chamber on single tiller of 20 day old plants (5 newly emerged adult female per chamber). Ten chambers were replicated for each treatment. The base of each plant was encircled by filter paper disk dyed with bromocresol green. After 24 hrs, the filter papers were collected and total area of blue–green spots (resulting from honeydew deposition) was measured using Motic Picture Advance program for Windows.

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Figure 6 Honeydew excretion experiment

a. The experiment was placed in a conditional control room

b. Honeydew deposit chamber and filter paper dyed with Bromocresol green inside

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4. Antibiosis to nymphal survival and development period

Seeds were sowed on tissue paper soaking in water then transplanted to hydroponic tray using sponge cube as medium. After 7 days, the seedlings were transferred into test tube, one seedling on each tube. The experiment was conducted in a control climate room. Liquid fertilizers were applied to seedling after transferring and at 3 days interval. Ten first instars nymphs were infested on tested lines in a test tube include susceptible check. Seedlings were changed new ones at 2 days interval. The number of nymphs that reached adulthood was counted and the percentage of nymphal survival was calculated. Ten tubes were replicated for each treatment. For development period of nymphs, only one of 2nd instars nymph were infested on tested lines in a test tube include susceptible check, started then to observe daily for ecdysis and the number of days that reached to adult stage as development period. Growth index on each line was computed from the data obtained from the experiment from nymphal survival and development period as percent of survived nymphs divided by the developmental period of nymphs.

5. Assessment of plant tolerance

Seeds were pregerminated with tissue paper on a 10 x 10 cm of plastic tray, transplanted into modified cage for experiment then. The fifty pregerminated seeds then were planted on tissue paper as media (1 cm of media flooded with water). During the seedling growing, liquid fertilizers were applied to seedling after transplantation and at 3 days intervals. The level of tolerance on seedling was studied by releasing 50 first instars nymphs on seedlings. A control plant without insects was maintained for each line. When TN1 plants (susceptible check) started to wilt then stopped the experiment and collected insects from all the lines. The insects from infestation were oven dried at 70°C for 48 h and weighed. The infested and uninfested plants were removed from cage with their media, and air dried for 3 h, then oven dried at 70°C for 48 h and weighed. Evaluation of tolerant level was calculated by using functional plant loss index, tolerance index and plant dry weight loss per mg of insect dry weight produced as described by Panda and Heinrichs (1983).

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Figure 7 Antibiosis and the assessment of tolerance experiment

a. Development period and Nymphal survival experiment were conducted in a conditional control room

b. The assessment of tolerance experiment was conducted in a greenhouse

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6. Data handling and statistical analysis

For the data obtained from antixenosis against the BPH nymphs in the rice seedlings experiment were converted into proportion of insects that settled on each line in a chamber. Hypothesis test were applied to prove the experiment following;

H_0: The proportion of insect settlement in a chamber is 1:1 without disturbance of host plant

H_1: The proportion of insect settlement in a chamber is not 1:1 within disturbance of host plant.

When X^2*–test* < *P–value* , H_0 is accepted and H_1 is rejected. While X^2*–test* >*P–value,* H_0 is rejected and H_1 is accepted.

$$X^2 = \sum (O - E)^2/E$$

Where *O* is observed data, *E* is expected data and *P–value* ≥ 0.3481 at *df* = 1

GI, FPLI and TI were calculated using the following formula as described by Panda and Heinrichs (1983).

GI = Percentage of nymphs survived/The developmental period of nymphs

FPLI = [1–(Dry weight of infested plant/Dry weight of uninfested plant)] x 100

TI = BPH dry weight on test line/BPH dry weight on susceptible check, KDML105

The data were analyzed by the ANOVA. Means were compared by the test of least significant difference (P > 0.05, LSD test)

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RESULTS AND DISCUSSION

Results

Mechanisms of resistance to brown planthopper, *Nilaparvata lugens* (Stål) on two sets of rice resistant gene, *Bph3* and *QTLs* (chromosomes *Qbph6,* and *Qbph12*) were investigated and compared with their parent cultivars KDML105, Rathu Heenati, Abhaya and susceptible check TN1. The Bph3 resistant gene was derived from UBNKD6–56 and UBNKD4–283 from BC_3F_7 progenies of KDML105 (KD) and Rathu Heenati (RH) crosses. The resistant QTLs (Qbhp6 and Qbph12) were obtained from two selected lines, KPSKD17–173 and KPSKD55–220, carrying *Qbph6, 12* resistant *QTLs*, and unidentified gene derived from BC_4F_8 progenies of KDML105 and Abhaya (AB) crosses.

1. Antixenosis of rice seedling

The proportion of insects in a chamber with 2 choices of rice plants should be closely to 1:1 if there is no preference in terms of rice varieties. Figures 8A – 8C shows proportions of *N. lugens* between the same rice varieties. The differences of proportions of *N. lugens* are minimized after 48 hours of infestation. The proportions were confirmed by the chi–square test for non–preference of *N. lugens* on the same rice varieties (Table 3).

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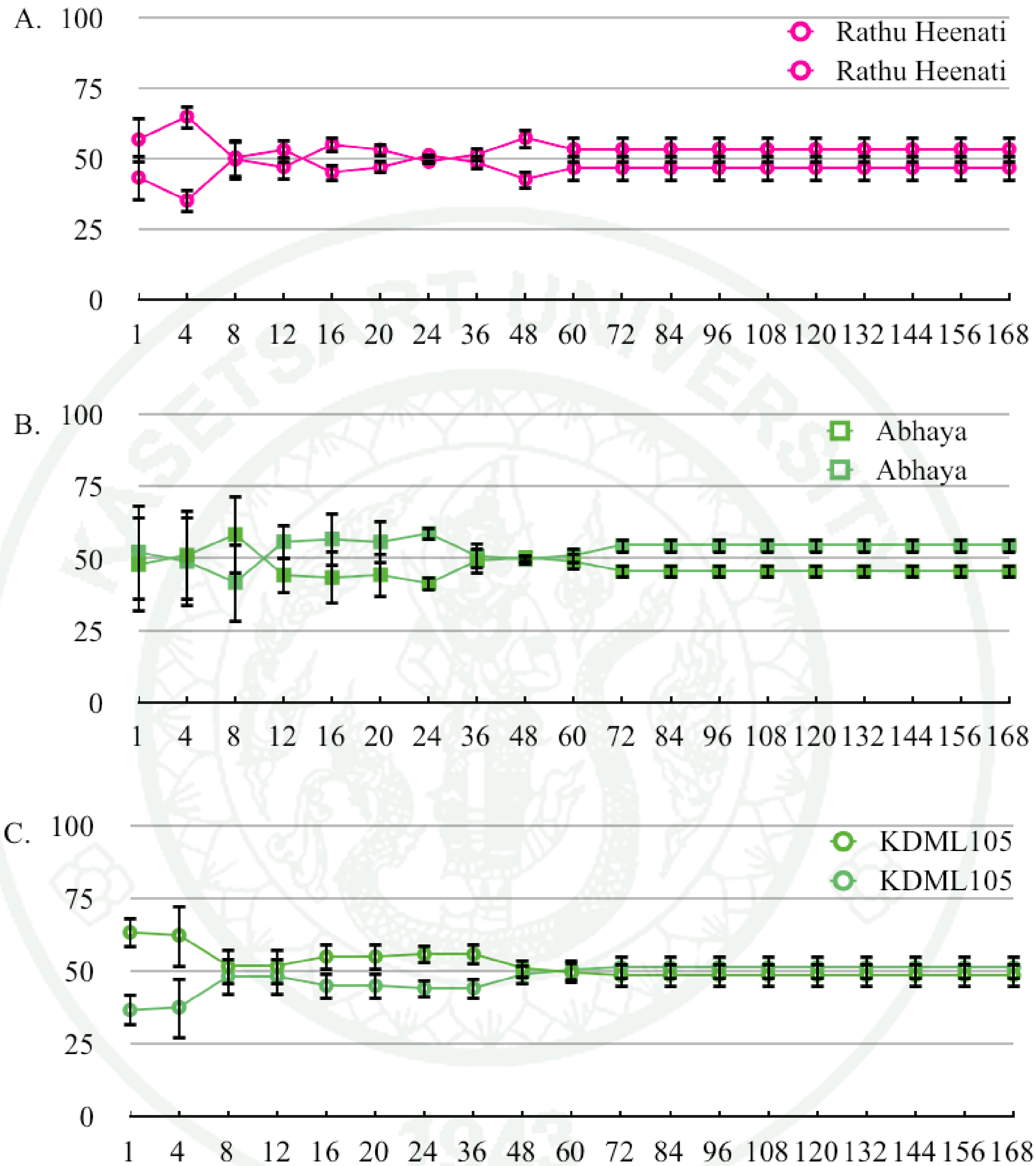

Figure 8 The proportion of *N. lugens* in a chamber with the same rice varieties. The X–axis showed hours after infestation and the Y–axis showed % of *N. lugens* settling on seedling in a chamber. Vertical bars indicated the standard error of six replications.

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Table 3 Preference test of *N. lugens* on the same rice cultivars at *P–values* ≥ 3.481

HAI[1]	*Chi*–square values[2]		
	Rathu Heenati vs. Rathu Heenati	Abhaya vs. Abhaya	KDML105 vs. KDML105
1	1.83	0.18	*7.11*
4	*8.79*	0.05	*6.11*
8	0.00	2.78	0.11
12	0.38	1.34	0.11
16	0.96	1.78	1.00
20	0.39	1.31	1.00
24	0.05	3.02	1.36
36	0.08	0.03	1.36
48	2.15	0.00	0.05
60	0.44	0.05	0.01
72	0.44	0.81	0.07
84	0.44	0.81	0.07
96	0.44	0.81	0.07
108	0.44	0.81	0.07
120	0.44	0.81	0.07
132	0.44	0.81	0.07
144	0.44	0.81	0.07
156	0.44	0.81	0.07
168	0.44	0.81	0.07

[1] Hour after infestation

[2] Number italicized indicates preference of *N. lugens* with *P*–value ≥ 3.481.

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In the comparison among KD and 4 introgression lines (Figures 9A – 9D), at the beginning of insect orientation, the results showed that most insects clustered on UBNKD6–56 (22%), KPSKD17–173 (51%) and KPSKD55–220 (23%) that H_0 was rejected in preference of H_1 (Table 4). The higher proportions were found in UBNKD4–283 which were not significant difference to KD and the H_0, the non–preference in rice varieties was accepted.

The insects gradually moved to KD after first 4 hours, and evidently stayed on the KD more than on the introgression lines. In addition, the results rejected H_0 and accepted H_1, however some proportions of insects still remained on the introgression lines. During the 12 and 24 hours, the insects more preferred orienting to KD and finally totally settlement on KD till the end of experiment in UBNKD6–56, UBNKD4–283 and KPSKD55–220, dissimilarly on KPSKD17–173 which showed some proportions of insects that kept settlement on this line.

In the comparison of introgression lines and their donors, at the initial time of releasing, the proportion of insects was not significantly different in UBNKD6–56 (Figure 10A) and the highly significant difference of proportion was observed from UBNKD4–283 (Figure 10B) which accepted and rejected H_0 (Table 5), respectively. Both of KPSKD17–173 and KPSKD55–220 were observed the higher proportion of insects at 1 hour after infestation (Figures 10C and 10D).

The insects variably oriented among two varieties in a chamber during first 24 hours. After 1 day of releasing, the insect more preferred to settle on UBNKD6–56 and UBN4–283 than RH which is their donor till the end of experiment. Comparison of KPSKD17–173 and KPSKD55–220 against their donor (AB) showed that insects more significantly preferred on these two introgression lines than on AB at the initial time of releasing. The insects continuously oriented and settled on introgression lines and the proportion was not significant difference during first 24 hours. After 1 day, the insects oriented to settle on introgression lines and continuously settled on them in higher proportion than AB till the end of experiment.

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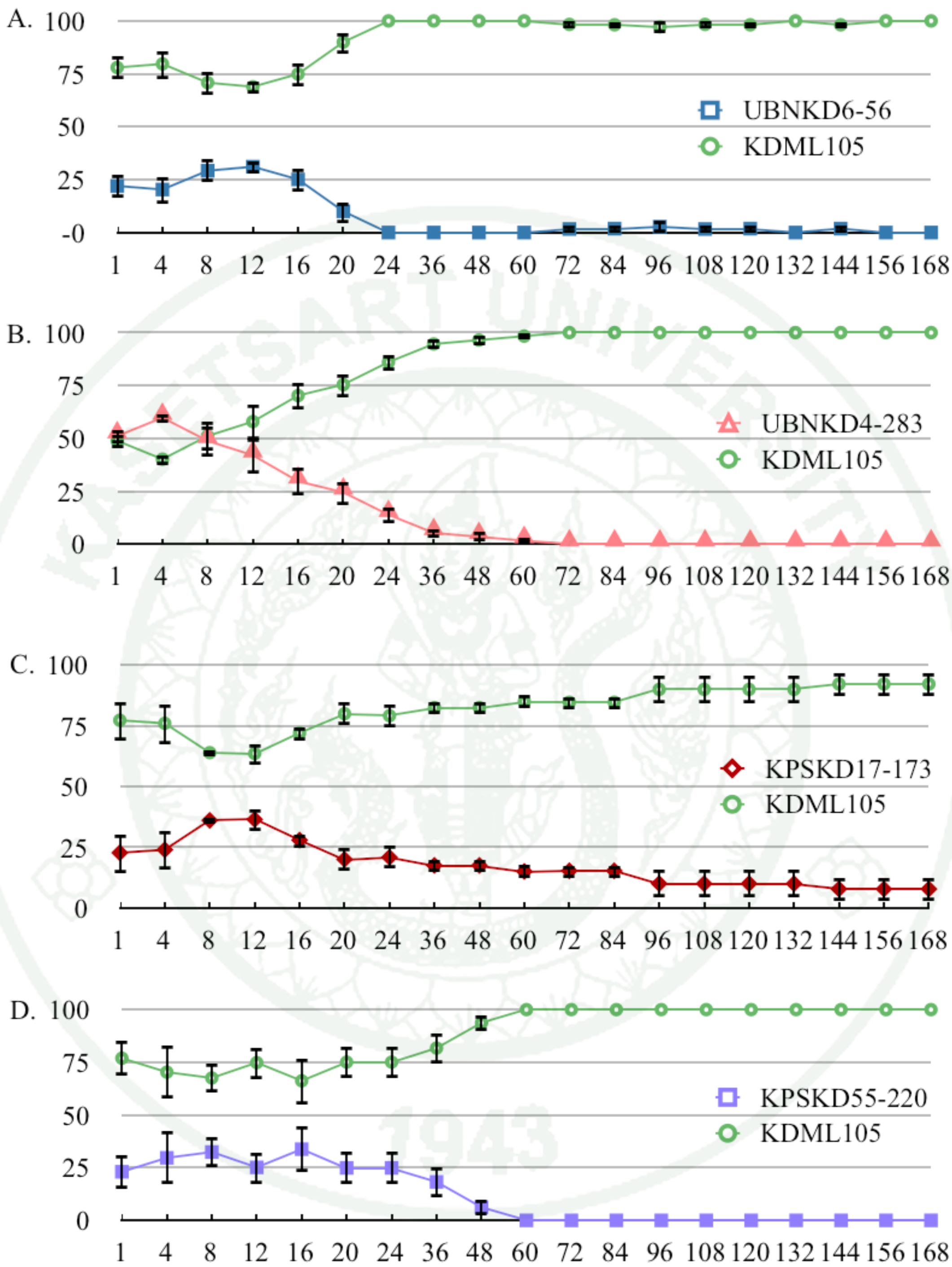

Figure 9 The proportion of *N. lugens* settling on selected lines against KDML105. The X –axis showed hours after infestation and the Y–axis showed % of *N. lugens* settling on seedling in a chamber. Vertical bars indicated the standard error of six replications.

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Table 4 The preference test of nymphs in the comparison of tested lines and KDML105, at *P–values* ≥ 3.481

HAI[1]	*Chi*–square values[2]			
	UBNKD6–56 vs. KDML105	UBNKD4–283 vs. KDML105	KPSKD17–173 vs. KDML105	KPSKD55–220 vs. KDML105
1	*31.31*	0.05	*29.79*	*29.06*
4	*35.24*	*3.85*	*27.13*	*16.54*
8	*17.33*	0.05	*7.79*	*12.35*
12	*14.27*	2.54	*7.39*	*24.72*
16	*24.82*	*16.40*	*19.51*	*10.53*
20	*63.94*	*25.93*	*36.00*	*25.22*
24	*100.00*	*52.03*	*34.03*	*25.22*
36	*100.00*	*79.67*	*42.25*	*40.33*
48	*100.00*	*86.42*	*42.25*	*76.66*
60	*100.00*	*93.44*	*49.00*	*100.00*
72	*93.44*	*100.00*	*48.23*	*100.00*
84	*92.73*	*100.00*	*48.23*	*100.00*
96	*89.20*	*100.00*	*64.00*	*100.00*
108	*93.44*	*100.00*	*64.00*	*100.00*
120	*92.73*	*100.00*	*64.00*	*100.00*
132	*100.00*	*100.00*	*64.00*	*100.00*
144	*92.73*	*100.00*	*71.31*	*100.00*
156	*100.00*	*100.00*	*71.31*	*100.00*
168	*100.00*	*100.00*	*71.31*	*100.00*

[1] Hour after infestation

[2] Number italicized indicates preference of *N. lugens* with *P*–value ≥ 3.481.

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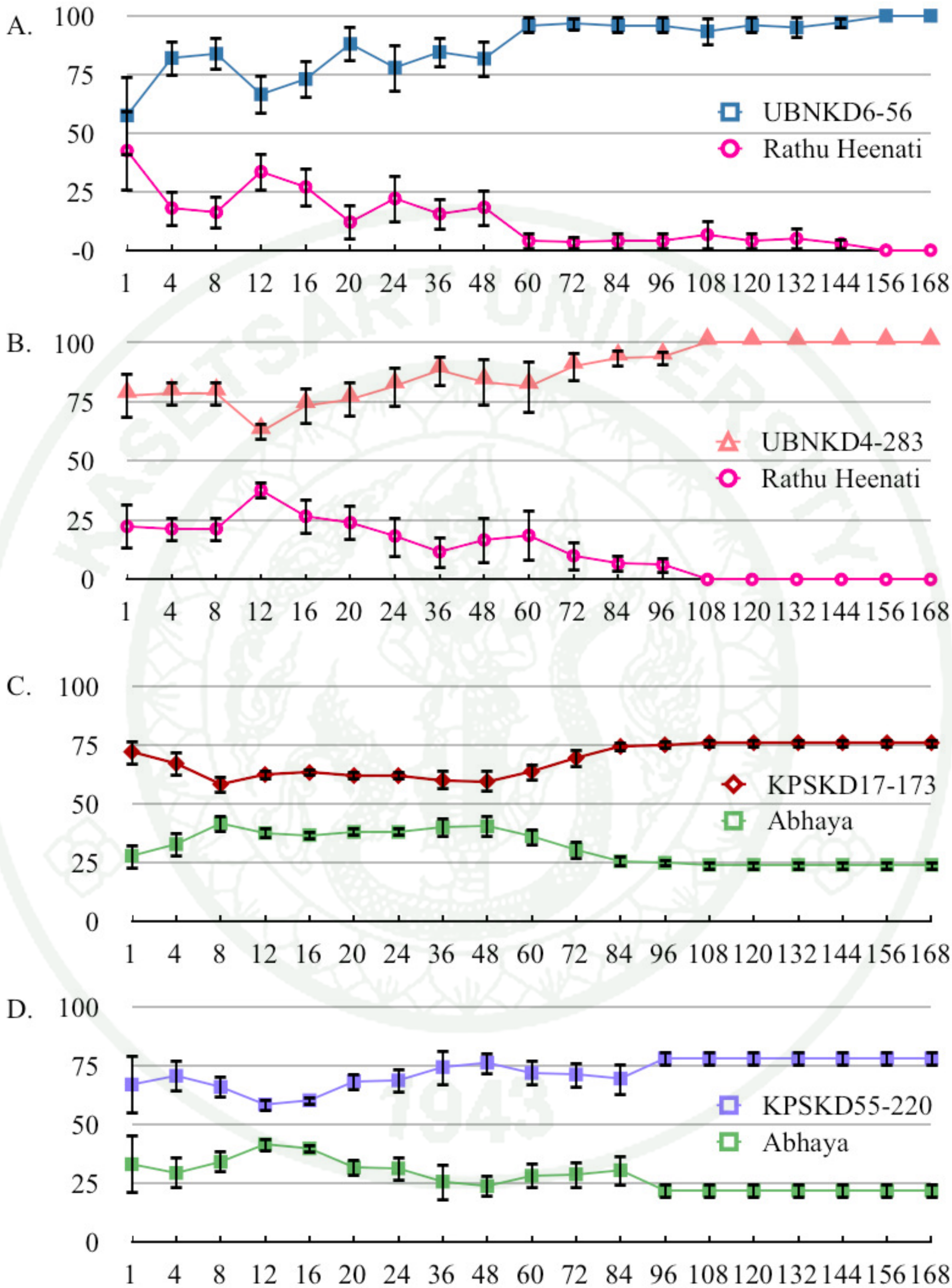

Figure 10 The propotion of *N. lugens* settling on selected lines against their donors (Rathu heenati and Abhaya) . The X–axis showed hours after infestation and the Y–axis showed % of *N. lugens* settling on seedling in a chamber. Vertical bars indicated the standard error of six replications

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Table 5 The preference of nymphs in the comparison of selected lines and their donors, at *P–values* ≥ 3.481

HAI[1]	*Chi*–square values[2]			
	UBNKD6–56 vs. Rathu Heenati	UBNKD4–283 vs. Rathu Heenati	KPSKD17–173 vs. Abhaya	KPSKD55–220 vs. Abhaya
1	2.25	*30.51*	*19.61*	*11.51*
4	*40.96*	*32.76*	*11.76*	*17.13*
8	*45.72*	*32.76*	2.78	*10.18*
12	*10.90*	*6.08*	*6.19*	2.86
16	*21.16*	*21.78*	*7.11*	*4.19*
20	*57.76*	*27.04*	*5.76*	*13.44*
24	*31.15*	*40.11*	*5.76*	*14.03*
36	*47.61*	*58.78*	*4.00*	*23.77*
48	*40.23*	*44.44*	*3.65*	*27.56*
60	*84.64*	*39.51*	*7.59*	*19.14*
72	*87.11*	*64.00*	*15.30*	*18.06*
84	*84.64*	*75.11*	*23.90*	*15.23*
96	*84.64*	*76.77*	*25.22*	*31.64*
108	*75.11*	*100.00*	*27.04*	*31.64*
120	*84.64*	*100.00*	*27.04*	*31.64*
132	*81.00*	*100.00*	*27.04*	*31.64*
144	*88.90*	*100.00*	*27.04*	*31.64*
156	*100.00*	*100.00*	*27.04*	*31.64*
168	*100.00*	*100.00*	*27.04*	*31.64*

[1] Hour after infestation

[2] Number italicized indicates preference of *N. lugens* with *P*–value ≥ 3.481.

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Antixenosis between UBNKD6–56 and UBNKD4–283 were compared and the result showed that the insects preferred to settling on UBNKD4–283 than on UBNKD6–56 (Figure 11A) at first hour of infestation that rejected H_0 and accepted H_1 (Table 6). During 4 to 12 hours, insects variably oriented among these two introgression lines in a chamber. After 12 hours of infestation, the proportion of insects was continuously higher in UBNKD4–283, the big significant difference was not found through the end of experiment.

The results of comparison between KPSKD17–173 and KPSKD55–220 showed the proportion of insect settlement was higher in KPSKD17–173 at first hour of infestation, however the big significant different was observed (Figure 11B) and H_0 was rejected and H_1 was accepted (Table 6). During 4 to 12 hours, the variable orienting was observed. After 12 hours of infestation, the proportion of insects was continuously higher in KPSKD17–173 through the end of experiment; however the big significant difference were not found.

Antixenotic factor to the BPH on RH and AB, donors of introgression lines, were compared in this current study (Figure 11C). At the first hour after releasing, the proportions of insect settling on two varieties were not significantly different and H_0 could be accepted (Table 6). The insects gradually oriented to settle on AB and the slight difference of proportion was observed during 4 to 20 hours then moved to AB in the higher proportion than on RH through the end of experiment and H_1 could be accepted.

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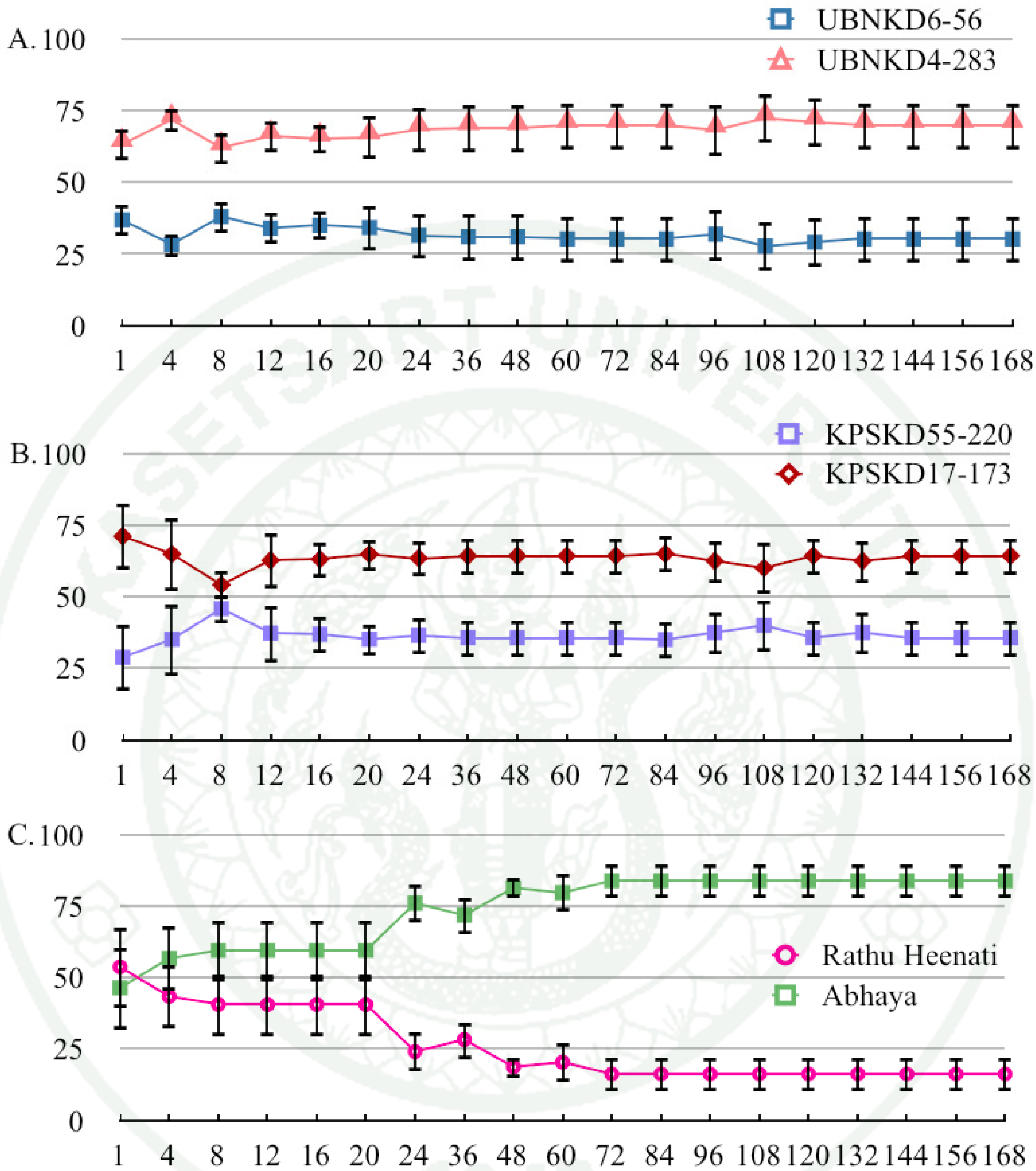

Figure 11 The proportion of *N. lugens* the selected lines and donors. The X–axis showed hours after infestation and the Y–axis showed % of *N. lugens* settling on seedling in a chamber. Vertical bars indicated the standard error of six replications.

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Table 6 The prefernce test of nymphs in the comparison among the tested lines and the comparison of donors, at *P–values* ≥ 3.481

HAI[1]	*Chi*–square values[2]		
	UBNKD6–56 vs. UBNKD4–283	KPSKD17–173 vs. KPSKD55–220	Rathu Heenati vs. Abhaya
1	*6.94*	*17.86*	0.54
4	*18.92*	*8.86*	1.78
8	*5.76*	*0.69*	3.61
12	*10.33*	*6.46*	3.61
16	*9.11*	*6.82*	3.61
20	*10.03*	*8.81*	3.61
24	*13.80*	*7.24*	*26.94*
36	*14.48*	*8.16*	*18.98*
48	*14.48*	*8.16*	*39.21*
60	*15.72*	*8.16*	*35.15*
72	*15.72*	*8.16*	*45.72*
84	*15.72*	*9.00*	*45.72*
96	*13.18*	*6.25*	*45.72*
108	*19.93*	*4.00*	*45.72*
120	*17.53*	*8.16*	*45.72*
132	*15.72*	*6.25*	*45.72*
144	*15.72*	*8.16*	*45.72*
156	*15.72*	*8.16*	*45.72*
168	*15.72*	*8.16*	*45.72*

[1] Hour after infestation

[2] Number italicized indicates preference of *N. lugens* with *P*–value ≥ 3.481.

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2. Area of honeydew excretion on tillering

The rate of honeydew excretion was measured in order to assess the feeding rate of sap–sucking insects. The amount of food intake is directly proportional to the amount of honeydew excreted by the BPH (Figure 12).

The data on the honeydew excreted on the filter paper by one adult female BPH fed on UBNKD6–56, UBNKD4–283, KPSKD17–173, KPSKD55–220 and their donors (RH, AB) including the susceptible KD and TN1, as measured in square millimeters, revealed differences in the rate of honeydew excretion (Table 7 and Figure 12). After 24 hours, the lowest honeydew excretion was recorded from UBNKD4–283 in value of 6.16 mm^2, followed by RH, UBNKD6–56 and AB with values of 6.82, 8.71 and 8.80 mm^2, respectively, and the significant difference was not found against the lowest one. KPSKD17–173, KPSKD55–220 and TN1 showed the moderate area of honeydew excretion with values of 14.86, 17.95 and 26.28 mm^2, respectively. Both of KPSKD17–173 and KPSKD55–220 were not significantly different against the lowest honeydew excretion group. The highest honeydew excretion was obtained from KDML105 with the value of 40.72 mm^2.

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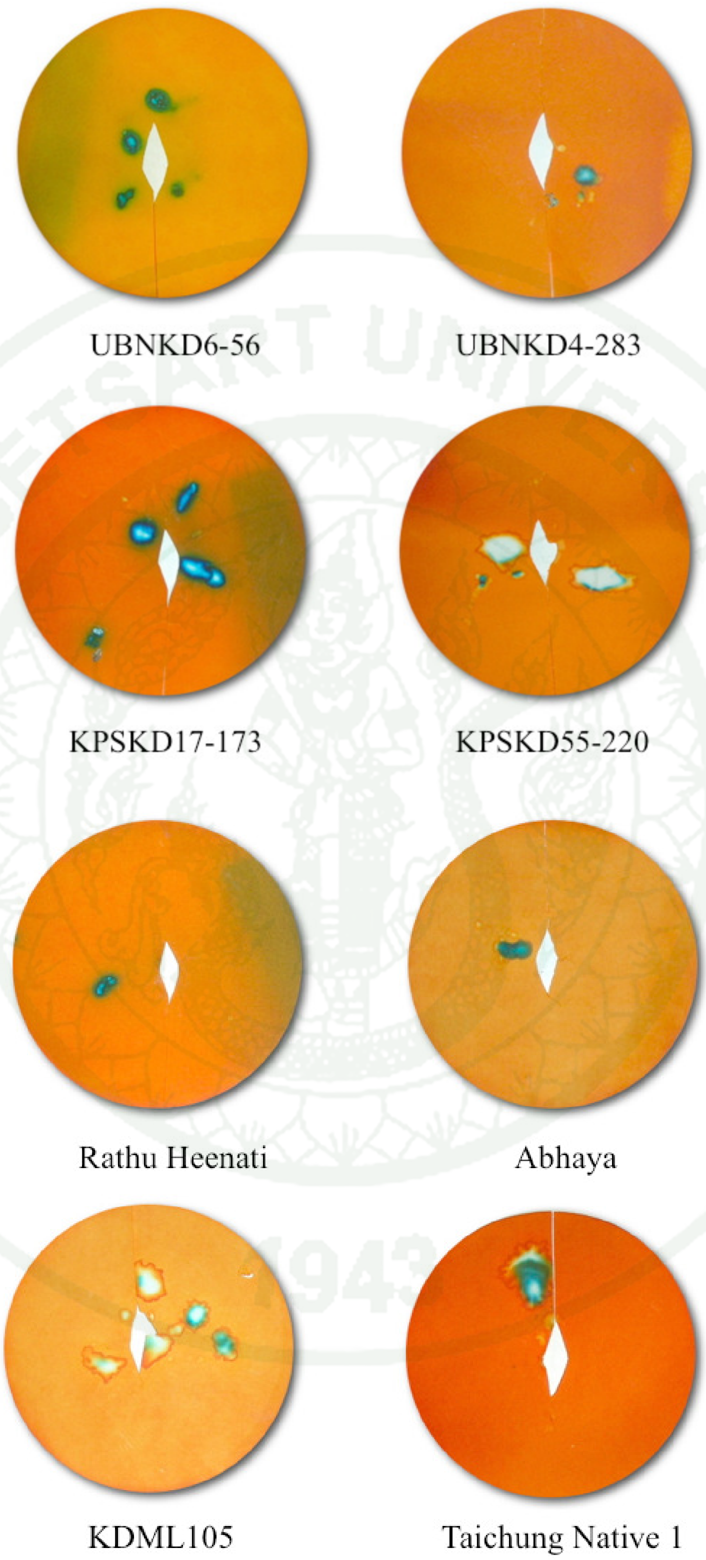

Figure 12 Honeydew spot deposited on filter paper dyed with Bromocresol green after one female of *N. lugens* fed on 30 days old rice plant for 24 hours.

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Table 7 Honeydew excretion area, developmental period, percentage of nymphal survival and growth index.

Lines	Antibiosis (mean ± S.E.[Hm])			
	Area of honeydew (mm^2)[1]	Development period (days)[2]	Nymphal survival (%)[2]	Growth index[3]
UBNKD6–56	8.71±2.97[a]	21±0.6[a]	41.90±2.4[a]	2.08±0.1[a]
UBNKD4–283	6.16±5.29[a]	23±0.2[a]	50.38±3.1[a]	2.18±0.1[a]
KPSKD17–173	14.86±4.33[ab]	17±0.4[b]	62.04±2.2[c]	3.61±0.1[b]
KPSKD55–220	17.95±2.90[ab]	18±0.4[b]	62.55±2.6[c]	3.47±0.1[b]
Rathu Heenati	6.82±2.03[a]	22±0.2[a]	57.03±2.9[b]	2.63±0.2[a]
Abhaya	8.80±1.40[a]	17±0.4[b]	58.91±1.5[b]	3.53±0.1[b]
KDML105	40.72±5.94[c]	10±0.6[c]	75.60±4.4[d]	7.78±0.5[c]
TN1	26.28±5.08[b]	10±0.4[c]	77.83±3.1[d]	7.47±0.3[c]
Grand mean	16.30	17	60.78	4.09
LSD	12.28	3.05	12.20	1.27
%CV	58.20	28.52	19.63	55.25

Means within a row followed by the same letter are not significantly different.

[1] Based on the average from 6 replications, each replication were infested by one adult female.

[2] Based on the average from 10 replications, each replication was infested by 10 of 1st to 2nd instars nymph.

[3] Based on the average from 10 replications and Growth index of BPH on each line was computed from the data obtained from the experiments on nymphal survival and developmental period as percentage of nymphs survived divided by the developmental period of nymphs.

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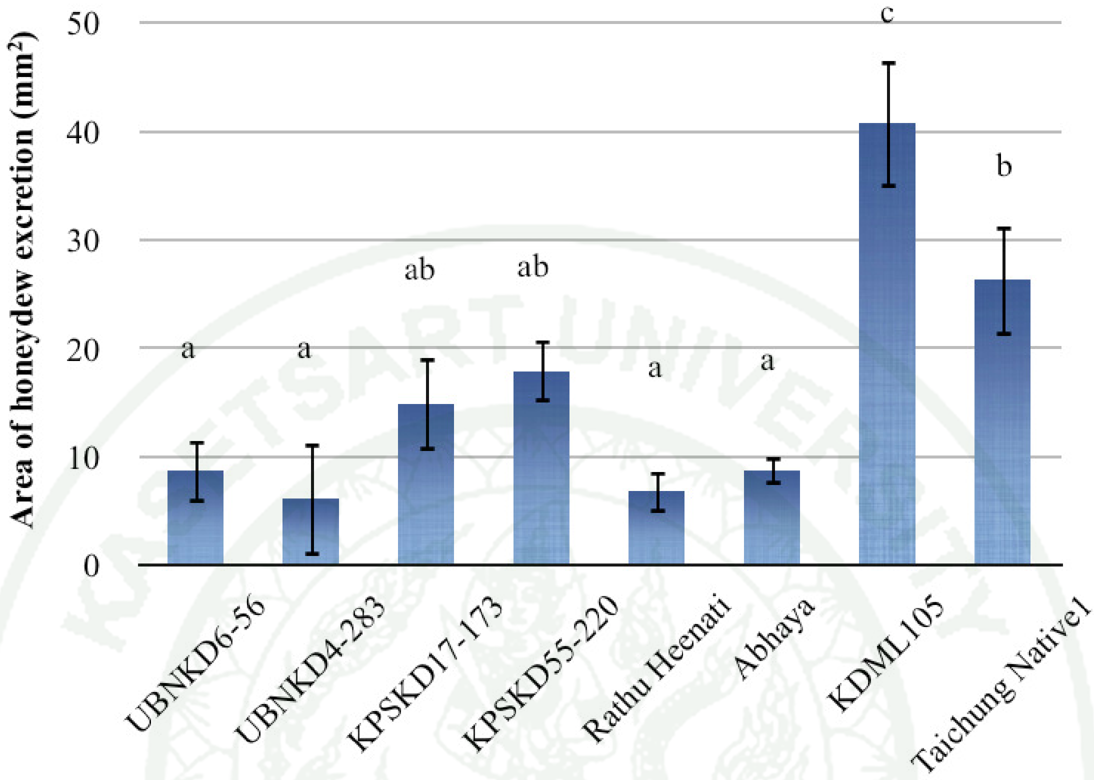

Figure 13 Comparison of honeydew excreted area obtained from 1 BPH from different lines at 24 hrs after infestation with vertical bars indicated the standard errors from 6 replications. Same letters above vertical bars indicated statistically non–significant differences ($P \geq 0.05$).

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3. Nymphal survival (NS), development period (DP) and growth index (GI)

Significantly higher percentage of nymphs becoming adulthoods was obtained from the susceptible check TN1 (77.38%) and KD (75.60%) (Table 7 and Figure 14 A), followed by KPSKD55–220, KPSKD17–173, AB and RH in values of 62.55%, 62.04%, 58.91% and 57.03%, respectively. The percentage of nymphal survival recorded from KPSKD17–173 and KPSKD55–220, were not significantly different among RH and AB. Almost of insects were died on UBNKD6–56 and UBNKD4–283, which had the lowest of survival rates in values of 41.90% and 50.38%, respectively, but the survival rate from UBNKD4–283 was not significantly different among KPSKD17–173 and KPSKD55–220.

The nymphal development period was more prolonged on resistant cultivars than on the susceptible TN1 and KD (Table 7 and Figure 14 B). Among the introgression lines of RH and AB, the highest nymphal development periods were observed from UBNKD4–283 and followed by UBNKD6–56, KPSKD55–220 and KPSKD17–173 with values of 23, 21, 18 and 17 days, respectively. Both of UBNKD6–56 and UBNKD4–283 were not significantly different against their donor RH, and similarly in KPSKD17–173 and KPSKD55–220, the recorded results were not significantly different against their donor AB. Both of RH and AB showed long development period but significant shorter in AB.

The lowest growth index (GI) of BPH was recorded from UBN6–56 (2.08) and it was not significantly different to UBN4–283 (2.18) and RH (2.63). The moderate growth index was obtained from KPSKD17–173 (3.61) and KPSKD55–220 (3.47) and they were not significantly different from their donor AB (3.53) (Table 7 and Figure 15).

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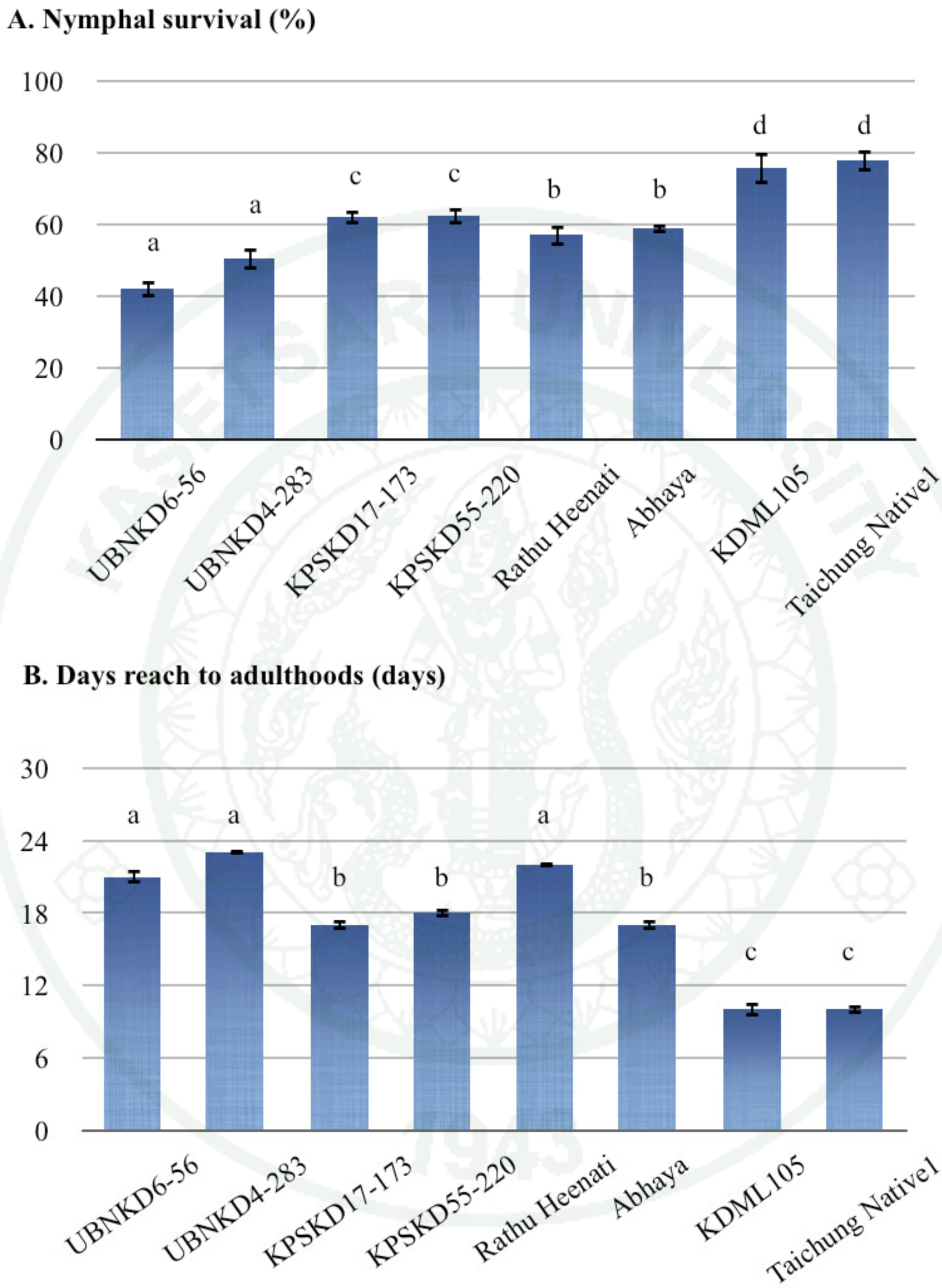

Figure 14 Comparison of nymphal survival (A) and developmental period (B) with vertical bars indicated the standard errors from 6 replications. Same letters vertical bars indicated statistically non–significant differences (P ≥ 0.05)

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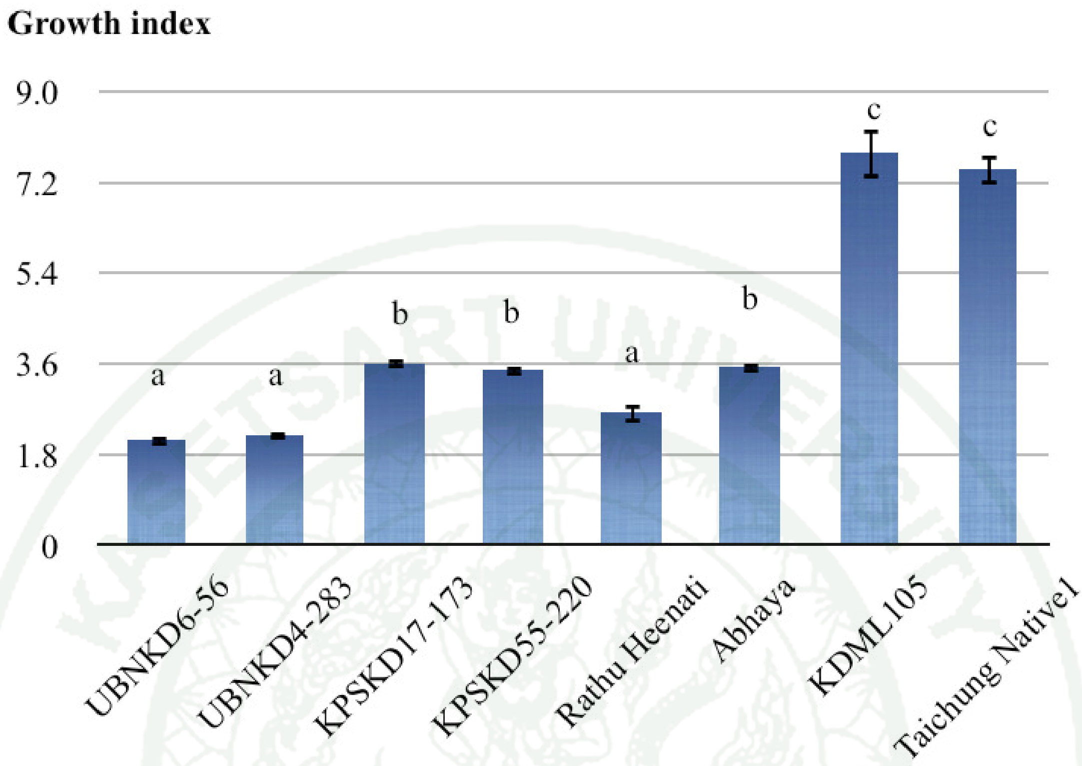

Figure 15 Comparison of growth index with vertical bars indicated the standard errors from 6 replications. Same letters above vertical bars indicated statistically non–significant differences ($P \geq 0.05$)

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4. Functional plant loss index (FPLI) and tolerance index (TI)

The standard evaluation system (SES) of rice damage by BPH was applied to this experiment as one of co–factors to obtain the FPLI. The experiment was stopped when the susceptible KD was definitely died after 7 days of infestation. The mean SES values of KD revealed the highest amount of damage from Ubon BPH with a mean SES value of 7.7 followed by KPSKD55–220, AB and KPSKD17–173 with mean SES values of 3.8, 2.2, and 1.3, respectively. The mean SES values of UBNKD6–56 and UBNKD4–283 revealed the lowest amount of damage with mean SES values of 0.1 and 0.1, respectively, and they were not significantly different against their donor RH with a mean SES value of 0.0 (Table 8).

The seedling height was also observed from the experiment and applied as the other co–factors to obtain the FPLI. The mean of plant height was highest in UBNKD6–56 (19.2) and UBNKD4–283 (18.3). The data obtained from the susceptible TN1 revealed the shortest of plant height (Table 8).

The Functional Plant Loss Index (FPLI) was lowest in RH (25.67%) and UBNKD4–283 (30.89%), and the significant differences were not found between them. The higher percentage was observed from UBNKD6–56 (39.42%) and significantly different to the lowest one, followed by KPSKD55–220 (48.36%), KPSKD17–173 (56.59%) and Abhaya (58.90%), respectively, the significant differences were not found between KPSKD17–173 and Abhaya. The highest of FPLI was obtained from KD (86.40%) and the susceptible TN1 (89.65%) (Table 8).

The tolerance index (TI) was obtained from BPH dry weight on test lines divided by BPH dry weight on susceptible TN1. The results revealed that UBNKD6–56 (0.16) and UBNKD4–283 (0.19) were not significantly different among their donors, RH (0.13) and Abhaya (0.14) which are the resistant cultivars to BPH damaged. Whereas KPSKD17–173 and KPSKD55–220 were obtained the higher values and significantly different against their donor AB but not including UBNKD4–283. The data obtained from TN1 and KD showed the lowest tolerance in values of

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0.58 and 0.94, respectively (Table 8).

The correlation of resistant mechanism among the parameters was analyzed to illustrate the correspondence between mechanisms (Table 9). Area of honeydew excretion correlated positively with nymphal survival, growth index, functional plant loss index and tolerance index (r = 0.83, $P < 0.05$; r = 0.93, $P < 0.001$; r = 0.86; $P >$ 0.05 and r = 0.97, $P < 0.001$, respectively). Nymphal survival correlated positively with growth index, functional plant loss index and tolerance index (r = 0.93, $P <$ 0.001; r = 0.86, $P > 0.05$ and r = 0.78; $P < 0.05$, respectively). Growth index correlated positively with functional plant loss index and tolerance index (r = 0.94, P < 0.001 and r = 0.93, $P < 0.001$, respectively). Functional plant loss index correlated positively with tolerance index (r = 0.83, $P < 0.01$). However, developmental period correlated negatively with all parameters, area of honeydew excretion, growth index, functional plant loss index and tolerance index (r = –0.90, $P < 0.01$; r = –0.90, $P <$ 0.05; r = –0.97; $P < 0.001$; r = –0.99, $P < 0.001$ and r = –0.86, $P < 0.001$, respectively).

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Table 8 Functional plant loss index and tolerance index

Lines	Tolerance (mean ± S.E.[Hm])			
	SES[1,2]	Plant height (cm)[2]	Functional plant loss index (%)[3]	Tolerance index[4]
UBNKD6–56	0.4±0.1[a]	19.2±0.3[a]	39.42±2.0[b]	0.16±0.01[a]
UBNKD4–283	0.3±0.1[a]	18.3±0.2[ab]	30.89±2.4[a]	0.19±0.01[ab]
KPSKD17–173	1.3±0.3[b]	12.4±0.4[d]	56.59±1.6[d]	0.25±0.01[b]
KPSKD55–220	3.8±0.3[d]	12.6±0.4[cd]	48.36±1.9[c]	0.23±0.01[b]
Rathu Heenati	2.2±0.3[c]	13.1±0.3[cd]	25.67±0.8[a]	0.13±0.03[a]
Abhaya	0.0±0.0[a]	17.9±0.3[b]	58.90±2.2[d]	0.14±0.01[a]
KDML105	7.7±0.4[e]	13.4±0.1[c]	86.40±4.2[e]	0.94±0.04[d]
TN1	9.0±0.0[f]	9.30±0.1[e]	89.65±0.0[e]	0.58±0.04[c]
Grand mean	3.1	14.40	54.48	0.33
LSD	0.7	1.84	13.62	0.65
% CV	27.0	15.60	43.49	12.18

Means within a row followed by the same letter are not significantly different.

[1] Standard evaluation system

[2] Based on the average from 8 replications, each replications were infested by ten of 3rd to 4th instars nymphs.

[3] Based on the average from 8 replications, and the data obtained from Functional plant loss index (FPLI) formula.

[4] Based on the average from 6 replications and the data obtained from Tolerance index formula.

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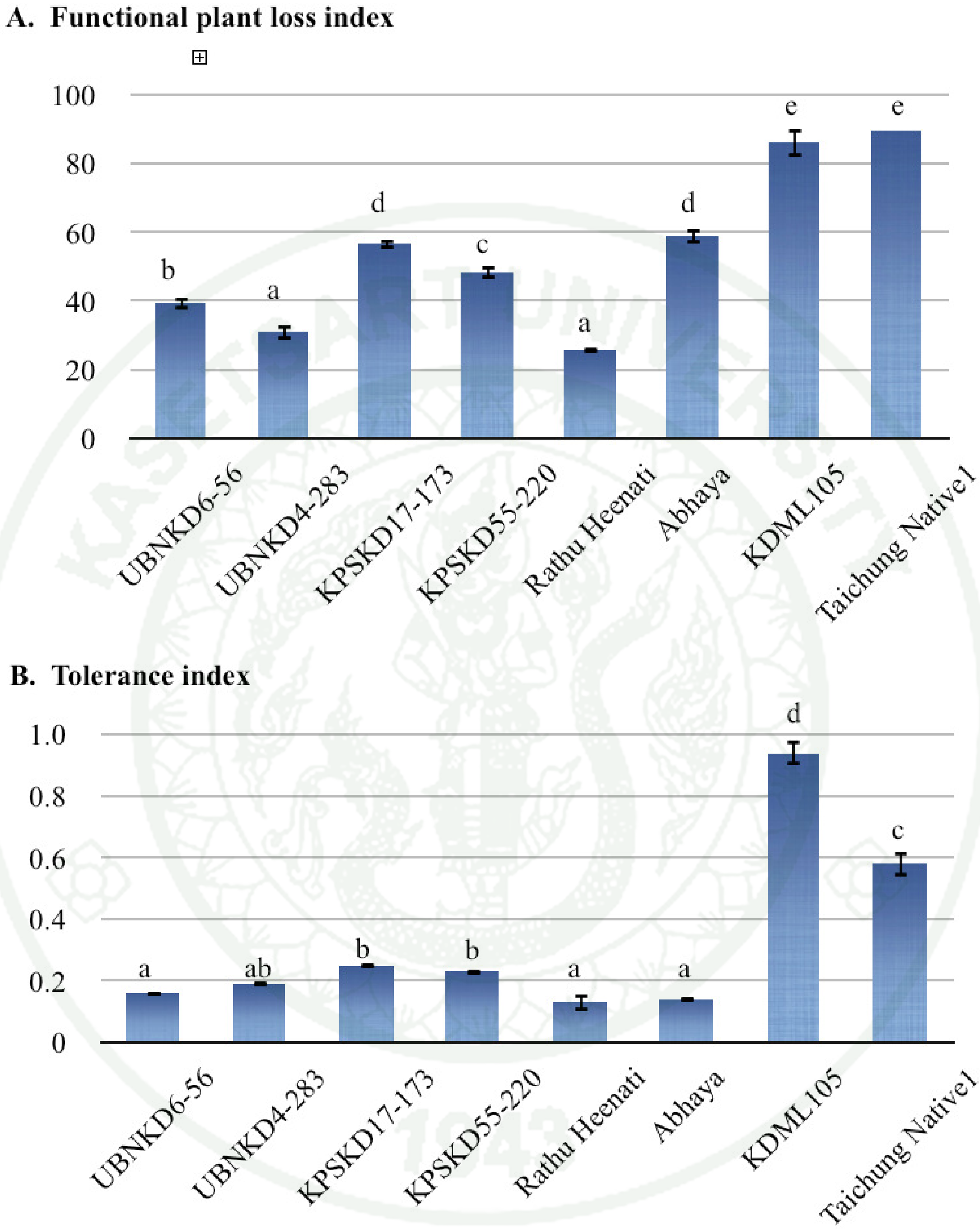

Figure 16 Comparison of functional plant loss index (A.) and tolerance index (B.) on different varieties with vertical bars indicated the standard errors from 6 replications. Same letters above vertical bars indicated statistically non–significant differences ($P \geq 0.05$).

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Table 9 Correlation coefficients among area of honeydew excretion (AHD), nymphal survival (NS), developmental period (DP), growth index (GI), functional plant loss index (FPLI), and tolerance index (TI)

Traits	AHD	NS	DP	GI	FPLI
NS	0.83*				
DP	–0.90**	–0.90*			
GI	0.93***	0.93***	–0.97***		
FPLI	0.86	0.86	–0.99***	0.94***	
TI	0.97***	0.78*	–0.86***	0.93***	0.83**

Significant differences at ***P < 0.001, **0.01, *0.05, respectively.

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Discussion

Study on mechanisms of resistance to brown planthopper, *Nilaparvata lugens* (Stål) on two sets of rice resistant gene, *Bph3* and *QTLs* (chromosomes *Qbph6,* and *Qbph12*), the *Bph3* resistant gene was derived from UBNKD6–56 and UBNKD4–283 from BC_3F_7 progenies of KDML105 (KD) and Rathu Heenati (RH) crosses, the resistant *QTLs* (*Qbhp6* and *Qbph12*) were obtained from two selected lines, KPSKD17–173 and KPSKD55–220, carrying *Qbph6,12* resistant *QTLs*, unidentified gene derived from BC_4F_8 progenies of KDML105 and Abhaya (AB) crosses revealed the three differences of mechanism, Anitixenosis, Anitbiosis and Tolerance

Hypothesis test of orienting of insects in the control set could be accepted H_0 which is the proportion of insect settlement in a chamber is 1:1 without disturbance of host plant. The current results revealed clearly that the insects settled freely without disturbance of normal olfaction or vision (Jung and Im, 2005) and accepted the preference–performance hypothesis. Therefore, this experiment fits to illustrate the antixenotic mechanism of host plant.

The behavioral observations at the initial times of antixenosis tests indicated that the nymphs did not settle freely. The comparison of introgression lines and KDML105 showed that the insects preferred orienting to KDML105 more than on UBNKD6–56, KPSKD17–173 and KPSKD55–220 accepted on UBNKD4–283 at the initial time after releasing. According to previous genotyping of these introgression lines, the fragrance was not found only in UBNKD6–56 which was not interested by BPH orienting, while UBN4–283 which is fragrant host plant and attracted the insect orienting. The insects were not attracted by KPSKD27 and KPKD28 at the initial time of behavioral observation, however they are fragrant choices. That kind of response has been observed in the previous studies (J. W. Kim, *et al.*, 1985; J.W. Kim and Kim, 1986; Park and Song, 1988).

Whereas, the comparison of introgression lines and their donor, RH and AB showed that the insects preferred orienting and settlement on introgression lines more

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than on RH and AB, although there were resistant gene/QTLs in every choice. Thus, this evidences suggested that there were must other factors effecting to insect preference accepted the fragrance or host–plant characteristic.

Rathu Heenati has no repellent chemical against planthoppers and only has common volatiles as released by susceptible cultivars. The feeding inhibition of this cultivar occurred when the insect started to ingest phloem sap (Jirapong Jairin, 2008; Khan and Saxena, 1984; Liu*, et al.*, 1994). There were several studies confirmed that the resistant mechanism in Rathu Heenati is associated with the phloem (Kimmins, 1989; Padgham*, et al.*, 1989; Stevenson*, et al.*, 1996)

It seems that the introgression lines did not derive the antixenotic property from their donors, Rathu Heenati and Abhaya. However, this kind of resistant mechanism is not any strong repellent factors that effect before insect's exploring on the surface of rice plant, and the preference of insect is determined through trials–and–errors process. Those kinds of process have been directly proved by observation of more active moving and probing not followed by sucking on resistant plants (Cook*, et al.*, 1987; Padgham and woodhead, 1989)

The present results revealed the lowest honeydew excretion in all selected lines at the same amount of honeydew obtained from Rathu Heenati and Abhaya. Therefore, the resistant varieties, Rathu Heenati and Abhaya have given some antifeedant properties to their progenies. However, the insects fed on the selected lines and resistant varieties, no longer alive of insect was not observed in any treatment within 24 hours. On the other hand, despite of small amounts of honeydew excretion were obtained from all selected lines and resistant varieties indicated that the insect could suck a small volume of phloem sap. The result means that the insect penetrated its stylet into the phloem sieve element of those lines (Alagar*, et al.*, 2007; Jung and Im, 2005).

The previous studies suggested that the mechanism of the reduced excretion of honeydew on resistant varieties has been presumed to be caused by the activity of

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antifeedants in phloem sap on insect feeding not by mechanicals barriers (J.W. Kim and Kim, 1986; Sogawa, 1973; Stevenson*, et al.*, 1996)

High mortality of insect is a criterion evaluating antibiosis in plant resistance to insect (Jung and Im, 2005; N. Panda and Khush, 1995). In the current study, the percentage of nymphal survival on UBNKD6–56 and UBNKD4–283 were lowest and even lower than Rathu Heenati. It is remarkable that the progenies of resistant variety and KMDL105 crossing have more resistant factor to antibiosis than resistant variety. It seems that donors have given some factors to their progenies which combine to the genetic background of KDML105.

N. lugens feeds on only phloem sap of rice (Khan and Saxena, 1984). Homopteran insects such as *N. lugens* directly pass a portion of sucked plant sap from anterior midgut to posterior midgut or anterior hindgut, using filter chamber, a specialized digestive system, without absorption (Ammar, 1985; Sogawa, 1982). The passed sap and its metabolites from the insect are mixed in hindgut and excreted outside as honeydew. Sucrose and amino acids are main components of rice phloem (Fukumorita and Chino, 1982). Therefore, analysis of sugar and amino acids in the honeydew excreted by *N. lugens* is a good method for deciding the possibility of phloem feeding and for identifying resistant and susceptible plants to the insect.

In the present study, it is remarkable that the development period of nymphs were longer on progenies and donors, on the contrary when nymphs fed on the susceptible TN1 and KDML105 that emerged earlier the new adults. However, we also founded that the insect fed on those progenies and donors were undersized and blackish of body. Additionally, growth index of the BPH obtained from nymphal survival and development period revealed agreeably in the results. The previous studies also reported that the BPH expressed as longer nymphal period, lower fecundity, and lower body weight of newly–emerged adults, shorter adult longevity, and decrease of population density (Jung and Im, 2005; J. W. Kim*, et al.*, 1985; J.W. Kim and Kim, 1986; Park and Song, 1988; Seo*, et al.*, 2010)

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From the results of antibiosis evaluation, it was presumed that the antibiotic factor is not ones like neurotoxicants to be able to destroy completely the viability of the insect after once ingested.

In the present study, the UBNKD6–56 and UBN4–283 had the lowest functional plant loss index and tolerance index compared with KPSKD17–173, KPSKD55–220 and KDML105. Similar to our study, previous research work carried out with a series of parameters to evaluate the degree of resistance (Alagar*, et al.*, 2007; Heinrichs*, et al.*, 1985; Niranjan Panda and Heinrichs, 1983; Soundararajan*, et al.*, 2004),

It seems that the donors have given the the tolerance to their progenies obviously. However, these introgression lines are not definitely tolerant to the BPH damaging, we still observed the slight damaging on them. These results are agreement with the area of honeydew excretion, nymphal survival, developmental period that activity of host–plant defense is the complex trait of resistant mechanism.

From the result, it is remarkable that when the insects had no choice to settle on the host–plant, they could feed only small amounts of phloem sap, theirs bodies turned blackish and undersized, and even increased the life span. It is well known that plant resistance to insects has evolved as a complex trait, and it results basically from three mechanisms: antixenosis, antibiosis and tolerance. Some insight can be gained by considering the effects of the resistant mechanisms in these introgression lines. Our results indicated that there is some level of antibiosis and tolerance in these introgression lines. Correlation coefficients among the parameters of resistant mechanisms, area of honeydew excretion, nymphal survival, growth index, functional plant loss index and tolerance index were correlated positively for each others excepted for developmental period which was correlated negatively to other parameters. Thus, these correlations demonstrate that antibiosis highly involves the tolerance resistant mechanism.

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CONCLUSION AND RECOMMENDATION

Conclusion

Insect resistance in rice has been recognized as a major tactic in the integrated control of rice pests. Because of the importance of the brown planthopper, the abundance of resistant donors among the cultivated rice and the efficiency of the screening method for evaluating breeding lines, breeders opted for transferring insect–resistant genes from traditional cultivars into improved breeding lines with a high yield potential. High–yielding resistant rice varieties have been extensively cultivated and have contributed to increased rice production in Asia. However, determining the mechanisms of resistance for those resistant cultivars need to be done.

This current study on mechanisms of resistance to brown planthopper, *Nilaparvata lugens* (Stål) of two introgression lines, UBNKD6–56 and UBN4–283, within *Bph3* resistant gene derived from BC_3F_7 progenies of KDML105 and Rathu Heenati crosses, and the others two introgression lines, KPSKD17–173 and KPSKD55–220, carrying *Qbph6,12* resistant *QTLs*, unknown genes derived from BC_4F_8 progenies of KDML105 and Abhaya crosses revealed that antixenosis was not play role in these introgression lines. There were other factors of introgression lines deriving from KDML105 that insects preferred them. Antibiosis and tolerance play role on these introgression lines and they are complex traits of resistant cultivars.

Because it appears that the resistant mechanisms to the *N. lugens* in these introgression lines have several advantageous features, it would be useful to transfer the gene/*QTL*s conferring this resistance to other rice varieties or in breeding program of KDML105.

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Recommendation

Although we have been successful to determine the mechanisms of resistance for those selected lines, the resistance level in vegetative and reproductive stage must be determined. The only one BPH population from Ubonratchathani province, north–eastern of Thailand was used for infestation in this study, so the other populations or even different biotypes should be applied for further study. Furthermore, understanding of rice plant mechanism of resistance, the nutritional value of phloem sap should be compared on the selected lines and KDML105. And it is not clear whether the resistance is constitutive or inducible, although the inducible has been suggested in an experiment about rice responses to *N. lugens* feeding and gene expression.

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APPENDIX

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Appendix Table 1 Standard evaluation system (SES) of rice for damage by the BPH

Grade of damage	Rating[1]	Symptom
0	HR	No damages
1	R	First leaf partially yellow
3	MR	First and second leaves partially yellow
5	MS	Marked yellowing; stunting
7	S	Severe wilting and stunting
9	HS	Plants die

[1] HR= Highly resistant; R= Resistant; MR= Moderately resistant; MS= Moderately susceptible; S= Susceptible; and HS= Highly susceptible.

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