GENOTYPIC SCREENING OF BROWN PLANTHOPPER (*Bph*) RESISTANCE GENES AND EVALUATION OF BPH RESISTANCE IN UPLAND RICE VARIETIES

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Thesis Title	Genotypic Screening of Brown Planthopper (*Bph*) Resistance Genes and Evaluation of BPH Resistance in Upland Rice Varieties
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ABSTRACT

Brow planthopper (*Nilaparvata lugens*; BPH) is the most dangerous pest in rice cultivation, which reduces crop yields. The use of BPH-resistant rice varieties has been used as an economical and environmentally friendly approach to BPH control. Upland rice varieties are an interesting source for developing BPH-resistant rice varieties. In this study, 143 upland rice varieties were genotyped for the presence of the five major *Bph* resistance genes (*bph2, Bph3*, *Bph14, Bph15*, and *Bph17*). The result showed that 139 varieties have at least one to five resistance genes. Five varieties, IR70175-51-2-1-1-2, Lai Chan, Beu Hmeu Mae La, Pi Ai Kor, and Khao Nieow Kla have all resistance genes. The allele frequencies of the *R* genes ranged from 33.57% to 61.54%. The Polymorphism information content value (PIC) ranged from 0.4460 (IN76-2) to 0.4984 (RM16626) with an average value of 0.47437, indicating that the markers were moderately informative for identifying genetic diversity of BPH resistance. Cluster analysis merged 143 upland rice varieties into two main clusters. The population structure was also examinated, which divided the upland rice into the SP1 and SP2 subpopulations. 92% of the variation was identified within the subpopulations, according to analysis of molecular variance (AMOVA), indicating that there was a significant gene

exchange between the two subpopulations. Additionally, an expression of BPH resistance in upland rice was evaluated using a six-level scoring system. The result showed that 11.19% moderately resistance, 29.37% moderately susceptible, 13.99% susceptible and 45.45% highly susceptible. The correlation shows that *Bph3* gene and BPH resistance are negatively correlated which could be implied that indicating that the *Bph3* gene is highly effective against BPH infestation. This study provides useful information about the BPH resistance varieties that can be useful in the breeding programs for the development of BPH resistance in rice.

Keywords: BPH, BPH Resistance Genes, Brown Planthopper, *Nilaparvata lugens*, Upland Rice

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CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important crop in the world. It is widely consumed as a staple food for one-third of the world's population, especially in Asia. In Thailand, rice production is classified into four ecosystems consisting of irrigated, rainfed lowland, deep water, and upland. The cultivation of upland rice is mostly located in North and Northeast of Thailand along upland, hilly slopes and mountainous areas. Increasing the yield of rice has been the main focus in the rice growing countries (Jene et al., 2015). Biotic stresses, e.g., fungi, bacteria, insects, and weeds cause losing approximate 52% of global rice production of which 21% by insect pests (Sai et al., 2013).

Among the biotic stresses, brown planthopper (*Nilaparvata lugens*; BPH) is the most serious monophagous pest of the rice cultivation area and is widespread in South, Southeast and East Asia, besides the South Pacific islands and Australia. BPH can damage rice and cause a loss of yield every year by directly feeding on rice that causes the wilting and drying in rice plants (called “Hopperburn”), and indirectly transmits diseases such as ragged stunt virus and grassy stunt virus. There are four biotypes of BPH population in rice. Biotype 1 was found in the East and Southeast Asia. Biotype 2 scourged in Vietnam and Indonesia (Khush, 1979). Biotype 3 which was created in the laboratory and biotype 4 was found in South Asia (Sai et al., 2013). The chemical control by the use of pesticide is the major approach for controlling BPH. However, the use of high amounts of pesticide exacerbates environmental problems such as pollution, killing beneficial insects, or creating BPH which are resistant to pesticides.

Rice resistance is considered the most economical and environmentally friendly approach for BPH management (Han et al., 2018). Host resistance of rice to BPH began in 1969 (Jing et al., 2017). Molecular markers have proved to be a powerful approach for genetic diversity and relatedness of most crop species that help in plant genetic

resource management (Sai et al., 2013). The molecular markers which are used to study the genetics of BPH resistance in rice include SSR, InDel, and SNP markers. Several host-plant resistance genes have been identified and used in the breeding program. More than 32 BPH resistance genes have been identified from cultivated and wild rice species (Han et al., 2018). Many resistance genes are located on chromosomes 3, 4, 6, and 12 (Jiang et al., 2018). Among 32 resistance genes, most of these genes are dominant whereas a few genes such as *bph4*, *bph5*, *bph7*, *bph8*, *bph19*, *bph25* and *bph29* are recessive (Jiang et al., 2018). Moreover, there are seven genes including *Bph9*, *Bph14*, *Bph17*, *Bph18*, *Bph26*, *bph29*, and *Bph32* have been cloned (Hu et al., 2016). Conventional breeding and marker-assisted selection (MAS) method have ability to develop BPH resistance varieties and their durability (Hu et al., 2016).

1.1 Objectives

1.1.1 To screen *Bph* resistance genes in upland rice varieties

1.1.2 To evaluate BPH resistance in upland rice varieties

1.1.3 To find the correlation between *Bph* resistance genes and phenotypic expression

1.2 Scope of Research

In this thesis, 143 upland rice varieties were studied both genotypic and phenotypic levels of BPH resistance. One hundred forty-three upland rice varieties were planted. DNA was extracted from young leaves of 143 upland rice varieties. First, five *Bph* resistance genes, *bph2*, *Bph3*, *Bph14*, *Bph15*, and *Bph17*, were examined in upland rice varieties to determine the number of *R* genes in each variety. Second, phenotypic expression was evaluated by infestation of upland rice with BPH at the seedling stage. Then, the correlation between *Bph* resistance genes and phenotype was analyzed.

CHAPTER 2

LITERATURE REVIEW

2.1 Rice

Rice belongs to the genus *Oryza* of the family Gramineae, the same as others cereal grains including wheat, oat, rye, barley, sorghum, corn or maze and millet. There are two species of domesticated rice, *Oryza sativa*, which originated in southern and eastern Asia, and *Oryza glaberrima*, which is native to Africa. The traits which are used to separate wild rice and domesticated rice include pericarp color, dormancy, shattering, panicle architecture, tiller number, mating type and number and size of seeds (Sweeney & McCouch, 2007). Among *Oryza sativa* or Asian rice can be geographically divided into three subspecies, subsp. *japonica*, subsp. *indica* and subsp. *javanica*. The japonica rice is cultivated in temperate climates with high latitudes such as Italy, Japan, Korea, Portugal, Spain as well as in high elevation areas such as the Yunnan and Guizhou provinces of China, Laos, Myanmar, and Vietnam. The indica rice is usually grown in tropical and subtropical regions, mostly in Southern Asia, including India, Southern China, Sri Lanka, Thailand, Vietnam (Lu et al., 2009). On the other hand, the javanica rice, or known as tropical japonica, is mainly found in Indonesia.

Rice is the most important crops in the Asia-Pacific region, particularly China, India, Indonesia, Thailand, and Japan. It is widely consumed as staple food for one-third of the world's population. The global production of rice is approximately more than 700 million tons per year with harvested areas up to 165 million hectares (Hassen et al., 2017). Over 90% of the world's total rice production are from the Asia-Pacific region including Bangladesh, China, India, Indonesia, Japan, Korea, Myanmar, Pakistan, the Philippines, Thailand, and Vietnam (FAO, 2018).

In Thailand, rice plays an important role and is viewed as the essence of Thai life. The main rice cultivated in Thailand are non-glutinous rice and glutinous rice or

sticky rice. Rice production in Thailand is classified into four ecosystems which include irrigated, rainfed lowland, deep water, and upland. Rainfed lowland rice is the dominant (75%), followed by irrigated rice (19%), deep water rice (5%), and upland rice (1%) (Damrongkiat, 2012). In addition, Thailand is geographically divided into four regions: central, north, northeast, and south. Rice cultivation covers in all regions each with different environments for rice-growing. The central region is a cultivated alluvial area which has excellent irrigation systems. This region is the main producer of rice which mostly produce non-glutinous rice. The size of the rice plantation is large in this area, with access to irrigation facilities, thus the farmers can grow two rice crops during the year (Rosentrater & Evers, 2018). The northeastern region is the largest rice plantation area of Thailand, accounting to nearly a half of the whole country. This region produces both of non-glutinous rice and glutinous rice. The rice land in Northeastern has limited irrigation, soil erosion and drought that are the main problems. The southern region has only 6% of the total rice plantation. The soil is acidic and has a delayed rainy season. The northern region is mostly mountainous. There are three types of rice are grown in this area divided by elevation which includes upland rice, lowland rice and terraced rice. Upland rice is the staple food for the hill tribe households which is grown in high hills and upland areas. Lowland rice is grown in lower valleys. Terraced rice is grown on where water is available (Maclean, 2002).

2.2 Upland Rice

Upland rice is grown in rainfed conditions, naturally well-drained soils without surface water accumulation, without phreatic water supply, and normally not bunded (Fageria, 2001). Thus, it is mainly grown in the fields without any irrigation facilities. Upland rice is approximately grown in 13% of the world rice area, mainly found in Asia, Africa, and Latin America (Gupta & O'Toole, 1986). The important producers of upland rice are Bangladesh, Cambodia, China, India, Indonesia, Laos, Myanmar, Thailand, and Vietnam (Arraudeau, 1995).

In Thailand, the upland rice varieties were selected and adapted from Temperate *japonica* to Tropical *japonica* due to the migration of ethnic groups from the southern

region of China through Myanmar and Laos to the border area in the north, northeast and west of Thailand (Damrongkiat, 2012). The upland rice is genetically diverse to survive under the climate variability and change. The ethnic group generally select and collect the varieties that were able to survive after the spread of diseases and/or pests, reaching to have the varieties that resistant to diseases and pests. In 1982, Rice Department collaborate worked with other government organizations to collect the traditionally rice varieties in every region of Thailand and found that there were 5,467 varieties of upland rice in Thailand that were mostly from the Northern region. Moreover, 12 upland rice varieties were later developed from selected traditional varieties and certified as suitable varieties for each region. However, the improving of new upland rice varieties from traditional *japonica* upland rice to have desirable characteristic in genetic and environment adaptability has limitation in high yield potential (Damrongkiat, 2012)

The cultivation of upland rice is mostly located in North, Northeast, and some areas of South along upland, hilly, slopes, and mountainous areas. According to the report of the Rice department in 2012, the total area of upland rice cultivation in Thailand was 668,486 rai including 343,461 rai in the Northern, 202,383 rai in Western border, 41,979 rai in Eastern border, 42,293 rai in Northeast, and 38,370 rai in South. The upland rice cultivation in Thailand can be divided into three categories based on the aims of product use; (1) Upland rice growing in mountainous areas by the ethnography which aims to produce sufficient productivity for all people in the family. This mainly occur in the upper north which is approximately 70% of the total cultivation area in Thailand. (2) Upland rice is mixed-grown with other products such as rubber and oil palm in order to have rice for consumption and to reduce the cost of purchasing rice. This occurs in the flat areas in the south. (3) Upland rice which is grown for trading. The varieties that have grown for trading will have high nutrition such as LeumPua, Chaw Lung 97, Niaw Dam Cham Mai Pai 49. These varieties are also aimed to develop to Geographical Indication product, as known as GI product (Damrongkiat, 2012).

Biology

Upland rice is medium-tall to tall, approximately height is 130 - 150 cm, with long and pale-green leaves, low to medium tillering ability, and long well-exerted panicles. The maturity generally ranges from 105 - 135 days (IRRI, 1979). Both glutinous and non-glutinous rice are found in the upland rice. Moreover, upland rice is drought tolerant, but it gives a low yield potential (Saito et al., 2018). Upland rice varieties also have higher resistance to blast disease compared with lowland varieties (Lestari et al., 2011). These serves as effective germplasm sources for breeding program.

2.3 Rice Insect Pests

Several insect pests impact rice and cause diseases and loss in yield. They can damage entire parts of the rice plant and all stages of plant growth. The insect pests consist of black bug, rice skipper, rice whorl maggot, rice thrips, mealy bug, mole cricket, ant, armyworm, green semilooper, green horned caterpillar, rice bug, field cricket, cutworm, rice caseworm, rice gall midge, rice hispa, stem borer, root grub, root aphid, rice leaf folder, grasshopper, green leafhopper, zigzag leafhopper, and planthopper (Pathak & Khan, 1994).

2.4 Brown Planthopper (BPH)

Brown planthopper (BPH), *Nilaparvata lugens* (Stål), is a migratory insect pest which has the piercing and sucking type mouth-parts. BPH has been classified into the order Hemiptera and belongs to Delphacidae family. The adults have a brown color in body and the wings are transparent. BPH adults have two forms based on their wings including macropterous (long wings) and brachypterous (short wing) (Xue et al., 2014). They prefer high temperature (25 – 28 ºC) for living.

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Hemiptera
Family: Delphacidae
Genus: *Nilaparvata*
Species: *Nilaparvata lugens*

Figure 2.1 Classification of BPH

BPH, the most serious monophagous pests of the rice cultivation, was firstly found in early 1960‘s when the insecticides were used as a major control. BPH is widespread in Australia, Bangladesh, Bhutan, Myanmar, Cambodia, China, Fiji, India, Indonesia, Japan, North and South Korea, Laos, Malaysia, Nepal, Pakistan, Papua New Guinea, Philippines, Sri Lanka, Taiwan, Thailand, and Vietnam (Heong & Hardy, 2009).

BPH causes severe yield losses in many countries. Large-scale damage has been reported in India, Indonesia, the Philippines, and Sri Lanka (IRRI, 1979). According to International Rice Research Institute (IRRI), BPH outbreaks have occurred in several Asian countries since 2004. In China, the damage caused losses of 1 million tons and 2.8 million tons in 2004 and 2005, respectively. Japan and Korea also found an outbreak of BPH in 2005 and 2006. In Indonesia, BPH has caused losses over tens of thousands of hectares since 2008.

In Thailand, there was no damage from the BPH before 1974. In the dry season, pest populations were high in the central plain area and some pest problems started in 1975 (IRRI, 1979). BPH was firstly found in Thailand in 1978 at Saraburi province. The outbreak occurred highly in rice RD7 variety and damaged several hectares. The outbreak areas of BPH cover 22 provinces in the irrigation area, including Sukhothai, Kamphaeng Phet, Nakhon Sawan, Uthai Thani, Suphan Buri, Kanchanaburi, Nakhon Pathom, Ratchaburi, Phetchaburi, Nonthaburi, Chachoengsao, Pathum Thani, Nakhon

Nayok, Ayutthaya, Saraburi, Ang Thong, Lop Buri, Sing Buri, Chanyat, Phichit, Phitsanulok, and Chiang Rai. In 2018, an outbreak of BPH damaged more than 8,727 Rai in seven provinces including Lampang, Phrae, Uttaradit, Phitsanulok, Phetchabun, Nakhon Ratchasima and Lop Buri (Rice department, 2018).

The main causes of BPH outbreak in Thailand include using high nitrogen fertilizer and planting susceptible varieties of rice such as Pathum Thani 1, Khao Dawk Mali 105, and RD 35. The rice department has suggested the farmers to plant resistant varieties to reduce the outbreak. The BPH resistant varieties are Phitsanulok 2, Suphan Buri 2, Suphan Buri 3, Suphan Buri 90, RD31, RD41, RD47, and RD49.

2.4.1 Life Cycle

BPH has gradual metamorphosis which consists of eggs, nymph, and adult (Figure 2.2). After mating, females BPH will lay a group of eggs into the sheaths or midrib of leaves. Brachypterous females can lay 300 to more than 700 eggs, whereas macropterous females lay about 100 eggs. After 5-9 days, the first instar nymphs will hatch which has a white color and turn purple brown within an hour. The nymphs will molt five times and then become adults (IRRI, 1979).

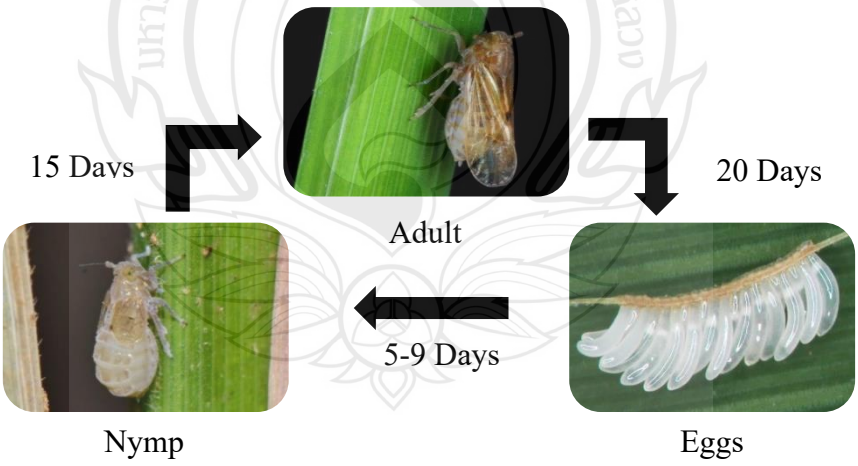


Figure 2.2 Life cycle of BPH

2.4.2 Biotype

The biotype refers to the populations or individuals with virulence to a previously resistant variety and has firstly studied since 1975 (IRRI, 1979). It has believed that developing new resistant variety is effective approach to control the BPH. However, new resistant variety causing the adaptation of new virulent BPH population to defense rice varieties (Lai et al., 2022). The precise nature of the adaptation in BPH is ambiguous. Some characteristics, such as morphological features, isozymes, and DNA polymorphisms, have been claimed as the differences between the biotype (Tang et al., 2010). The bacteria in BPH and expression of resistance genes are also possibly factors making the differences in BPH biotype.

2.4.2.1 Microbial symbiosis

The relationship between insects and the microbes has recently focused. Endosymbiont bacteria and yeast-like symbiont (YLS) are reported to be important for the nutrition, development, and reproduction of BPH. *Wolbachia* and *Arsenophonus* are mainly endosymbiotic bacteria on BPH found in abdominal fat and were able to be transmitted through maternal lines (Qu et al., 2013). Likewise, the study of Hongoh et al. (2000) that reported *Ascomycetes* symbionts, *Pichia*-like symbionts, and *Candida*-like symbionts are other mainly symbionts. The role of YLS in nymph survival rate, development and adult reproduction, and activities of transaminases in BPH on different resistant rice varieties were studied by Lu et al. (2004), the results revealed that the virulence of BPH populations to resistant rice varieties was associated to the abundance of YLS. BPH nymphs that do not contain YLS tend to have weight loss and slower growth compared to normal BPH nymphs. Some study reported that YLS may be play a role in metabolic pathways related to the growth and development of insect host. As demonstrated, the study of nitrogen recycling in BPH which showed that YLS plays an important role in amino acid metabolism through the recycling of uric acid and also capable provide the nutrients to BPH (Sasaki et al., 1996). Therefore, it actively illustrates that YSL possibly related to the variation of BPH virulence and its adaptation to rice resistance

2.4.2.2 The expression of resistant genes

The level of resistance is different in each part of rice. Jairin et al. (2007b) evaluated the level of BPH resistance in different stages of rice (seedling, vegetative, and flowering stage) and found the resistant to BPH in seedling and vegetative stage but susceptible in flowering stage. The study also showed that BPH were able to damage well on panicle necks and panicles. As well as the study of BPH resistance 11 varieties during flowering stage that revealed BPH could not feed the rice on leaf sheath but could feed on the uppermost internodes that elongate and emerge from the flag leaf sheath (Jairin et al., 2017). Therefore, the different level of BPH resistant genes in each part of rice could affect the feeding behavior of BPH causing the adaptation an evolution in BPH population.

BPH population can be mainly classified into four biotypes based on non-morphological traits such as adaptation and development to a specific host, feeding or oviposition behaviors. Biotype 1 and Biotype 2 have spread throughout Southeast and East Asia. Biotype 3 was produced in a laboratory in Philippines and biotype 4 occurs in the Indian subcontinent (Jena & Kim, 2010). The relationship between biotypes of BPH and *Bph* genes for resistance in rice shown in Table 1, the rice varieties that contain *Bph1* gene are reported that can resistant to Biotype 1 and 3 but Biotype 2 and 4 are susceptible. The *bph2* gene showed resistance to biotype 1 and 2 but susceptible to biotype 3 while the varieties that containing *Bph3*, *bph4*, *bph8,* and *Bph9* genes are resistance to all 4 biotypes. *bph5*, *Bph6,* and *bph7* genes are only resistance to biotype 4 (Brar et al., 2010).

Table 2.1 Relationship between BPH biotypes and BPH genes for resistance in rice

Varieties	Genes	Reaction to biotypes			
		1	**2**	**3**	**4**
Mudgo	*Bph1*	R	S	R	S
ASD 7	*bph2*	R	R	S	S
Rathu Heenati	*Bph3*	R	R	R	R
Babawee	*bph4*	R	R	R	R
ARC 10550	*bph5*	S	S	S	R
Swarnalata	*Bph6*	S	S	S	R
T12	*bph7*	S	S	S	R
Chin Saba	*bph8*	R	R	R	–
Balamawee	*Bph9*	R	R	R	–
TN1	*None*	S	S	S	S
Oryza officinalis (Accession 100896)	*Bph6, Bph13*	R	R	R	R
Oryza minuta (Accession 101141)	*Bph20, Bph21*	R	ND	ND	ND
Oryza latifolia (Accession B14)	*Bph12*	ND	R	ND	ND

Note R for (Resistance), S for (Susceptible), ND for (No data)

Source Jena and Kim (2010)

In Thailand, there are more than eight biotypes found in the field which trend to continuously increase. The ratio of biotype is different in each population. The study of Thanyasiriwat et al. (2009) in BPH population collected from Phitsanulok and Chainat province reported that seven biotypes were found in one population. Furthermore, the study of BPH population collected in the central region of Thailand reported that 31 biotypes were identified from the BPH population collected from

Lopburi, Suphanburi, Nakhon Nayok, and Ratchaburi. Among 31 biotypes, two mainly biotypes were found (biotype 3 and biotype 4) while 29 biotypes were unidentified biotypes (Prakobna et al., 2022).

2.4.3 Damage

Both of nymphs and adults of BPH can damage the rice by their piercing-sucking mouthparts in order to ingest phloem-sap. The plant will rapidly turn yellow, which soon becomes brown, and will dry up. Later, the rice will wilt. This is known as “hopper burn” (Du et al., 2009). The most susceptible time is from tillering to flowering. Hopper burns commonly occurs in non-glutinous rice. Eggs of BPH also damage the rice because they can block the water and food channels inside the plant. Additionally, BPH can indirectly damage rice by transmission. They are important vectors of rice grassy stunt virus and rice ragged stunt viruses (Pathak & Khan, 1994).

2.4.4 Cause of Outbreak

The outbreaks are mainly caused by the failure of the biological control function in the rice landscape. Normally, the number of BPH in the field is controlled by natural enemies, such as mirid bug, staphylinids, carabids. When the predators and parasitoids are absent, the BPH populations become uncontrolled and can therefore rapidly grow, which results in outbreaks (IRRI, 1979).

2.4.4.1 Insecticide misuse

The overuse or misuse of insecticide is the key factor in BPH outbreaks which mainly cause of reducing in predator and parasitoid. Therefore, insecticide applications cannot eliminate to BPH eggs that will later hatch into an enemy-free environment (Cheng, 2009).

2.4.4.2 Excessive fertilizer use

Excessive use of fertilizers can cause inequality nutrients and lower pest resistance. For example, the excess use of nitrogen fertilizer can increase the number of protein and decrease silicon content in rice plants, which promote the feeding and growth of BPH population (Rashid et al., 2016).

2.4.4.3 Capacity to adapt

BPH can be overcome resistant rice varieties, especially where BPH densities are higher than the number of plants. Moreover, BPH rapidly develop resistance to insecticides (IRRI, 1979).

2.5 *Bph* Resistance Genes

Over 32 *Bph* resistance genes have been identified (Han et al., 2018). *Bph1, bph2, Bph3, bph4, bph5, Bph6, bph7, bph8, bph9, Bph17, Bph19, bph25, Bph26, Bph28* and *Bph32* genes originated from *indica* varieties. *Bph10, Bph11, Bph12, Bph13, Bph14, Bph15, Bph16, Bph18, Bph20, Bph21, bph22, bph23, bph24, Bph27, bph29* and *Bph30* were identified from wild rice species (Jing et al., 2017). Many resistance genes are located on chromosomes 3, 4. 6, and 12 as shown in Table 2 (Jiang et al., 2018).

Among resistance genes, most of these genes are dominant whereas some genes such as *bph2, bph4, bph5, bph7, bph8, bph19, bph25* and *bph29* are recessive (Jiang et al., 2018). Moreover, there are seven genes including *Bph9, Bph14, Bph17, Bph18, Bph26, bph29, and Bph32* have been cloned (Hu at al., 2016). Molecular makers developed from these genes are used to assist the breeding programs to screen and develop the BPH resistant varieties. However, none of the molecular genetic methods have been applied for *bph5, bph7, Bph8, Bph22, Bph23,* and *Bph24.*

Table 2.2 Chromosome locations of BPH resistance genes in rice

Genes	Chromosome	Position (Mbp)	Donor	Dominant/ Recessive	References
Bph1	12L	24.00-25.00	Nori-PL3	Dominant	Sharma et al. (2003)
bph2	12L	13.21-22.13	ASD7	Recessive	Sun et al. (2006)
Bph3	6S	1.21–1.40	Rathu Heenati	Dominant	Jairin et al. (2007)
bph4	6S	1.20–1.76	Babawee	Recessive	Kawaguchi et al. (2001)
Bph6	4L	21.36–21.39	Swarnalata	Dominant	Qiu et al. (2010)
Bph9	12L	19.11–22.13	Kaharamana	Dominant	Su et al. (2006)
Bph12	4S	5.21–5.66	B14 (*Oryza latifolia*)	Dominant	Qiu et al. (2011)
Bph14	3L	35.70–35.72	B5 (*Oryza officinalis*)	Dominant	Du et al. (2009)
Bph15	4S	6.68–6.90	B5 (*Oryza officinalis*)	Dominant	Lv et al. (2014)
Bph17	4S	6.93–6.97	Rathu Heenati	Dominant	Sun et al. (2005)
Bph18	12L	22.09–23.32	IR65482-7-216 (*Oryza australiensis*)	Dominant	Jena et al. (2006)
Bph20	4S	8.20–9.60	IR71033-121-15 (*Oryza minuta*)	Dominant	Rahman et al. (2009)
Bph21	12L	23.28–24.41	IR71033-121-15 (*Oryza minuta*)	Dominant	Rahman et al. (2009)
Bph26	12L	22.87–22.88	ADR52	Dominant	Tamura et al. (2014)
Bph27	4L	19.12–19.20	GX2183 (*Oryza rufipogon*)	Dominant	Huang et al. (2012)
bph29	6S	0.48–0.49	RBPH54 (*Oryza rufipogon*)	Recessive	Wang et al. (2015)
Bph31	3L	ND	CR2711–76	Dominant	Prahalada et al. (2017)
Bph32	6S	ND	Ptb33	Dominant	Ren et al. (2016)

Note ND for (No data)

2.5.1 *Bph1*

The *Bph1* gene is one of the first BPH resistance genes which was identified in 1970. It is located on chromosome 12. The rice variety that contains *Bph1* was released in 1973 and generally grown in the Philippines, Indonesia, and Vietnam. *Bph1* variety was found to be susceptible to BPH Biotype 2 (Heong & Hardy, 2009).

2.5.2 *bph2*

The *bph2* genes is the first *Bph* resistance gene as *Bph1* and is located on chromosome 12. *bph2* variety was first identified in an *indica* breeding line Karsamba Red ASD7 (Sharma et al., 2003). Rice varieties with *bph2* are susceptible to BPH Biotype 4.

2.5.3 *Bph3*

The *Bph3* gene was released in 1982 in the Philippines (Heong & Hardy, 2009). It is mapped on chromosome 6 (Jairin et al., 2007a). *Bph3* plays a crucial role in developing the resistant variety because it shows high effective and broad-spectrum resistance to all BPH biotypes (Liu et al., 2016).

2.5.4 *bph4*

The *bph4* displays as recessive BPH resistance gene located on a short chromosome 6 and first identified in the Babawee variety. *bph4* was reported that allelic and closely linked to the *Bph3* gene (Kawaguchi et al., 2001). *bph4* was later assigned to chromosome 10 based on trisomic analysis (Ikeda & Kaneda, 1981). Jairin et al. (2010) mapped *bph4* in the same region as *Bph3* and reported that the dominance or recessiveness of *bph4* depends on the different genetic backgrounds.

2.5.5 *Bph6*

The *Bph6* was initially found in rice *indica* cultivar Swarnalata. It is resistant only to Biotype 4 of BPH. *Bph6* is located on the long arm of chromosome 4 between *bph12* and *bph18(t)* and displays antixenotic and antibiotic effects in Bph6-NIL plants (Qiu et al., 2010).

2.5.6 *Bph9*

The *Bph9* is located on chromosome12 between the RFLP marker OPRO4 and RAPD marker S2545 in Pokkali variety (Nemoto et al., 1989). It was also mapped in Kaharamana variety between SSR markers RM463 and RM5341 (Su et al., 2006). According to Wang et al. (2017), *Bph6* and *Bph9* were pyramided into 9311 through MAS. The results showed that the *Bph6* and *Bph9* pyramided line LuoYang69 had higher antixenotic and antibiosis effects on BPH compared with NIL-*Bph6* and NIL-

Bph9. Varieties containing *Bph9* can be valuable sources to develop BPH resistant varieties in breeding programs.

2.5.7 *Bph12*

The *Bph12* was firstly mapped to a 13.4 cM region on the short arm of chromosome 4 (Yang et al., 2002). Lately, *Bph12* was also mapped to a 1.9 cM region on chromosome 4, flanked by the RM16459 and RM1305 markers and also estimated the pyramiding effects of *Bph12* and *Bph6*. The results indicated the lowest insect survival rates in rice PYL-*Bph6* + *Bph12*. Moreover, the pyramiding of different genes resulted in stronger antixenotic and antibiotic effects on the BPH insects (Qiu et al., 2011).

2.5.8 *Bph14*

The *Bph14* was mapped onto rice chromosome 4. It confers stable resistant in different genetic backgrounds which is a good source for the development of BPH resistance varieties. According to Du et al. (2009), *Bph14* was cloned to encode a coiled-coil, nucleotide-binding, and leucine-rich repeat (CC-NB-LRR). *Bph14* carries a unique LRR domain that functions to recognize the insect invasion and activate the defense response. The expression of *Bph14* activates the salicylic acid (SA) pathway and leads to callose deposition in phloem cells and trypsin inhibitor production after infestation by BPH, resulting in reducing the feeding, growth rate, and longevity of the BPH.

2.5.9 *Bph15*

The *Bph15* is located on the short arm of chromosome 4 and has high potential to control the BPH. *Bph15* is one of the *Bph* resistance genes which is generally used in breeding program. Among the three dominant *Bph* resistance genes (*Bph14*, *Bph15*, *Bph18*) that have been introgressed into 9311 and hybrid rice, *Bph15* showed the greatest resistance effect against BPH (Lv et al., 2014).

2.5.10 *Bph17*

The *Bph17* was cloned from the Rathu Heenati variety and was firstly fine-mapped on short chromosome 4 between two SSR markers, RM8213 and RM5953, at

a distance of 3.6 cM and 3.2 cM respectively (Sun et al., 2005). *Bph17* is actually a cluster of three genes encoding plasma membrane-localized lectin receptor kinases (OsLecRK1 - OsLecRK3), which function in broad-spectrum, durable resistance and provide an important gene source for MAS (Hu et al., 2016).

2.5.11 *Bph18*

The *Bph18* was mapped between the R10289S and RM6869 markers on a long arm chromosome 12. It is a unique resistance gene derived by which encodes CC-NBS-NBS-LRR protein and displays BPH resistance. *Bph18* confers strong GUS expression in the vascular bundles of the leaf sheath, especially in phloem cells where the BPH attacks. Therefore, *Bph18* gene can be a new source of resistance which gives benefits in breeding programs (Ji et al., 2016).

2.5.12 *Bph20* and *Bph21*

The Both *Bph20* and *Bph21* were derived from *O.minuta*. *Bph20* locatea at 193.4 kb region on the short chromosome 4 while *Bph21* locates to 194 kb region on the long arm chromosome 12. They were the first resistance genes to be developed from *Oryza minuta* variety and are expected to be a valuable source of BPH resistance (Rahman, 2009).

2.5.13 *Bph26*

The *Bph26* is located on the long arm of chromosome 12 encoding a coiled-coil, nucleotide-binding, leucine-rich repeat (CC-NBS-LRR) protein, and mediated sucking inhibition in the phloem sieve element. *Bph26* is considered as *Bph2* on the basic of map positioning, gene sequence analysis, gene expression analysis, and feeding ability of the *Bph2*-virulent biotype (Tamura et al., 2014). In addition, the whole genome sequencing of the NIL-*Bph18* and NIL-*Bph26* conferred *Bph18* and *Bph26* located at the same locus, indicating that *Bph18* and *Bph26* may be the same genes with different alleles (Ji et al., 2016). Furthermore, several DNA and amino acid sequence differences were found between the *Bph18* and *Bph26* genes. This may be because of the difference in donor sources of both genes by which *Bph26* was from *O. sativa indica* variety ADR52 whereas *Bph18* was originated from *O. australiensis*.

2.5.14 *Bph27*

The *Bph27* derived from Guangxi wild rice, GX2183. It was initially mapped to a 17-cM region flanked by SSR markers, RM273 and RM471, on the long arm of the chromosome 4. *Bph27* mediated resistance involves both antixenosis (non-preference) and antibiosis (Huang et al., 2012).

2.5.15 *bph29*

The *bph29* gene is a recessive gene derived from *O. rufipogon* and was mapped to a 24 Kb region on short chromosome 6. *bph29* encoding the B3 DNA-binding domain confers BPH resistance (Hu et al., 2016). The expression of *bph29* restricted to the vascular tissues where BPH attacks. After BPH infestation, *bph9* activates the salicylic acid (SA) pathway suppresses the jasmonic acid/ethylene (JA/Et)-dependent pathway and induces callose deposition in phloem cells, resulting in antibiosis to BPH (Wang et al., 2015).

2.5.16 *Bph31*

The *Bph31* is a single dominant gene derived from the CR2711–76 variety. It was located on the long arm of chromosome 3 between PA26 and RM2334 markers. CR2711–76 variety containing *Bph31* could against BPH populations in the Philippines. The results from bioassay demonstrated that CR2711–76 dominates all mechanisms of resistance including antibiosis, antixenosis, and tolerance (Prahalada et al., 2017).

2.5.17 *Bph32*

The *Bph32* is a dominant gene against BPH which stables and provides a potential for rice defense against pests. It was cloned from the rice variety Ptb33 in a 190 kb interval flanked by RM19291 and RM8072 markers on the short arm of chromosome 6. *Bph32* encodes a short consensus repeat (SCR) domain-containing protein that presents an antibiosis resistance to BPH and is restricted in the plasma membrane. *Bph32* is strongly expressed in the leaf sheaths (Ren et al., 2016).

2.6 Polymorphic Genetic Markers

Genetic markers have been originally used to evaluate genetic variation between two or more individuals. From morphological markers, via isozyme marker to DNA markers, they have evolved. Genetic markers play a key role in plant breeding to characterize plant germplasm, gene isolation, and marker-assisted selection. Genetic markers consist primarily of polymorphisms which are discontinuous genetic variations among individuals, groups, or populations. At the DNA level, Polymorphisms can result from a change in a single base pair or several base pairs, and repeated sequences (Teama, 2018). The main principle of marker selection is the ability to detect Polymorphisms (Serrote et al., 2020). Heterozygosity (Nei & Roychoudhury, 1974) and the Polymorphism Information Content: PIC (Botstein et al., 1980) are two indexes that can be used to determine the degree of Polymorphisms.

2.6.1 Heterozygosity

Heterozygosity refers to the percentage of gene loci that are heterozygous in an average individual of a given population. The heterozygosity value ranges from zero (without heterozygosity) to 1 (high number of alleles with equal frequency) (Chesnokov & Artemyeva, 2015). The simplest formula of heterozygosity is followed as:

$$H = 1 - \sum_{i=1}^{l} P_i^2$$

where P_i is the frequency of the i^{th} allele among the total number of alleles l

2.6.2 Polymorphism Information Content (PIC)

PIC is commonly used as indicator to quantitatively measure polymorphism of genetic markers for linkage analysis. The PIC value was developed by Botstein et al. (1980) for the case of a rare dominant disease. Theoretically, the range of PIC value for dominant and codominant makers are different. For the dominant marker, PIC value ranges from zero of monomorphic markers to 0.5 of markers present in 50% of

individuals and absent in the remaining 50% (Serrote et al., 2020). While the PIC value of codominant marker varies between zero and one. The PIC value can be calculated by following formula Botstein et al. (1980):

$$PIC = 1 - \sum_{i=1}^{n} P_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

where P_i and P_j are the frequencies of i^{th} and j^{th} alleles for the selected marker, respectively.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 BPH Colonies

BPH colonies were received from the Chiang Rai rice research center in Phan district, Chiang Rai province, Thailand. The colonies were then grown under natural conditions with the susceptible variety KDML105 in a greenhouse at Mae Fah Luang University to multiply the number of BPH in the population.

3.2 Evaluation of BPH Resistance

The phenotypic screening was conducted by using Completely randomized design (CRD) with three replications of three seedlings each. Rathu Heenati, Phitsanulok 2, RD23, RD 39, KMDL 105, and TN1 were used as the controls in the experiment. The seeds from individual rice varieties were sown in a plug tray. At the second-leaf stage, the seedlings were infested with 10 BPH. When all the seedling of the TN1 variety were almost dead, the seedling data were recorded. According to IRRI (1996), BPH resistance was evaluated with the six-scale standard of scoring system as the following: 0 = no damage; 1 = small damage; 3 = first and second leaves are yellowing; 5 = pronounced yellowing and stunting; 7 = almost entirely wilting, but still alive; 9 = the plant completely died. All plants were then scored as high resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S), and high susceptible (HS) (Table 3.1).

Table 3.1 The six-scale standard of scoring system and resistance levels

Scale	Signs on rice	Resistance levels
0	No damage	High resistant (HR)
1	Small damage	Resistant (R)
3	First and second leaves are yellowing	Moderately resistant (MR)
5	Pronounced yellowing and stunting	Moderately susceptible (MS)
7	Almost entirely wilting, but still alive	Susceptible (S)
9	The plant completely died	High susceptible (HS)

3.3 Plant Materials

Forty-six varieties of upland rice were collected from the farmers in Chiang Rai province and 97 varieties were kindly provided by Asst.Prof.Dr. Vaiphot Kanjoo from Phayao University, Thailand (Table 3.3). Rathu Heenati, Phitsanulok 2, RD23, RD39 and KMDL 105 were used as the controls derived from the Phitsanulok Rice Research Center. The other control TN1 variety was derived from the Pathum Thani Rice Research Center (Table 3.2).

Table 3.2 Six control varieties for BPH evaluation

Accession No.	Varieties	Sources
16CR0102	KDML105	Phitsanulok
12CR0104	Phitsanulok2	Phitsanulok
12CR0105	RD23	Phitsanulok
16CR0106	RD39	Phitsanulok
17CR107	Rathu Heenati	Phitsanulok
18CR154	TN1	Pathum Thani

Table 3.3 One hundred and forty-three upland rice varieties

Accession No.	Varieties	Sources	Accession No.	Varieties	Sources
12CR016	Khao' Jao Doi 1	Chiang Rai	18CR148	Doi Chang 148	Chiang Rai
18CR123	Wa Ji Da Noo	Chiang Rai	18CR149	Doi Chang 149	Chiang Rai
18CR124	Lee Su Ja	Chiang Rai	18CR150	Neaw Doi	Chiang Rai
18CR125	La Ta Na	Chiang Rai	18CR152	Ja Chue Chue	Chiang Rai
18CR127	Young Ger	Chiang Rai	18CR153	Wa La Nee Gu Su	Chiang Rai
18CR128	Ar Hmee Ma Ta Me	Chiang Rai	18CR165A	Ja Fu Ma	Chiang Rai
18CR129	Wa Chi Ku	Chiang Rai	18CR039	Ja Bue Ma	Chiang Rai
18CR130	Hwa Jue Ja Jue	Chiang Rai	18CR166	Ja Hroi	Chiang Rai
18CR131	Ja Nae Nae	Chiang Rai	18CR167	Ja No Nae	Chiang Rai
18CR132	Ta Ter Ma Ja 1	Chiang Rai	18CR168 (1)	Ja Gu Na	Chiang Rai
18CR133	Ta Ter Ma Ja 2	Chiang Rai	18CR168 (2)	Unknown168	Chiang Rai
18CR134	Ta Ter Ma Ja 3	Chiang Rai	18CR169(1)	Unknown169	Chiang Rai
18CR136	Ta Ter Ma Ja 5	Chiang Rai	18CR169 (2)	Ja Bue Ma169	Chiang Rai
18CR138	Khao Kam Doi Lan	Chiang Rai	18CR171	Ja Ye Gui171	Chiang Rai
18CR139	Doi Lan 1	Chiang Rai	18CR172	Ja Ye Gui172	Chiang Rai
18CR140	Hom Ma Li Doi	Chiang Rai	18CR173	Ja No Kor Hao	Chiang Rai
18CR141	Ta Ter Ma Ja 6	Chiang Rai	18CR174	Ja Gue Lei	Chiang Rai
18CR142	Doi Lan 2	Chiang Rai	18CR175	Ja Na Toy	Chiang Rai
18CR143	Khao Lai	Chiang Rai	18CR176	Kor Lei	Chiang Rai
18CR144	Wa La Go	Chiang Rai	18CR177	Unknown177	Chiang Rai
18CR145	Khao Tia	Chiang Rai	19CR178	Unknown178	Chiang Rai
18CR146	Ja Ma See	Chiang Rai	19CR179	Unknown179	Chiang Rai
18CR147	Doi Chang 147	Chiang Rai	19CR180	Unknown180	Chiang Rai

Table 3.3 (continued)

Accession No.	Varieties	Sources	Accession No.	Varieties	Sources
UP-2	Situ Patenggang	Phayao	UP-54	IR13240-108-2-2-3	Phayao
UP-5	SPRLR 85104-62-1-1	Phayao	UP-55	IR5675-81-2-3	Phayao
UP-14	Mali Namnao	Phayao	UP-58	Bue Wa	Phayao
UP-27	IR78914-B-22-B-B-B	Phayao	UP-59	Khao Ta Chee Long2	Phayao
UP-28	IR78875-190-B-1-3	Phayao	UP-70	Khao Rai Ka Reang	Phayao
UP-29	IR78878-53-2-2-2	Phayao	UP-108	2R-40	Phayao
UP-30	IR82310-B-B-67-2	Phayao	UP-110	2R-43	Phayao
UP-32	IR81413-B-B-75-3	Phayao	UP-135	Khao Pang Kam Noi	Phayao
UP-33	IR81423-B-B-111-3	Phayao	UP-140	Ja Su	Phayao
UP-34	IR78877-048-B-B-2	Phayao	UP-143	Lai San	Phayao
UP-35	IR70181-32-PMI-1-1-4-2	Phayao	UP-145	La Ngae	Phayao
UP-36	IR73000-98-1-2-1	Phayao	UP-148	Bue Ko	Phayao
UP-38	IR70175-51-2-1-1-2	Phayao	UP-151	Bue Sor Mee	Phayao
UP-40	IR84179-B-403	Phayao	UP-160	Lai Chan	Phayao
UP-41	IR81423-B-B-152-1	Phayao	UP-163	Ka Reang Kao	Phayao
UP-44	IR74371-54-1-1	Phayao	UP-208	Bue Por Lor Lay Ko	Phayao
UP-45	IR79971-B-338-2-2	Phayao	UP-215	Khao Lai Pang Kwam Noi	Phayao
UP-47	IR78875-176-B-2-B	Phayao	UP-244	Khao Maled Yao Hnong Keaw	Phayao
UP-48	IR71700-247-1-1-2	Phayao	UP-291	Ja No Mei	Phayao
UP-49	IR81025-B-327-3	Phayao	UP-292	Nai Jong Hwa	Phayao
UP-50	IR78875-207-B-1-B	Phayao	UP-293	Beu Chi	Phayao
UP-51	PSL85051-14-2-1-2	Phayao	UP-295	Jao Ho	Phayao
UP-52	CNT86095-42-2-3	Phayao	UP-298	IR78875-15-B-3-B	Phayao
UP-53	UNKNOWN UP-53	Phayao	UP-299	IR81063-B-94-U3-4	Phayao

Table 3.3 (continued)

Accession No.	Varieties	Sources	Accession No.	Varieties	Sources
UP-302	Heng Wa Ra Nong	Phayao	UP-357	Ber Sue Ha	Phayao
UP-305	Kaset Poo	Phayao	UP-364	Beu So	Phayao
UP-308	2R-10	Phayao	UP-367	Khao Man	Phayao
UP-313	Tee Hmee Jar	Phayao	UP-371	Beu Sor La So Som Kong	Phayao
UP-314	Lai Nok Khum	Phayao	UP-376	Bee Ai Kor Kae	Phayao
UP-315	Huey Lang	Phayao	UP-389	Ba Dai Koi Ngam	Phayao
UP-317	Beu Hmeu Mae La	Phayao	UP-415	Pi Ai Su Mae Pang1	Phayao
UP-318	Beu Su Mae Rad Par Kae	Phayao	UP-422	Khao Sang Loi	Phayao
UP-319	Fueng Kaum Pang Kwam Noi	Phayao	UP-424	Sue No Na	Phayao
UP-320	Beu Hmeu Po Mae Kwang Noi	Phayao	UP-429	Or Sae Nai Huey San Nai	Phayao
UP-321	Beu Por Mae Pang	Phayao	UP-436	Khaokeo-4	Phayao
UP-322	Beu Nor Pae Sob Kong	Phayao	UP-247	Khao Mun Huey San Nai	Phayao
UP-323	Khao Jao Doi Mae Ou Kor	Phayao	UP-248	Ja Nor Toy Huey San Nai	Phayao
UP-324	Nieow Jak Pa Mar	Phayao	UP-251	Leuang Rai Pui Ngam	Phayao
UP-325	Ja Nor Mei Ae Ko	Phayao	UP-252	Lai San Pung Yam	Phayao
UP-326	Beu Ku Pa Tong	Phayao	UP-258	Khao Lang Hua Lan	Phayao
UP-327	Kang Mo Ba	Phayao	UP-271	Khao Fueng Kham	Phayao
UP-331	Leuang Tum	Phayao	UP-290	Pi Ai Par Ngo Mae Rad Noi	Phayao
UP-334	Prae Kao Chue	Phayao	UP-463	Khao Hwaw	Phayao
UP-335	Khao Nieow Hom Lee Sor	Phayao	UP-465	Beu Po Lo Hnong Kang	Phayao
UP-340	CPAC08016	Phayao	UP-467	Pi Ai Kor	Phayao
UP-342	CPAC08020	Phayao	UP-469	Khao Nieow Kla	Phayao
UP-349	CPAC1008	Phayao	UP-471	Seu No Nu	Phayao
UP-353	Kor Dang	Phayao	UP-492	Khao Rai Mae Hong Son	Phayao
UP-354	Sew Dang	Phayao			

3.4 DNA Extraction

Genomic DNA was extracted from fresh young leaves using Tiangen DNA extraction kit (TIANGEN Biotech (Beijing) Co., Ltd., China) according to the manufacturer protocol. The extracted DNA was spectrophotometrically assayed for quantity using a Nanodrop spectrophotometer (Thermo Scientific™ NanoDrop, USA) and the quality was checked by 0.8% agarose gel electrophoresis. The DNA was diluted to concentration of 50 ng/µl and stored at -20ºC for further analysis.

3.5 Genotypic Screening of *Bph* Resistance Genes

Four SSR primers and one InDel primer (Table 3.4) were used for PCR amplification. PCR reaction was performed in 20 µl volume using Eppendorf gradient thermocycler (Marshall Scientific, LLC). The reaction mixture contained 100 ng of template DNA, 5pM each forward and reverse primers, 2mM each dNTPs, 10X PCR buffer (10 mM Tris HCl, 50 mM KCl, 0.01mg/ml gelatin and 1.5mM MgCl2), 1 µl of DMSO, and 1unit of Taq polymerase (Thermo Fisher Scientific, USA). PCR was performed as following: 1 cycle at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 30 seconds, 30 seconds for annealing at 55°C – 63.5°C, and 72°C for 30 seconds and final extension at 72°C for 10 minutes. The amplified products were separated by 4% of agarose gel electrophoresis in 1x TAE buffer (40 mM Tris-Acetate, 1 mM EDTA) at 100V for 40 minutes. The gel was then visualized under UV light using gel documentation (Bio-Rad, Germany).

Table 3.4 Primer sets and condition for PCR to detect genetic diversity

Gene	Primer	Chromosome	Sequences (5' – 3')	Type	T_a (^oC)	Expected size (bp)		References
						Resistant allele	Susceptible allele	
bph2	RM463	12	F: 5'- TTCCCCTCCTTTTATGGTGC -3' R: 5'- TGTTCTCCTCAGTCACTGCG -3'	SSR	55	200	210	Sun et al. (2006)
Bph3	RM588	3	F: 5'- TCTTGCTGTGCTGTTAGTGTACG -3' R: 5'- GCAGGACATAAATACTAGGCATGG -3'	SSR	58	90	110	Jairin et al. (2007)
Bph14	IN76-2	3	F: 5'- CTGCTGCTGCTCTCGTATTG -3' R: 5'- CAGGGAAGCTCCAAGAACAG -3'	InDel	*60.5*	*190*	*180*	Du et al. (2009)
Bph15	RM261	4	F: 5'- CTACTTCTCCCCTTGTGTCG -3' R: 5'- TGTACCATCGCCAAATCTCC -3'	SSR	58	130	120	Hu et al. (2015)
Bph17	RM16626	4	F: 5'- ACATGATTGCTGGCTTGCTTACC -3' R: 5'- GCCACGCAGTGTTGTTTCAGC -3'	SSR	*58*	200	190	Sun et al. (2005)

Note SSR for (Simple-sequence repeats), InDel for (Insertion-Deletion)

3.6 Correlation Between *R* Genes and Phenotypic Expression

The correlation between *R* genes and phenotypic expression was analyzed using *R* program. When a significant effect was observed, the mean was separated by Spearman correlation ($p < 0.05$).

3.7 Polymorphism Information Content (PIC)

Based on the genotypic characteristics, unique polymorphic bands of markers were scored, with a present allele scored as 1 and an absent allele scored as 0. Polymorphic information content (PIC) was estimated according to the formula of Anderson et al. (1993). The formula was as follows: $PIC = 1-\Sigma_j P_{ij}^2$, where P_{ij} is the frequency of the j^{th} allele for the locus summed over all alleles for the locus. The PIC of each marker was then calculated using the following formula:

$$PIC = \left(\sum_{i=1}^{n} PIC_i\right)/n$$

Where *n* is the number of bands

3.8 Population Structure

Population structure was analyzed based on five markers associated with five *Bph* resistance genes using the Bayesian Markov Chain Monte Carlo (MCMC) model in STRUCTURE version 2.3.4 software to interpreting genetic structure and classify clusters of 143 rice varieties (Sooklim et al., 2022). The actual subpopulations (*K*) were measured by running the program with different K values. In a range from $K = 1$ to $K = 10$, 10 independent iterations per *K* were performed. The Burn-in time and the number of MCMC replications were fixed at 500,000 for each run. The mean posterior

probability [ln(PD)] obtained for each *K* was plotted to find the plateau of *ΔK* values, which was then used to determine the most likely *K* value using Structure Harvester software (Yadav et al., 2019). The number of subpopulations was used for the analysis of molecular variance (AMOVA) to estimate the total molecular variance within and between subpopulations, fixation index (*Fst*) and *Nm* values (haploid number of migrants) using GenAlex version 6.503 software (Peakall & Smouse, 2012). The dendrogram of 143 upland rice varieties was generated based on genetic similarity using sequential agglomerative hierarchical nesting (SAHN) method with 10,000 bootstraps by UPGMA (unweighted pair group method using arithmetic average) in *R* program (Sooklim et al., 2022).

CHAPTER 4

RESULTS

4.1 Evaluation of BPH Resistance

Upland rice varieties were tested for BPH resistance at seedling stage. The seedlings were infested with 10 mature BPH in each replicate. The resistant control variety was Rathu Heenati, and the susceptible control variety was TN1. Four low land rice varieties including Pitsanulok2, RD23, RD39, and KDML105 were also tested for comparison. After the variety TN1 was almost dead, BPH resistance was evaluated according to the IRRI (2002) six-scale standard of scoring system. The resistance level was divided into 6 levels: highly resistant (0), resistant (1), moderately resistant (3), moderately susceptible (5), susceptible (7), and highly susceptible (9) (Figure. 4.1).

Score	0	1	3	5	7	9
						
Signs	No damage	Slightly damage	First and second leaves are yellow	Yellowing and stunning	Almost die	Completely die

Figure 4.1 The sign of BPH infestation in 6 scores

As the result, the evaluation of BPH resistance of upland rice from Chiang rai and Phayao were categorized into four groups; 16 varieties exhibited moderately resistance (MR; 11.19%), 43 varieties (MS; 30.07%) were moderately susceptible (score 5) and 20 varieties (S; 13.99%) were susceptible (score 7). However, as indicated in Figure 4.2, 64 varieties (HS; 45.76%) expressed highly susceptible (score 9). According to low land rice varieties, Pitsanulok2 and RD23 showed moderately resistance. RD39 varieties was moderately susceptible and KDML105 exhibited highly susceptible.

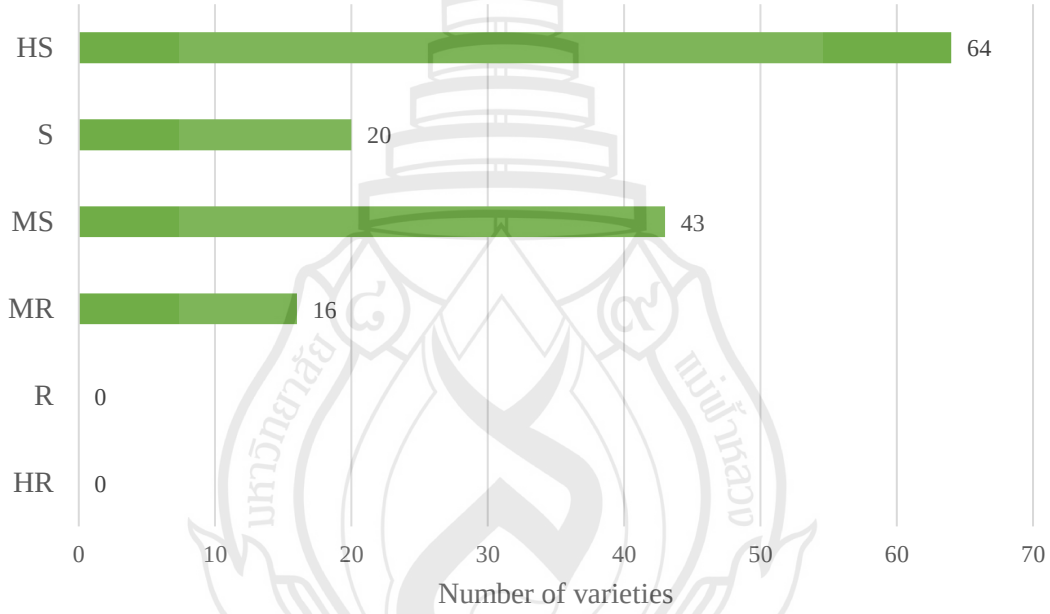


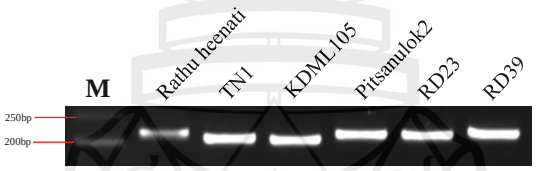
Figure 4.2 The evaluation of BPH resistance among 143 upland rice varieties

4.2 Genotypic Screening of *Bph* Resistance Genes

4.2.1 Identification Of *bph2* Gene

Six control varieties and 143 upland rice varieties were screened with RM463 marker, which is linked to the *bph2* resistance gene. The result showed that 88 varieties had the BPH resistance allele (200 bp fragment) and 55 varieties had the susceptible allele (210 bp fragment) as shown in Figure 4.3.

(A)



(B)

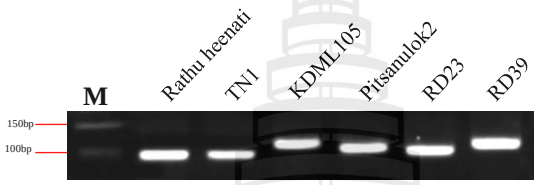
Note (A) The control varieties including Rathu heenati, TN1, KDML105, Pitsanulok2, RD23, and RD39 respectively. (B) 10 upland rice varieties.

Figure 4.3 PCR amplification of RM463 marker

4.2.2 Identification of *Bph3* Gene

The RM588 marker is linked to the *Bph3* resistance gene. The results showed 63 varieties had a 90 bp fragment of resistance allele and 85 varieties had a 110 bp fragment of susceptible allele (Figure 4.4). Additionally, five varieties showed heterozygous for both resistance and susceptible alleles.

(A)



(B)

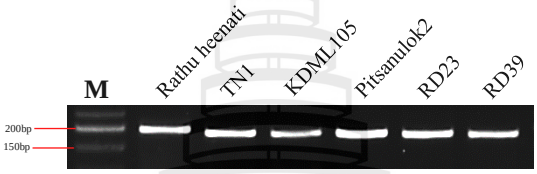
Note (A) The control varieties including Rathu heenati, TN1, KDML105, Pitsanulok2, RD23, and RD39 respectively. (B) 10 upland rice varieties.

Figure 4.4 PCR amplification of RM588 marker

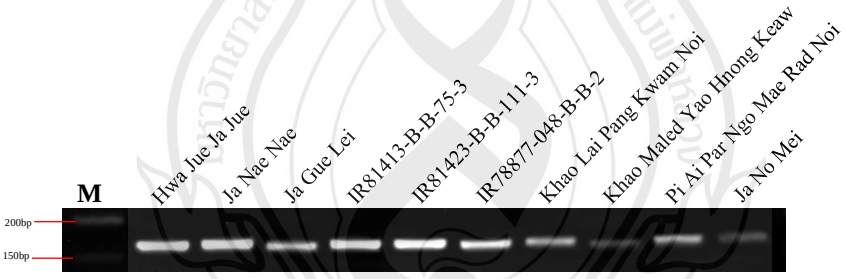
4.2.3 Identification of *Bph14* Gene

The Indel marker IN76-2 was used for screening *Bph14* resistance gene. Putative 190 bp and 180 bp fragments were amplified for resistance and susceptible allele, respectively. Forty-eight varieties showed resistance allele, while 95 varieties showed a susceptible allele (Figure 4.5).

(A)



(B)



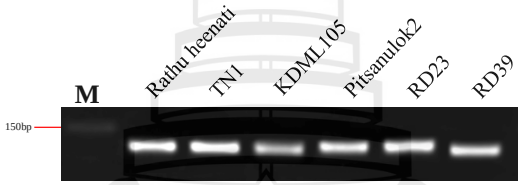
Note (A) The control varieties including Rathu heenati, TN1, KDML105, Pitsanulok2, RD23, and RD39 respectively. (B) 10 upland rice varieties.

Figure 4.5 PCR amplification of IN76-2 marker

4.2.4 Identification of *Bph15* Gene

The RM261 marker has been reported that linked to *Bph15* resistance gene and yielded 130 bp for the resistance allele and 120 bp for the susceptible allele. Among 143 varieties, 90 varieties showed a resistance allele while 53 varieties showed a susceptible allele (Figure 4.6)

(A)



(B)

Note (A) The control varieties including Rathu heenati, TN1, KDML105, Pitsanulok2, RD23, and RD39 respectively. (B) 10 upland rice varieties.

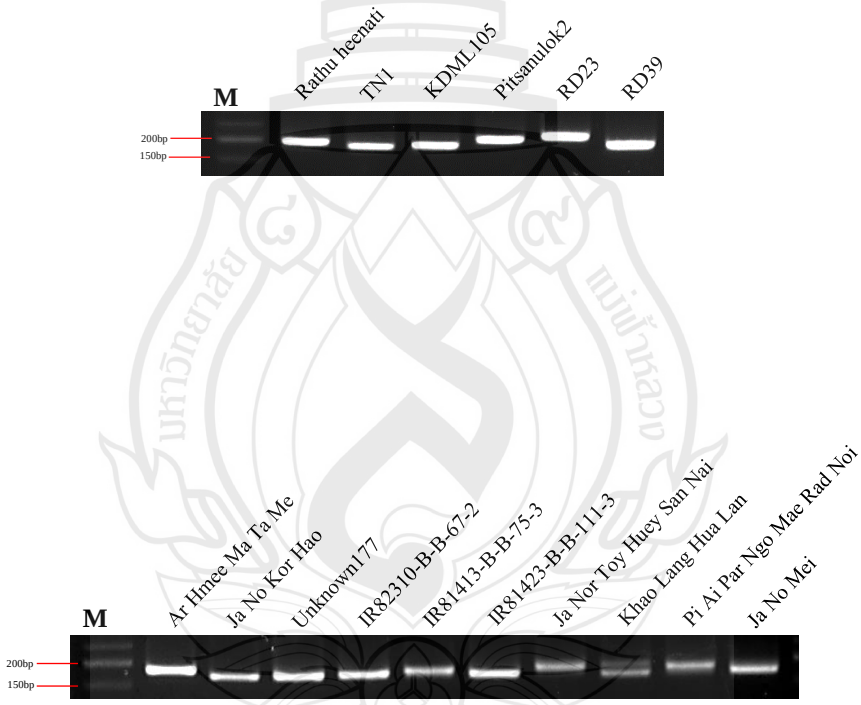
Figure 4.6 PCR amplification of RM261 marker

4.2.5 Identification of *Bph17* Gene

The RM16626 marker which produced 200 bp and 190 bp fragments as the resistance and susceptible alleles, respectively, was used to amplify the *Bph17*. The results showed that 67 varieties had a resistance allele and 76 varieties had a susceptible allele (Figure 4.7).

(A)

(B)



Note (A) The control varieties including Rathu heenati, TN1, KDML105, Pitsanulok2, RD23, and RD39 respectively. (B) 10 upland rice varieties

Figure 4.7 PCR amplification of RM16626 marker

4.2.6 The Pattern of *R* Genes

According to the previous results, five varieties (3.50%) including IR70175-51-2-1-1-2, Lai Chan, Beu Hmeu Mae La, Pi Ai Kor, Khao Nieow Kla, found carried five *Bph* genes, 19 varieties (13.29%) carried four genes, 48 varieties (33.57%) carried three genes, 44 varieties (30.77%) contained two genes and 23 varieties (16.08%) consisted of one gene for *Bph* resistance. However, 4 varieties (2.80%), including Ja Na Toy, Unknown179, Lai San and Ber Sue Ha, found no genes for *Bph* resistance. The gene frequency of five *Bph* genes ranged from 33.57% to 62.94%. Additionally, 143 varieties of upland rice were classified into 27 patterns based on the distribution of allele which shows genetic variation in a population as shown in Table 4.1.

Table 4.1 The allele distribution of *bph2, Bph3, Bph14, Bph15,* and *Bph17* genes in upland rice varieties

Pattern	Resistance genes					Varieties
	bph2	***Bph3***	***Bph14***	***Bph15***	***Bph17***	
5*R*	*R*	*R*	*R*	*R*	*R*	5 varieties: IR70175-51-2-1-1-2, Lai Chan, Beu Hmeu Mae La, Pi Ai Kor, Khao Nieow Kla
4*R*1*S* *(bph2, Bph3, Bph14, Bph15)*	*R*	*R*	*R*	*R*		8 varieties: IR74371-54-1-1, IR79971-B-338-2-2, Khao Rai Ka Reang, 2R-40, 2R-43, Bue Por Lor Lay Ko, Fueng Kaum Pang Kwam Noi, Beu Hmeu Po Mae Kwang Noi
4*R*1*S* *(bph2, Bph14, Bph15, Bph17)*	*R*		*R*	*R*	*R*	6 varieties: Ja Gue Lei, Pi Ai Par Ngo Mae Rad Noi, Nai Jong Hwa, Huey Lang, Beu Su Mae Rad Par Kae, Beu Po Lo Hnong Kang
4*R*1*S* *(bph2, Bph3, Bph15, Bph17)*	*R*	*R*		*R*	*R*	1 variety: IR82310-B-B-67-2
4*R*1*S* *(bph2, Bph3, Bph14, Bph17)*	*R*	*R*	*R*		*R*	4 varieties: IR70181-32-PMI-1-1-4-2, IR84179-B-403, Seu No Nu, Khao Rai Mae Hong Son
3*R*2*S* *(bph2, Bph3, Bph14)*	*R*	*R*	*R*			2 varieties: Doi Chang 149, IR81423-B-B-152-1
3*R*2*S* *(bph2, Bph3, Bph15)*	*R*	*R*		*R*		17 varieties: Wa Ji Da Noo, Doi Lan 139, Doi Lan 142, IR78877-048-B-B-2, IR73000-98-1-2-1, IR78875-176-B-2-B, IR78875-207-B-1-B, CNT86095-42-2-3, IR5675-81-2-3, Khao Ta Chee Long2, Khao Lang Hua Lan, IR81063-B-94-U3-4, 2R-10, Tee Hmee Jar, Lai Nok Khum, Beu Nor Pae Sob Kong, Nieow Jak Pa Mar
3*R*2*S* *(bph2, Bph3, Bph17)*	*R*	*R*			*R*	4 varieties: Young Ger, Wa Chi Ku, IR81413-B-B-75-3, IR81025-B-327-3
3*R*2*S* *(bph2, Bph14, Bph15)*	*R*		*R*	*R*		2 varieties: Ka Reang Kao, Khao Maled Yao Hnong Keaw

Table 4.1. (continued)

Pattern	Resistance genes					Varieties
	bph2	***Bph3***	***Bph14***	***Bph15***	***Bph17***	
3*R*2*S* *(bph2, Bph14, Bph17)*	*R*		*R*		*R*	9 varieties: Khao Jao Doi 1, Khao Mun Huey San Nai, Ja Nor Toy Huey San Nai, Beu So, Beu Sor La So Som Kong, Bee Ai Kor Kae, Ba Dai Koi Ngam, Pi Ai Su Mae Pang1, Khao Sang Loi
3*R*2*S* *(bph2, Bph15, Bph17)*	*R*			*R*	*R*	8 varieties: Khao Fueng Kham, Jao Ho, Heng Wa Ra Nong, Kang Mo Ba, Leuang Tum, Prae Kao Chue, Khao Nieow Hom Lee Sor, CPAC1008(Khao rai5)
3*R*2*S* *(Bph3, Bph14, Bph15)*		*R*	*R*	*R*		3 varieties: Ja Su, La Ngae, CPAC08016
3*R*2*S* *(Bph3, Bph15, Bph17)*		*R*		*R*	*R*	1 variety: Unknown177
3*R*2*S* *(Bph14, Bph15, Bph17)*			*R*	*R*	*R*	2 varieties: Bue Sor Mee, Khao Hwaw
2*R*3*S* *(bph2, Bph3)*	*R*	*R*				4 varieties: La Ta Na, Situ Patenggang, IR78878-53-2-2-2, IR71700-247-1-1-2
2*R*3*S* *(bph2, Bph14)*	*R*		*R*			1 variety: Beu Chi
2*R*3*S* *(bph2, Bph15)*	*R*			*R*		6 varieties: Ta Ter Ma Ja 5, Khao Kam Doi Lan 138, IR78875-190-B-1-3, Ja No Mei, Khao Jao Doi Mae Ou Kor, CPAC08020
2*R*3*S* *(bph2, Bph17)*	*R*				*R*	7 varieties: Su Ja, Ja Gu Na, Unknown169, Ja Ye Gui172, Beu Por Mae Pang, Kor Dang, Khao Man

Table 4.1. (continued)

Pattern	Resistance genes					Varieties
	bph2	***Bph3***	***Bph14***	***Bph15***	***Bph17***	
1*R*4*S* *(bph2)*	*R*					3 varieties: Neaw Doi, Wa La Nee Gu Su, Mali Namnao
1*R*4*S* *(Bph3)*		*R*				4 varieties: Ta Ter Ma Ja 1, Ta Ter Ma Ja 6, SPRLR 85104-62-1-1, IR81423-B-B-111-3
1*R*4*S* *(Bph15)*				*R*		8 varieties: Ta Ter Ma Ja 2, Khao Lai, Khao Tia, Ja Ma See, Doi Chang147, Unknown180, Bue Wa, Khaokeo-4
1*R*4*S* *(Bph17)*					*R*	8 varieties: Hom Ma Li Doi, Ja Fu Ma, Ja Hroi, Ja No Nae, Ja Bue Ma169, Kor Lei, Leuang Rai Pui Ngam, Sew Dang
5*S*						4 varieties: Ja Nae Nae, Ja Chue Chue, Unknown168, Khao Lai Pang Kwam Noi

Note *R* for (Resistance allele), *S* for (Susceptible allele)

4.3 The Correlation Between *R* Genes and Phenotypic Expression

Only the gene *Bph3* was negatively correlated (-0.185) with BPH resistance response, whereas other genes, *Bph14* and *Bph15*, were significantly correlated with BPH resistance, according to analysis of Spearman rank correlations between the genotype of various *Bph* resistance genes in 143 upland rice varieties and their resistance to BPH (Table 4.2).

Table 4.2 Spearman rank correlation between genotype and BPH resistance of upland rice varieties

***Bph* resistance genes**	***bph2***	***Bph3***	***Bph14***	***Bph15***	***Bph17***
BPH resistance	0.0760	-0.1852	0.1952	0.1953	0.0789
P-value	0.3639	0.0257*	0.0186*	0.0185*	0.3458

Note *, correlation is significant at the 0.05 level

4.4 Allelic Diversity of *Bph* Resistance Genes

The Polymorphism Information Content (PIC) value is used to measure the probability that two randomly chosen alleles from a population are prominent. The PIC values ranged from 0.4460 (IN76-2) to 0.4984 (RM16626) with an average of 0.47437, which were intermediately informative according to the Anderson et al., 1993 formula (Table 4.3).

Table 4.3 PIC values of 143 upland rice varieties

Accession No.	Varieties	*Bph* resistance genes						Resistance score
		bph2	***Bph3***	***Bph14***	***Bph15***	***Bph17***	**Total**	
12CR016	Khao' Jao Doi 1	1	0	1	0	1	3	5
18CR123	Wa Ji Da Noo	1	1	0	1	0	3	3
18CR124	Lee Su Ja	1	0	0	0	1	2	3
18CR125	La Ta Na	1	1	0	0	0	2	9
18CR127	Young Ger	1	1	0	0	1	3	5
18CR128	Ar Hmee Ma Ta Me	0	1	0	1	0	2	3
18CR129	Wa Chi Ku	1	1	0	0	1	3	3
18CR130	Hwa Jue Ja Jue	0	0	0	1	1	2	5
18CR131	Ja Nae Nae	0	0	0	0	0	0	5
18CR132	Ta Ter Ma Ja 1	0	1	0	0	0	1	3
18CR133	Ta Ter Ma Ja 2	0	0	0	1	0	1	3
18CR134	Ta Ter Ma Ja 3	0	1	0	1	0	2	3
18CR136	Ta Ter Ma Ja 5	1	0	0	1	0	2	5
18CR138	Khao Kam Doi Lan	1	0	0	1	0	2	5
18CR139	Doi Lan 1	1	1	0	1	0	3	5
18CR140	Hom Ma Li Doi	0	0	0	0	1	1	5
18CR141	Ta Ter Ma Ja 6	0	1	0	0	0	1	5
18CR142	Doi Lan 2	1	1	0	1	0	3	5
18CR143	Khao Lai	0	0	0	1	0	1	3
18CR144	Wa La Go	0	1	0	1	0	2	3
18CR145	Khao Tia	0	0	0	1	0	1	3

Table 4.3 (continued)

Accession No.	Varieties	*Bph* resistance genes						Resistance score
		bph2	*Bph3*	*Bph14*	*Bph15*	*Bph17*	Total	
18CR146	Ja Ma See	0	0	0	1	0	1	5
18CR147	Doi Chang 147	0	0	0	1	0	1	9
18CR148	Doi Chang 148	0	0	0	1	1	2	3
18CR149	Doi Chang 149	1	1	1	0	0	3	9
18CR150	Neaw Doi	1	0	0	0	0	1	3
18CR152	Ja Chue Chue	0	0	0	0	0	0	5
18CR153	Wa La Nee Gu Su	1	0	0	0	0	1	3
18CR165A	Ja Fu Ma	0	0	0	0	1	1	9
18CR039	Ja Bue Ma	0	0	0	1	1	2	9
18CR166	Ja Hroi	0	0	0	0	1	1	7
18CR167	Ja No Nae	0	0	0	0	1	1	9
18CR168 (1)	Ja Gu Na	1	0	0	0	1	2	9
18CR168 (2)	Unknown168	0	0	0	0	0	0	5
18CR169(1)	Unknown169	1	0	0	0	1	2	7
18CR169 (2)	Ja Bue Ma169	0	0	0	0	1	1	5
18CR171	Ja Ye Gui171	0	0	0	1	1	2	9
18CR172	Ja Ye Gui172	1	0	0	0	1	2	5
18CR173	Ja No Kor Hao	0	0	1	1	0	2	5
18CR174	Ja Gue Lei	1	0	1	1	1	4	5
18CR175	Ja Na Toy	0	0	1	0	1	2	5
18CR176	Kor Lei	0	0	0	0	1	1	5

Table 4.3 (continued)

Accession No.	Varieties	*Bph* resistance genes						Resistance score
		bph2	***Bph3***	***Bph14***	***Bph15***	***Bph17***	**Total**	
18CR177	Unknown177	0	1	0	1	1	3	5
19CR178	Unknown178	0	0	0	1	1	2	5
19CR179	Unknown179	0	0	1	0	1	2	5
19CR180	Unknown180	0	0	0	1	0	1	5
UP-2	Situ Patenggang	1	1	0	0	0	2	7
UP-5	SPRLR 85104-62-1-1	0	1	0	0	0	1	5
UP-14	Mali Namnao	1	0	0	0	0	1	5
UP-27	IR78914-B-22-B-B-B	0	1	0	1	0	2	5
UP-28	IR78875-190-B-1-3	1	0	0	1	0	2	5
UP-29	IR78878-53-2-2-2	1	1	0	0	0	2	5
UP-30	IR82310-B-B-67-2	1	1	0	1	1	4	7
UP-32	IR81413-B-B-75-3	1	1	0	0	1	3	5
UP-33	IR81423-B-B-111-3	0	1	0	0	0	1	7
UP-34	IR78877-048-B-B-2	1	1	0	1	0	3	5
UP-35	IR70181-32-PMI-1-1-4-2	1	1	1	0	1	4	5
UP-36	IR73000-98-1-2-1	1	1	1	0	0	3	7
UP-38	IR70175-51-2-1-1-2	1	1	1	1	1	5	7
UP-40	IR84179-B-403	1	1	1	0	1	4	5
UP-41	IR81423-B-B-152-1	1	1	1	0	0	3	5
UP-44	IR74371-54-1-1	1	1	1	1	0	4	5
UP-45	IR79971-B-338-2-2	1	1	1	1	0	4	5

Table 4.3 (continued)

Accession No.	Varieties	*Bph* resistance genes						Resistance score
		bph2	***Bph3***	***Bph14***	***Bph15***	***Bph17***	**Total**	
UP-47	IR78875-176-B-2-B	1	1	0	1	0	3	5
UP-48	IR71700-247-1-1-2	1	1	0	0	0	2	5
UP-49	IR81025-B-327-3	1	1	0	0	1	3	5
UP-50	IR78875-207-B-1-B	1	1	0	1	0	3	7
UP-51	PSL85051-14-2-1-2	0	1	0	1	0	2	9
UP-52	CNT86095-42-2-3	1	1	0	1	0	3	7
UP-53	UNKNOWN UP-53	0	1	0	1	0	2	9
UP-54	IR13240-108-2-2-3	0	0	0	1	1	2	7
UP-55	IR5675-81-2-3	1	1	0	1	0	3	9
UP-58	Bue Wa	0	0	0	1	0	1	9
UP-59	Khao Ta Chee Long2	1	1	0	1	0	3	9
UP-70	Khao Rai Ka Reang	1	1	1	1	0	4	5
UP-108	2R-40	1	1	1	1	0	4	9
UP-110	2R-43	1	1	1	1	0	4	9
UP-135	Khao Pang Kam Noi	0	0	1	1	0	2	9
UP-140	Ja Su	0	1	1	1	0	3	5
UP-143	Lai San	0	0	1	0	1	2	9
UP-145	La Ngae	0	1	1	1	0	3	9
UP-148	Bue Ko	0	0	1	1	0	2	9
UP-151	Bue Sor Mee	0	0	1	1	1	3	9

Table 4.3 (continued)

Accession No.	Varieties	*Bph* resistance genes						Resistance score
		bph2	*Bph3*	*Bph14*	*Bph15*	*Bph17*	Total	
UP-160	Lai Chan	1	1	1	1	1	5	9
UP-163	Ka Reang Kao	1	0	1	1	0	3	9
UP-208	Bue Por Lor Lay Ko	1	1	1	1	0	4	9
UP-215	Khao Lai Pang Kwam Noi	0	0	0	0	0	0	9
UP-244	Khao Maled Yao Hnong Keaw	1	0	1	1	0	3	9
UP-247	Khao Mun Huey San Nai	1	0	1	0	1	3	9
UP-248	Ja Nor Toy Huey San Nai	1	0	1	0	1	3	9
UP-251	Leuang Rai Pui Ngam	0	0	0	0	1	1	9
UP-252	Lai San Pung Yam	0	0	0	1	1	2	9
UP-258	Khao Lang Hua Lan	1	1	0	1	0	3	9
UP-271	Khao Fueng Kham	1	0	0	1	1	3	9
UP-290	Pi Ai Par Ngo Mae Rad Noi	1	0	1	1	1	4	9
UP-291	Ja No Mei	1	0	0	1	0	2	7
UP-292	Nai Jong Hwa	1	0	1	1	1	4	9
UP-293	Beu Chi	1	0	1	0	0	2	7
UP-295	Jao Ho	1	0	0	1	1	3	9
UP-298	IR78875-15-B-3-B	0	1	0	1	0	2	5
UP-299	IR81063-B-94-U3-4	1	1	0	1	0	3	5
UP-302	Heng Wa Ra Nong	1	0	0	1	1	3	9
UP-305	Kaset Poo	0	1	0	1	0	2	9
UP-308	2R-10	1	1	0	1	0	3	9
UP-313	Tee Hmee Jar	1	1	0	1	0	3	9

Table 4.3 (continued)

Accession No.	Varieties	*Bph* resistance genes						Resistance score
		bph2	*Bph3*	*Bph14*	*Bph15*	*Bph17*	Total	
UP-314	Lai Nok Khum	1	1	0	1	0	3	9
UP-315	Huey Lang	1	0	1	1	1	4	9
UP-317	Beu Hmeu Mae La	1	1	1	1	1	5	9
UP-318	Beu Su Mae Rad Par Kae	1	0	1	1	1	4	9
UP-319	Fueng Kaum Pang Kwam Noi	1	1	1	1	0	4	9
UP-320	Beu Hmeu Po Mae Kwang Noi	1	1	1	1	0	4	9
UP-321	Beu Por Mae Pang	1	0	0	0	1	2	9
UP-322	Beu Nor Pae Sob Kong	1	1	0	1	0	3	7
UP-323	Khao Jao Doi Mae Ou Kor	1	0	0	1	0	2	9
UP-324	Nieow Jak Pa Mar	1	1	0	1	0	3	9
UP-325	Ja Nor Mei Ae Ko	0	1	0	1	0	2	9
UP-326	Beu Ku Pa Tong	0	1	0	1	0	2	9
UP-327	Kang Mo Ba	1	0	0	1	1	3	9
UP-331	Leuang Tum	1	0	0	1	1	3	7
UP-334	Prae Kao Chue	1	0	0	1	1	3	9
UP-335	Khao Nieow Hom Lee Sor	1	0	0	1	1	3	9
UP-340	CPAC08016	1	0	1	1	0	3	9
UP-342	CPAC08020	1	0	0	1	0	2	9
UP-349	CPAC1008	1	0	0	1	1	3	9
UP-353	Kor Dang	1	0	0	0	1	2	7
UP-354	Sew Dang	0	0	0	0	1	1	9
UP-357	Ber Sue Ha	0	0	1	0	1	2	7

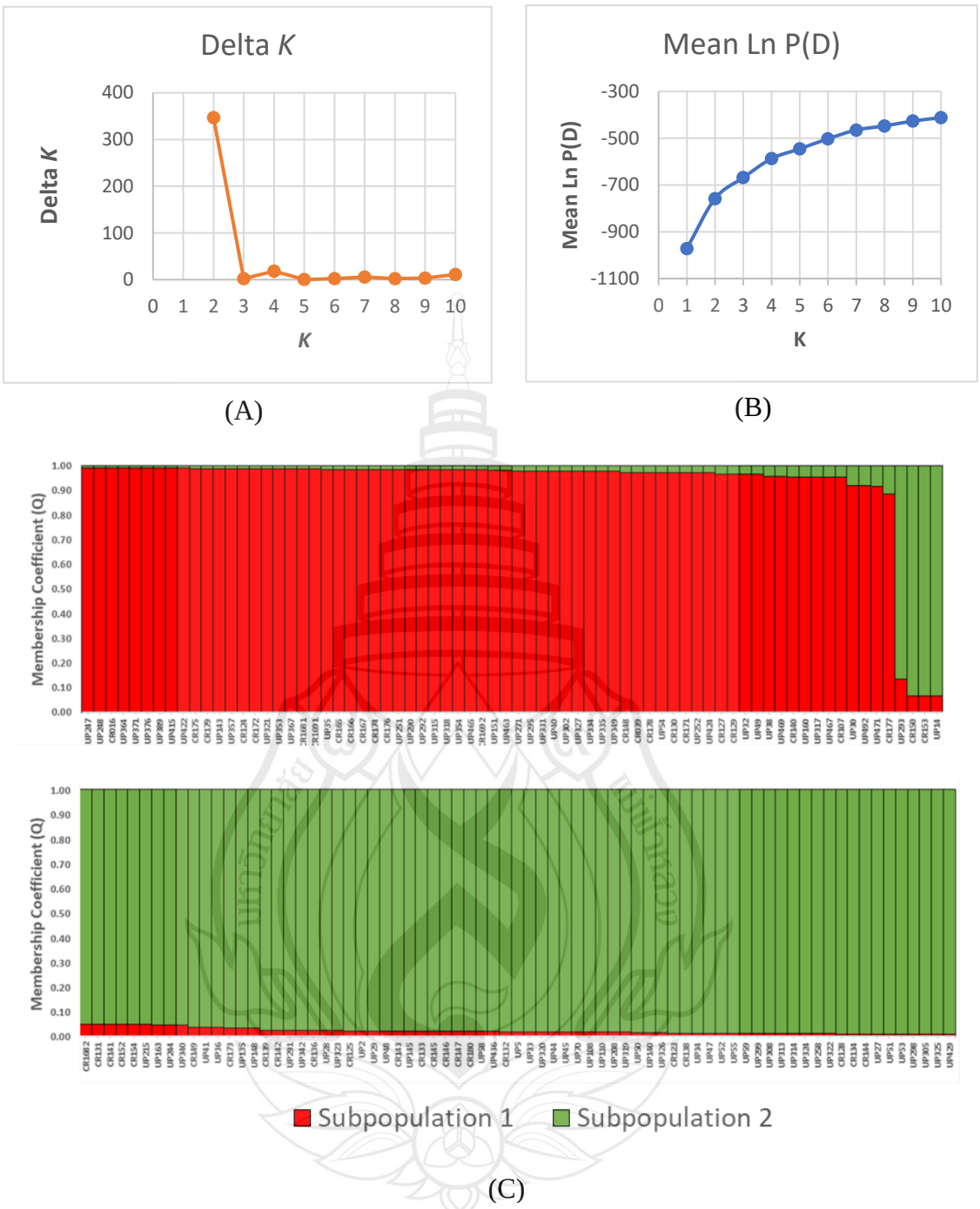
Table 4.3 (continued)

Accession No.	Varieties	*Bph* resistance genes						Resistance score
		bph2	***Bph3***	***Bph14***	***Bph15***	***Bph17***	**Total**	
UP-364	Beu So	1	0	1	0	1	3	9
UP-367	Khao Man	1	0	0	0	1	2	5
UP-371	Beu Sor La So Som Kong	1	0	1	0	1	3	9
UP-376	Bee Ai Kor Kae	1	0	1	0	1	3	7
UP-389	Ba Dai Koi Ngam	1	0	1	0	1	3	3
UP-415	Pi Ai Su Mae Pang1	1	0	1	0	1	3	9
UP-422	Khao Sang Loi	1	0	1	0	1	3	9
UP-424	Sue No Na	0	0	0	1	1	2	9
UP-429	Or Sae Nai Huey San Nai	0	1	0	1	0	2	5
UP-436	Khaokeo-4	0	0	0	1	0	1	9
UP-463	Khao Hwaw	0	0	1	1	1	3	7
UP-465	Beu Po Lo Hnong Kang	1	0	1	1	1	4	7
UP-467	Pi Ai Kor	1	1	1	1	1	5	3
UP-469	Khao Nieow Kla	1	1	1	1	1	5	9
UP-471	Seu No Nu	1	1	0	1	1	4	3
UP-492	Khao Rai Mae Hong Son	1	1	0	1	1	4	5
R gene frequency (%)		61.54	44.06	33.54	62.94	46.85		
PIC value		0.4734	0.4876	0.4460	0.4665	0.4984		

Note the resistance genes are scored as 1 (presence) and 0 (absence) of the amplicon bands

4.5 Analysis of Molecular Variance (AMOVA)

All 143 upland rice varieties were scored to estimate the population structure for BPH resistance based on five markers associated with five *Bph* resistance genes using STRUCTURE software. Based on the genotypic data, the *K* value was used to estimate the number of subpopulations. To determine the optimal *K* value, the number of clusters (*K*) was plotted against ΔK, where ad hoc $K = 2$ (Figure 4.8A). LnP(D) increased continuously and gradually as *K* increased for all value (Figure 4.8B). The optimal *K* value showed that 143 upland rice varieties were divided into two subpopulations (subpopulation 1: SP1 and subpopulation 2: SP2). A small group called SP1 (red color; Figure 4.8C) had 68 varieties (46.90%), of which 33.82% and 66.18% were from the provinces of Chiang rai and Phayao, respectively. In contrast, SP2 (green color; Figure 4.8C) included 77 upland rice varieties (53.10%), of which 32.67% were from Chiang rai and 67.53% were from Phayao. The number of *R* genes varied from one to five genes in both subpopulations. In both subpopulations, the mean fixation index (*Fst*) of both subpopulations were 0.2148 and 0.4218, respectively, indicating that both subpopulations had significant diversity (Fst > 0.15) (Table 4.5). Furthermore, the expected heterozygosity (*He*) of SP1 (0.362) was higher than SP2 (0.347), indicating that SP1 was more diverse than SP2 (Table 4.4).



Note (A) The average log-likelihood of *K* value against *K*, (B) The relationship between *ΔK* and *K* showing the maximum peak at *K* = 2, (C) The estimation of population structure of 143 upland rice varieties on *K* = 2.

Figure 4.8 Population structure of 143 upland rice varieties

Table 4.4 Population structure results of 143 upland rice varieties for the inferred clusters, fixation index (*Fst*), expected heterozygosity (*He*) and number of genotypes determined to subpopulation

Subpopulation	**Inferred clusters**	**Mean *Fst***	**Expected heterozygosity (*He*)**	**No. of genotypes**
Subpopulation 1	0.482	0.2148	0.362	68
Subpopulation 2	0.465	0.4218	0.3486	77

Analysis of molecular variance (AMOVA) was used to calculated the genetic diversity within and among populations. The result revealed that genetic variation among subpopulations was 7%, whereas only 1% variation was detected within populations (Table 12). In addition, 3.410 *Nm* values and 0.068 *Fst* values were determined in agreement with the genetic distance analysis of Nei (Table 4.5).

Table 4.5 Analysis of Molecular Variance (AMOVA) for the 143 upland rice varieties

Source	***df***	**SS**	**MS**	**Est. var.**	**% mol var.**
Among Population	1	14.479	14.479	0.085	7%
Among Individuals	143	327.825	2.292	1.139	92%
Within Individuals	145	2.000	0.014	0.014	1%
Total	289	344.303		1.238	100%
Fixation index (*Fst*)	0.068				
Nm (Heploid)	3.410				

Note *df* for (Degree of freedom), SS for (Sum of squares), MS for (Means squares), Est. var for (Estimated variance), % mol var for (Percentage molecular variance)

4.6 Cluster Analysis

The phylogenetic tree based on genetic dissimilarity segregated 143 upland rice varieties into two main clusters (Cluster I and II) (Figure 4.8). Cluster I contained 100 varieties with a similarity coefficient of 0.35. In cluster I, there were five subclusters: I-A, I-B, I-C, I-D, and I-E. Of these I-A and I-B contained the varieties mainly carrying *Bph15* and *Bph3* genes, respectively. Subcluster I-C contained the varieties carrying the *Bph15* and *Bph17* genes. There were 26 varieties in subcluster I-D, the majority of which had the *bph2*, *Bph3*, *Bph14*, and *Bph15* genes. Five varieties including IR70175-51-2-1-1-2, Lai Chan, Beu Hmeu Mae La, Pi Ai Kor, Khao Nieow Kla, were grouped in subcluster I-D. These varieties also had all five *R* genes. The smallest subcluster, I-E, had only five varieties with a similarity coefficient of 0.70. Two *Bph* resistance genes, *Bph14* and *Bph15*, were the principally genes of this subcluster. Cluster II contained 45 varieties with a similarity coefficient of 0.43, which were divided into two subclusters: II -A and II -B. The 26 varieties in the subcluster II-A mainly had the *bph2* and *Bph17* genes, while the three varieties, Neaw Doi, Wa La Nee Gu Su, and Mali Namnao, contained only *bph2* gene. Most of the varieties in subcluster II -B had only the *Bph17* gene. In contrast, four varieties, Ja Nae Nae, Ja Chue Chue, Unknown168, and Khao Lai Pang Kwam Noi, which lacked any of the *Bph* resistance genes, were similarly grouped together in the subcluster II -B.

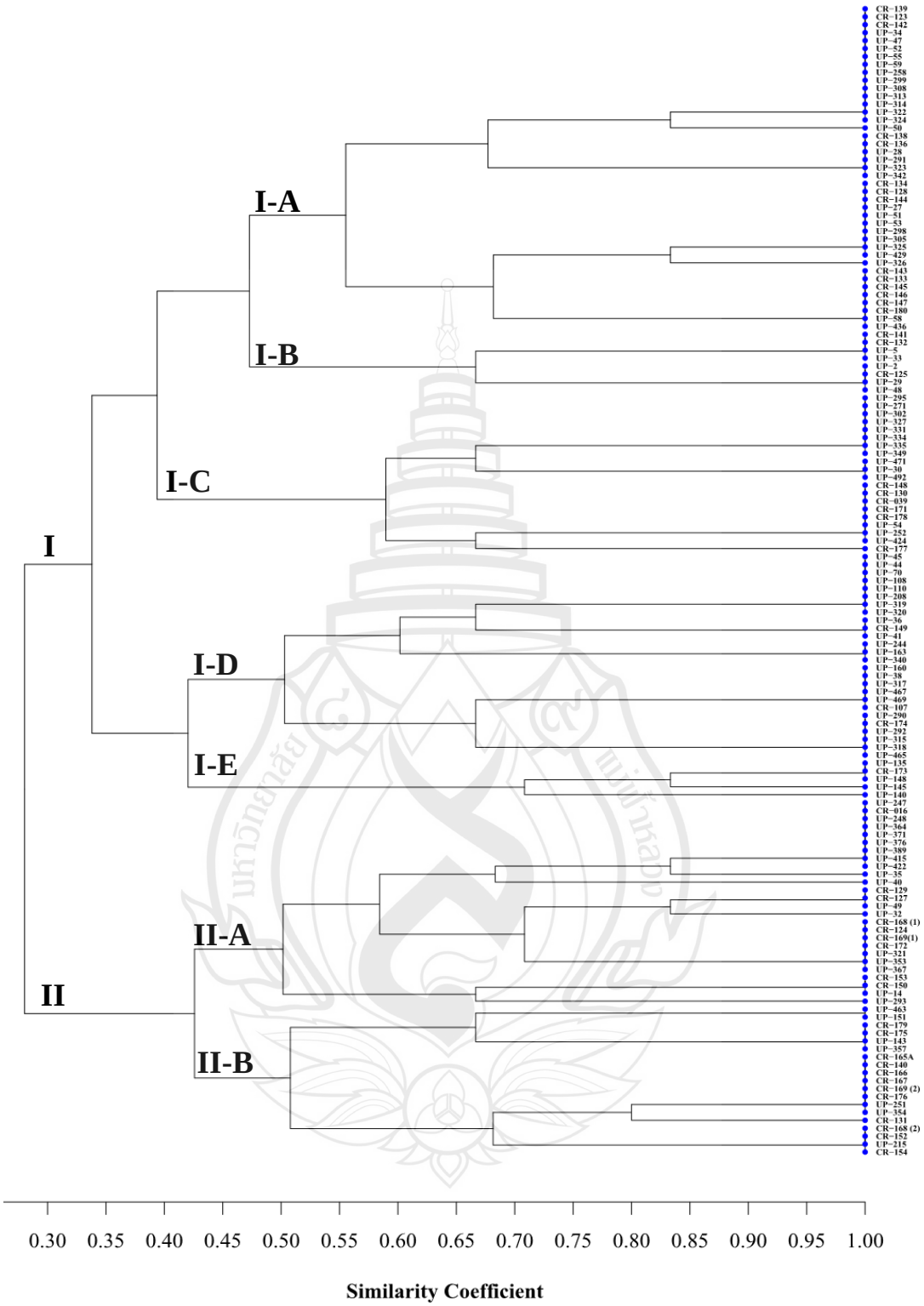


Figure 4.9 The dendrogram depicting the genetic diversity of 143 upland rice varieties

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 Discussion

BPH has become the most serious pests of rice for an innumerable year, causing negative impact on rice production. The control of BPH is mainly in the use of insecticide, which can cause of the contamination in the environment, production, and also induce BPH to rapidly develop resistance to insecticides (Kim et al., 2022). The developing of BPH resistant varieties have been the most economical and environmentally friendly approach for the BPH control. However, the resistant varieties generally lose resistance due to the adaptation evolution BPH (Kumar et al., 2020). Therefore, identification of *Bph* resistance genes in new source of rice variety which can be good germplasm to develop resistant varieties is continuously needed for breeding program.

Of the 143 upland rice varieties, 3.50% carried all five *Bph* genes, 13.29% contained four genes, 33.57% contained three genes, 30.77% contained two genes, and 16.08% contained a single gene. Based on the allele distribution, the gene pattern could be divided into 27 patterns. This substantiated that the BPH resistance genotypes are vary among rice varieties. Phenotypic expression in four low land rice varieties showed that Pitsanulok2 and RD23 were resistant while RD39 and KDML105 were in susceptible level. The results were related to the recorded information of Rice Department that Pitsanulok2 and RD23 are the BPH resistance varieties while RD39 and KDML105 were susceptible varieties. Among 143 varieties, the results showed that 16 varieties had moderate resistance to BPH, with the variety Pi Ai Kor containing five *Bph* genes. Most other varieties, including Wa Ji Da Noo, Ar Hmee Ma Ta Me, Ta Ter Ma Ja 3, Wa La Go, Seu No Nu, Doi Chang 148, Ba Dai Koi Ngam, Wa Chi Ku, and Lee Su Ja contained more than one gene. Similarly, previous study of Anant et al (2021)

in 104 farmers' varieties (FVs) found that 18 were highly resistant, 22 as moderately resistant and 20 as moderately susceptible. Moreover, 22 varieties were categorized as highly susceptible. This confirmed that BPH resistance is a quantitative trait controlled by multiple genes (Park et al., 2020). However, Ta Ter Ma Ja 2, Khao Lai, Khao Tia, Ta Ter Ma Ja 1, Neaw Doi, and Wa La Nee Gu Su were found only 1 gene. This also suggested that the number of R genes has no effect on phenotypic level. Additionally, the result of 16 varieties showed the resistance level as Rathu heenati variety (resistance control). The result conflicted with previous studies which reported that Rathu heenati is strong BPH resistance variety and generally used as a donor in breeding programs (Kusumawati et al., 2018). According to the experiment of Jairin et al. (2007b), Rathu heenati was used as the donor variety for *Bph3* gene, which showing strong and broad-spectrum resistance against all biotype of BPH populations in Thailand. However, the identified and utilized of BPH resistant rice varieties possibly caused of the appearance new biotype of BPH populations that can adapt to those resistant varieties. There are more than eight biotypes have found in Thailand. The adaptation and the appearance biotype of BPH has described to be induced by a combination of genetic factors and other factors which caused the change on feeding or oviposition behaviors of BPH such as microbial symbiosis found in BPH abdominal fat (Jairin, 2021). The biotype is also different in each population with varied ratio. Jairin et al. (2014) investigated the biotypes of collected BPH population in seven rice varieties (Mudgo, ASD7, Rathu heenati, Babawee, Chainat1, Pathum Thani1, and RD49) and found that all of rice varieties were susceptible to BPH biotype 8. Therefore, the mixed biotype of BPH population in this research could be possibly cause of moderately resistant shown in Rathu heenati variety.

According to the correlation between the *R* genes and phenotypic expression, the *Bph3* gene showed the highest efficacy against BPH infestation. This is consistent with the results of Liu et al. (2016), who reported that the *Bph3* gene exhibited highly effective and broad-spectrum resistance against all BPH biotypes. He et al. (2020) induced four *Bph* resistance genes (*Bph3/14/18/32*) into the cultivar Guang 8B and found that the *Bph3* gene exhibited the highest efficacy among the other three genes. On the other hand, the *Bph14* gene, which encodes for a coiled-coil, nucleotide-binding and leucine-rich repeat (CC-NB-LRR), showed low efficacy against BPH. The result

was consistent with Xu (2013) finding that the varieties carrying the *Bph14* gene had a moderately susceptible to BPH. The result was related to Xu (2013), who found moderate susceptibility to BPH in the variety with Bph14 gene. As well as who found moderate susceptibility to BPH in the variety with Bph14 gene as well as Han et al. (2018) who evaluated the BPH resistance in rice varieties BR4831 (breeding line of *Bph14* and *Bph15*), HF106 (single-gene introgression lines carry *Bph14*), and C602 (single-gene introgression lines carry *Bph15*) and found the moderately susceptible in rice introgression lines carry only *Bph14* and *Bph15*. In contrast, the pyramid of *Bph14/Bph15* was transferred to rice varieties to improve resistance and exhibited strong antibiotic resistance to BPH. This confirmed that the BPH resistance in rice is controlled by multiple genes and the pyramiding of two or more genes could improve the ability resistance.

PIC Value is generally used as an indicator of the ability to detect polymorphism of markers for linkage analysis. The PIC is useful in selecting markers for genetic studies (Serrote et al., 2020). Based on the study by Botstein et al. (1980), markers are classified into three levels: (1) The highly informative marker are those with a PIC value more than 0.5 (PIC>0.5). (2) Medium informative markers were those with PIC value between 0.25 and 0.50 (0.50 > PIC > 0.25). (3) The markers with PIC value less than 0.25 (PIC < 0.25) were low informative (Luo et al., 2019). As a result, the PIC value of this study ranged from 0.4460 to 0.4984 with an average of 0.4744, confirming that the markers used in this study were moderately informative to detect the genetic diversity of BPH resistance loci. The PIC values were higher than the report from Anant et al. (2021) which ranged from 0.00 to 0.413 in similar markers. It should be emphasized that numerous factors such as species behavior, genetic diversity and size of collection, genotypic approach, and the location of markers used affect the PIC values (Singh et al., 2013).

Population among upland rice varieties was recognized by *Fst* value. *Fst* is a value that can be a measure of population diversity based on genetic structure. According to Mohammadi and Prasanna (2003), *Fst* value can be considered as *Fst* > 0.25 for high differentiation, 0.25 > *Fst* > 0.15 for medium differentiation and *Fst* < 0.05 for low differentiation. As a result, *Fst* values of SP1 and SP2 were 0.2148 and 0.4218, respectively, indicating significant divergence within both subpopulations.

However, a low *Fst* value (0.068) was found between the two subpopulations, indicating a low genetic divergence between the two subpopulations.

According to the AMOVA analysis shows that 92% of the variation was found between subpopulations. The results were similar to those of Anant et al.'s study (2021) which showed 83% variation among individuals and 17% among populations. This indicated that there is high genetic exchange between individuals (SP1 and SP2).

5.2 Conclusion

In this study, BPH resistance was evaluated the genotype and phenotype in 143 upland rice varieties to investigate the correlation between *R* genes and the expression of resistance. The variation of *Bph* genes found in upland rice varieties with some varieties being moderately resistant. Cluster analysis grouped the upland rice varieties into two main groups based on genetic variation. The genetic diversity among populations was high, according to population structure. AMOVA analysis was able to confirm the exchange of genes between SP1 and SP2 with 93% variation. This suggests that upland rice is a valuable source of *Bph* resistance for the future rice breeding.

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APPENDICES

APPENDIX A

GENOTYPIC SCORING

Table A1 The score of amplicon bands

Accession Number	Varieties	RM463 (b*ph2*)		RM588 (*Bph3*)		IN76-2 (*Bph14*)		RM26 (*Bph15*)		RM16626 (*Bph17*)	
		R	S	R	S	R	S	R	S	R	S
		200	210	90	110	190	180	130	120	200	190
12CR016	Khao' Jao Doi 1	1	0	0	1	1	0	0	1	1	0
18CR123	Wa Ji Da Noo	1	0	1	0	0	1	1	0	0	1
18CR124	Lee Su Ja	1	0	0	1	0	1	0	1	1	0
18CR125	La Ta Na	1	0	1	0	0	1	0	1	0	1
18CR127	Young Ger	1	0	1	0	0	1	0	1	1	0
18CR128	Ar Hmee Ma Ta Me	0	1	1	0	0	1	1	0	0	1
18CR129	Wa Chi Ku	1	0	1	0	0	1	0	1	1	0
18CR130	Hwa Jue Ja Jue	0	1	0	1	0	1	1	0	1	0
18CR131	Ja Nae Nae	0	1	0	1	0	1	0	1	0	0
18CR132	Ta Ter Ma Ja 1	0	1	1		0	1	0	1	0	1
18CR133	Ta Ter Ma Ja 2	0	1	0	1	0	1	1	0	0	1
18CR134	Ta Ter Ma Ja 3	0	1	1		0	1	1	0	0	1
18CR136	Ta Ter Ma Ja 5	1	0	0	1	0	1	1	0	0	1
18CR138	Khao Kam Doi Lan	1	0	0	1	0	1	1	0	0	1
18CR139	Doi Lan 1	1	0	1	0	0	1	1	0	0	1
18CR140	Hom Ma Li Doi	0	1	0	1	0	1	0	1	1	0
18CR141	Ta Ter Ma Ja 6	0	1	1	0	0	1	0	1	0	1
18CR142	Doi Lan 2	1	0	1	0	0	1	1	0	0	1
18CR143	Khao Lai	0	1	0	1	0	1	1	0	0	1
18CR144	Wa La Go	0	1	1	0	0	1	1	0	0	1
18CR145	Khao Tia	0	1	0	1	0	1	1	0	0	1
18CR146	Ja Ma See	0	1	0	1	0	1	1	0	0	1
18CR147	Doi Chang 147	0	1	0	1	0	1	1	0	0	1
18CR148	Doi Chang 148	0	1	0	1	0	1	1	0	1	0

Table A1 (continued)

Accession Number	Varieties	RM463 (b*ph2*)		RM588 (*Bph3*)		IN76-2 (*Bph14*)		RM26 (*Bph15*)		RM16626 (*Bph17*)	
		R	S	R	S	R	S	R	S	R	S
		200	210	90	110	190	180	130	120	200	190
18CR149	Doi Chang 149	1	0	1	0	1	0	0	1	0	1
18CR150	Neaw Doi	1	0	0	1	0	1	0	1	0	1
18CR152	Ja Chue Chue	0	1	0	1	0	1	0	1	0	1
18CR153	Wa La Nee Gu Su	1	0	0	1	0	1	0	1	0	1
18CR165A	Ja Fu Ma	0	1	0	1	0	1	0	1	1	0
18CR039	Ja Bue Ma	0	1	0	1	0	1	1	0	1	0
18CR166	Ja Hroi	0	1	0	1	0	1	0	1	1	0
18CR167	Ja No Nae	0	1	0	1	0	1	0	1	1	0
18CR168 (1)	Ja Gu Na	1	0	0	1	0	1	0	1	1	0
18CR168 (2)	Unknown168	0	1	0	1	0	1	0	1	0	1
18CR169 (1)	Unknown169	1	0	0	1	0	1	0	1	1	0
18CR169 (2)	Ja Bue Ma169	0	1	0	1	0	1	0	1	1	0
18CR171	Ja Ye Gui171	0	1	0	1	0	1	1	0	1	0
18CR172	Ja Ye Gui172	1	0	0	1	0	1	0	1	1	0
18CR173	Ja No Kor Hao	0	1	0	1	1	0	1	0	0	1
18CR174	Ja Gue Lei	1	0	0	1	1	0	1	0	1	0
18CR175	Ja Na Toy	0	1	0	1	1	0	0	1	1	0
18CR176	Kor Lei	0	1	0	1	0	1	0	1	1	0
18CR177	Unknown177	0	1	1	0	0	1	1	0	1	0
19CR178	Unknown178	0	1	0	1	0	1	1	0	1	0
19CR179	Unknown179	0	1	0	1	1	0	0	1	1	0
19CR180	Unknown180	0	1	0	1	0	1	1	0	0	1
UP-2	Situ Patenggang	1	0	1	0	0	1	0	1	0	1
UP-5	SPRLR 85104-62-1-1	0	1	1	0	0	1	0	1	0	1
UP-14	Mali Namnao	1	0	0	1	0	1	0	1	0	1
UP-27	IR78914-B-22-B-B-B	0	1	1	0	0	1	1	0	0	1
UP-28	IR78875-190-B-1-3	1	0	0	1	0	1	1	0	0	1
UP-29	IR78878-53-2-2-2	1	0	1	0	0	1	0	1	0	1
UP-30	IR82310-B-B-67-2	1	0	1	0	0	1	1	0	1	0
UP-32	IR81413-B-B-75-3	1	0	1	1	0	1	0	1	1	0
UP-33	IR81423-B-B-111-3		1	1	0	0	1	0	1	0	1
UP-34	IR78877-048-B-B-2	1	0	1	0	0	1	1	0	0	1

Table A1 (continued)

Accession Number	Varieties	RM463 (b*ph2*)		RM588 (*Bph3*)		IN76-2 (*Bph14*)		RM26 (*Bph15*)		RM16626 (*Bph17*)	
		R	S	R	S	R	S	R	S	R	S
		200	210	90	110	190	180	130	120	200	190
UP-35	IR70181-32-PMI-1-1-4-2	1	0	1	1	1	0	0	1	1	0
UP-36	IR73000-98-1-2-1	1	0	1	0	1	0	0	1	0	1
UP-38	IR70175-51-2-1-1-2	1	0	1	0	1	0	1	0	1	0
UP-40	IR84179-B-403	1	0	1	0	1	0	0	1	1	0
UP-41	IR81423-B-B-152-1	1	0	1	0	1	0	0	1	0	1
UP-44	IR74371-54-1-1	1	0	1	0	1	0	1	0	0	1
UP-45	IR79971-B-338-2-2	1	0	1	0	1	0	1	0	0	1
UP-47	IR78875-176-B-2-B	1	0	1	0	0	1	1	0	0	1
UP-48	IR71700-247-1-1-2	1	0	1	0	0	1	0	1	0	1
UP-49	IR81025-B-327-3	1	0	1	0	0	1	0	1	1	0
UP-50	IR78875-207-B-1-B	1	0	1	1	0	1	1	0	0	1
UP-51	PSL85051-14-2-1-2	0	1	1	0	0	1	1	0	0	1
UP-52	CNT86095-42-2-3	1	0	1	0	0	1	1	0	0	1
UP-53	UNKNOWN UP-53	0	1	1	0	0	1	1	0	0	1
UP-54	IR13240-108-2-2-3	0	1	0	1	0	1	1	0	1	0
UP-55	IR5675-81-2-3	1	0	1	0	0	1	1	0	0	1
UP-58	Bue Wa	0	1	0	1	0	1	1	0	0	1
UP-59	Khao Ta Chee Long2	1	0	1	0	0	1	1	0	0	1
UP-70	Khao Rai Ka Reang	1	0	1	0	1	0	1	0	0	1
UP-108	2R-40	1	0	1	0	1	0	1	0	0	1
UP-110	2R-43	1	0	1	0	1	0	1	0	0	1
UP-135	Khao Pang Kam Noi	0	1	0	1	1	0	1	0	0	1
UP-140	Ja Su	0	1	1	0	1	0	1	0	0	1
UP-143	Lai San	0	1	0	1	1	0	0	1	1	0
UP-145	La Ngae	0	1	1	1	1	0	1	0	0	1
UP-148	Bue Ko	0	1	0	1	1	0	1	0	0	1
UP-151	Bue Sor Mee	0	1	0	1	1	0	1	0	1	0
UP-160	Lai Chan	1	0	1	0	1	0	1	0	1	0
UP-163	Ka Reang Kao	1	0	0	1	1	0	1	0	0	1
UP-208	Bue Por Lor Lay Ko	1	0	1	0	1	0	1	0	0	1
UP-215	Khao Lai Pang Kwam Noi	0	1	0	1	0	1	0	1	0	1
UP-244	Khao Maled Yao Hnong Keaw	1	0	0	1	1	0	1	0	0	1

Table A1 (continued)

Accession Number	Varieties	RM463 (b*ph2*)		RM588 (*Bph3*)		IN76-2 (*Bph14*)		RM26 (*Bph15*)		RM16626 (*Bph17*)	
		R	S	R	S	R	S	R	S	R	S
		200	210	90	110	190	180	130	120	200	190
UP-247	Khao Mun Huey San Nai	1	0	0	1	1	0	0	1	1	0
UP-248	Ja Nor Toy Huey San Nai	1	0	0	1	1	0	0	1	1	0
UP-251	Leuang Rai Pui Ngam	0	1	0	1	0	1	0	1	1	0
UP-252	Lai San Pung Yam	0	1	0	1	0	1	1	0	1	0
UP-258	Khao Lang Hua Lan	1	0	1	0	0	1	1	0	0	1
UP-271	Khao Fueng Kham	1	0	0	1	0	1	1	0	1	0
UP-290	Pi Ai Par Ngo Mae Rad Noi	1	0	0	1	1	0	1	0	1	0
UP-291	Ja No Mei	1	0	0	1	0	1	1	0	0	1
UP-292	Nai Jong Hwa	1	0	0	1	1	0	1	0	1	0
UP-293	Beu Chi	1	0	0	1	1	0	0	1	0	1
UP-295	Jao Ho	1	0	0	1	0	1	1	0	1	0
UP-298	IR78875-15-B-3-B	0	1	1	0	0	1	1	0	0	1
UP-299	IR81063-B-94-U3-4	1	0	1	0	0	1	1	0	0	1
UP-302	Heng Wa Ra Nong	1	0	0	1	0	1	1	0	1	0
UP-305	Kaset Poo	0	1	1	0	0	1	1	0	0	1
UP-308	2R-10	1	0	1	0	0	1	1	0	0	1
UP-313	Tee Hmee Jar	1	0	1	0	0	1	1	0	0	1
UP-314	Lai Nok Khum	1	0	1	0	0	1	1	0	0	1
UP-315	Huey Lang	1	0	0	1	1	0	1	0	1	0
UP-317	Beu Hmeu Mae La	1	0	1	0	1	0	1	0	1	0
UP-318	Beu Su Mae Rad Par Kae	1	0	0	1	1	0	1	0	1	0
UP-319	Fueng Kaum Pang Kwam Noi	1	0	1	0	1	0	1	0	0	1
UP-320	Beu Hmeu Po Mae Kwang Noi	1	0	1	0	1	0	1	0	0	1
UP-321	Beu Por Mae Pang	1	0	0	1	0	1	0	1	1	0
UP-322	Beu Nor Pae Sob Kong	1	0	1	0	0	1	1	0	0	1
UP-323	Khao Jao Doi Mae Ou Kor	1	0	0	1	0	1	1	0	0	1
UP-324	Nieow Jak Pa Mar	1	0	1	0	0	1	1	0	0	1
UP-325	Ja Nor Mei Ae Ko	0	1	1	0	0	1	1	0	0	1
UP-326	Beu Ku Pa Tong	0	1	1	1	0	1	1	0	0	1
UP-327	Kang Mo Ba	1	0	0	1	0	1	1	0	1	0
UP-331	Leuang Tum	1	0	0	1	0	1	1	0	1	0

Table A1 (continued)

Accession Number	Varieties	RM463 (b*ph2*)		RM588 (*Bph3*)		IN76-2 (*Bph14*)		RM26 (*Bph15*)		RM16626 (*Bph17*)	
		R	S	R	S	R	S	R	S	R	S
		200	210	90	110	190	180	130	120	200	190
UP-334	Prae Kao Chue	1	0	0	1	0	1	1	0	1	0
UP-335	Khao Nieow Hom Lee Sor	1	0	0	1	0	1	1	0	1	0
UP-340	CPAC08016	1	0	0	1	1	0	1	0	0	1
UP-342	CPAC08020	1	0	0	1	0	1	1	0	0	1
UP-349	CPAC1008	1	0	0	1	0	1	1	0	1	0
UP-353	Kor Dang	1	0	0	1	0	1	0	1	1	0
UP-354	Sew Dang	0	1	0	1	0	1	0	1	1	0
UP-357	Ber Sue Ha	0	1	0	1	1	0	0	1	1	0
UP-364	Beu So	1	0	0	1	1	0	0	1	1	0
UP-367	Khao Man	1	0	0	1	0	1	0	1	1	0
UP-371	Beu Sor La So Som Kong	1	0	0	1	1	0	0	1	1	0
UP-376	Bee Ai Kor Kae	1	0	0	1	1	0	0	1	1	0
UP-389	Ba Dai Koi Ngam	1	0	0	1	1	0	0	1	1	0
UP-415	Pi Ai Su Mae Pang1	1	0	0	1	1	0	0	1	1	0
UP-422	Khao Sang Loi	1	0	0	1	1	0	0	1	1	0
UP-424	Sue No Na	0	1	0	1	0	1	1	0	1	0
UP-429	Or Sae Nai Huey San Nai	0	1	1	0	0	1	1	0	0	1
UP-436	Khaokeo-4	0	1	0	1	0	1	1	0	0	1
UP-463	Khao Hwaw	0	1	0	1	1	0	1	0	1	0
UP-465	Beu Po Lo Hnong Kang	1	0	0	1	1	0	1	0	1	0
UP-467	Pi Ai Kor	1	0	1	0	1	0	1	0	1	0
UP-469	Khao Nieow Kla	1	0	1	0	1	0	1	0	1	0
UP-471	Seu No Nu	1	0	1	0	0	1	1	0	1	0
UP-492	Khao Rai Mae Hong Son	1	0	1	0	0	1	1	0	1	0

APPENDIX B

BPH EVALUATION

Table B1 The recorded data of six score scales of BPH Evaluation

Accession Number	Variety	Replication	Score of a seedling					Mode	Final mode
			1	2	3	4	5		
12CR016	Khao' Jao Doi 1	1	5	5	5	5	5	5	5
		2	5	5	5			5	
		3	5	5	5			5	
18CR123	Wa Ji Da Noo	1	3	3	3	3	3	3	3
		2	9	9	9	9	9	9	
		3	3	3	3	3	3	3	
18CR124	Lee Su Ja	1	3	3	3	3	3	3	3
		2	9	3	3	3	3	3	
		3	5	5	3	3	3	3	
18CR125	La Ta Na	1	9	9	3	3	3	3	9
		2	5	9	9	9	9	9	
		3	9	9	9	5	5	9	
18CR127	Young Ger	1	5	5	5	5	5	5	5
		2	7	5	5	5	5	5	
		3	9	9	9	5	5	9	
18CR128	Ar Hmee Ma Ta Me	1	9	9	9	9	9	9	3
		2	9	3	5	3	3	3	
		3	3	3	3	9	9	3	
18CR129	Wa Chi Ku	1	5	5	5	5	5	5	3
		2	5	3	3	3	3	3	
		3	3	3	3	3	3	3	

Table B1 (continued)

Accession Number	Variety	Replication	Score of a seedling					Mode	Final mode
			1	2	3	4	5		
18CR130	Hwa Jue Ja Jue	1	3	3	3	3	3	3	5
		2	5	5	5			5	
		3	7	9	5	5	5	5	
18CR131	Ja Nae Nae	1	5	5	5	9		5	5
		2	5	5	5	3	3	5	
		3	3	3	3	5		3	
18CR132	Ta Ter Ma Ja 1	1	9	9	9	9	9	9	3
		2	3	3	3	3	9	3	
		3	3	3	3	3	3	3	
18CR133	Ta Ter Ma Ja 2	1	9	9	9	5	5	9	3
		2	3	3	3	3	3	3	
		3	5	3	3	3	3	3	
18CR134	Ta Ter Ma Ja 3	1	5	5	5	5	5	5	3
		2	9	5	3	3	3	3	
		3	3	3	3	3	3	3	
18CR136	Ta Ter Ma Ja 5	1	5	5	5	5	5	5	5
		2	5	5	5			5	
		3	5	5	5			5	
18CR138	Khao Kam Doi Lan	1	9	5	5	5	5	5	5
		2	7	9	5	5	5	5	
		3	9	5	5			5	
18CR139	Doi Lan 1	1	5	5	5	5	5	5	5
		2	3	3	3	3	3	3	
		3	5	5	5	5	5	5	
18CR140	Hom Ma Li Doi	1	9	9	9	9	5	9	5
		2	5	5	5	5	5	5	
		3	5	5	5	5	5	5	
18CR141	Ta Ter Ma Ja 6	1	9	5	5	5	5	5	5
		2	5	5	5	9	9	5	
		3	5	5	5			5	
18CR142	Doi Lan 2	1	9	9	9	9	9	9	5
		2	9	5	5	5	5	5	
		3	3	3	5	5	5	5	
18CR143	Khao Lai	1	9	9	5			9	3
		2	5	3	3	3	3	3	
		3	5	5	3	3	3	3	

Table B1 (continued)

Accession Number	Variety	Replication	Score of a seedling					Mode	Final mode
			1	2	3	4	5		
18CR144	Wa La Go	1	3	3	3	3	3	3	3
		2	3	3	3			3	
		3	5	9	9	9		9	
18CR145	Khao Tia	1	5	5	5	5	5	5	3
		2	3	3	3	3	3	3	
		3	3	3	3	3	3	3	
18CR146	Ja Ma See	1	9	7	5	5	5	5	5
		2	3	3	3	3	3	3	
		3	9	9	5	5	5	5	
18CR147	Doi Chang 147	1	9	9	9	5	5	9	9
		2	9	9	9	9	9	9	
		3	7	7	9	9	9	9	
18CR148	Doi Chang 148	1	3	3	3	3	3	3	3
		2	5	5	5	5	5	5	
		3	3	3	3	3	3	3	
18CR149	Doi Chang 149	1	9	9	9	9	9	9	9
		2	7	9	9	9	9	9	
		3	7	9	9	9	9	9	
18CR150	Neaw Doi	1	3	3	3	3	3	3	3
		2	3	3	3	3	3	3	
		3	3	3	3	3	3	3	
18CR152	Ja Chue Chue	1	9	9	9	5	5	9	5
		2	9	5	5			5	
		3	5	5	5	5	5	5	
18CR153	Wa La Nee Gu Su	1	5	5	3	3	3	3	3
		2	3	3	3	3	3	3	
		3	7	9	9			9	
18CR165A	Ja Fu Ma	1	7	7	9			7	9
		2	9	5	9			9	
		3	7	9	9			9	
18CR039	Ja Bue Ma	1	9	9	9			9	9
		2	9	9	9			9	
		3	9	9	9			9	
18CR166	Ja Hroi	1	7	7	9			7	7
		2	5	7	7			7	
		3	9	9	7			9	

Table B1 (continued)

Accession Number	Variety	Replication	Score of a seedling					Mode	Final mode
			1	2	3	4	5		
18CR167	Ja No Nae	1	9	9	7				9
		2	9	9	9				
		3	9	9	9				
18CR168 (1)	Ja Gu Na	1	9	9	5				9
		2	9	9	9				
		3	7	7	9				
18CR168 (2)	Unknown168	1	9	9	9				5
		2	5	9	5				
		3	5	5	5				
18CR169 (1)	Unknown169	1	7	7	9				7
		2	7	5	7				
		3	9	7	7				
18CR169 (2)	Ja Bue Ma169	1	5	5	9				5
		2	5	9	5				
		3	7	7	9				
18CR171	Ja Ye Gui171	1	7	7	9				9
		2	9	5	9				
		3	9	5	9				
18CR172	Ja Ye Gui172	1	5	5	9				5
		2	5	5	9				
		3	9	9	9				
18CR173	Ja No Kor Hao	1	5	9	5				5
		2	5	5	5				
		3	9	5	5				
18CR174	Ja Gue Lei	1	5	5	5				5
		2	5	5	7				
		3	5	5	5				
18CR175	Ja Na Toy	1	9	5	9				5
		2	9	5	5				
		3	7	5	5				
18CR176	Kor Lei	1	5	5	9				5
		2	5	5	5				
		3	5	5	5				
18CR177	Unknown177	1	5	5	5				5
		2	9	5	5				
		3	7	5	5				

Table B1 (continued)

Accession Number	Variety	Replication	Score of a seedling					Mode	Final mode
			1	2	3	4	5		
19CR178	Unknown178	1	5	5	5			5	7
		2	7	9	7			7	
		3	7	9	7			7	
19CR179	Unknown179	1	5	5	5			5	5
		2	7	5	5			5	
		3	5	5	7			5	
19CR180	Unknown180	1	9	9	7			9	9
		2	9	9	7			9	
		3	9	5	5			5	
UP-2	Situ Patenggang	1	7	7	7	5	5	7	5
		2	5	5	5	5	5	5	
		3	9	9	5	5	5	5	
UP-5	SPRLR 85104-62-1-1	1	5	5	5	5	5	5	5
		2	5	7	7	7	7	7	
		3	5	5	5	5	5	5	
UP-14	Mali Namnao	1	5	5	5	5	5	5	5
		2	7	7	7	5	5	7	
		3	5	5	5	5	5	5	
UP-27	IR78914-B-22-B-B-B	1	9	9	9	7	7	9	5
		2	5	5	5	5	5	5	
		3	5	5	5	5	5	5	
UP-28	IR78875-190-B-1-3	1	9	9	9	9	9	9	7
		2	9	9	7	7	7	7	
		3	5	5	7	7	7	7	
UP-29	IR78878-53-2-2-2	1	7	7	7	7	7	7	5
		2	9	9	5	5	5	5	
		3	5	5	5	5	7	5	
UP-30	IR82310-B-B-67-2	1	5	5	9	9	9	9	5
		2	5	5	5	5	5	5	
		3	5	5	5	5	5	5	
UP-32	IR81413-B-B-75-3	1	5	5	5	9	9	5	5
		2	5	5	5	7	7	5	
		3	5	5	5	5	5	5	
UP-33	IR81423-B-B-111-3	1	9	9	7	7	7	7	5
		2	5	5	5			5	
		3	5	5	5	5	5	5	

Table B1 (continued)

Accession Number	Variety	Replication	Score of a seedling					Mode	Final mode
			1	2	3	4	5		
UP-35	IR70181-32-PMI-1-1-4-2	1	5	9	7	7	7	7	7
		2	9	9	9	9	7	9	
		3	9	5	7	7	7	7	
UP-36	IR73000-98-1-2-1	1	5	5	5	7	7	5	5
		2	5	5	5	5	5	5	
		3	5	5	5	5	5	5	
UP-38	IR70175-51-2-1-1-2	1	9	9	9	9	5	9	7
		2	7	5	7			7	
		3	5	7	7			7	
UP-40	IR84179-B-403	1	7	7	7			7	5
		2	5	5	5	5	5	5	
		3	5	5	5			5	
UP-41	IR81423-B-B-152-1	1	9	9	9			9	5
		2	9	7	5	5	5	5	
		3	5	5	5			5	
UP-44	IR74371-54-1-1	1	7	5	5	5	5	5	7
		2	7	7	7	7	5	7	
		3	5	7	7	7	7	7	
UP-45	IR79971-B-338-2-2	1	7	5	5	5	5	5	7
		2	9	7	7	7	5	7	
		3	9	9	7	7	7	7	
UP-47	IR78875-176-B-2-B	1	5	5	5			5	5
		2	7	9	9	9	9	9	
		3	5	5	5	5	5	5	
UP-48	IR71700-247-1-1-2	1	5	5	5			5	5
		2	7	7	7	5	5	7	
		3	5	5	5	5	5	5	
UP-49	IR81025-B-327-3	1	5	5	5	9	9	5	5
		2	5	5	5	5	5	5	
		3	9	5	5	5	5	5	
UP-50	IR78875-207-B-1-B	1	7	9	5	5	5	5	7
		2	7	7	7	9	9	7	
		3	9	7	7	7	5	7	
UP-51	PSL85051-14-2-1-2	1	7	7	9	9	9	9	9
		2	5	5	5			5	
		3	9	9	9	9	9	9	

Table B1 (continued)

Accession Number	Variety	Replication	Score of a seedling					Mode	Final mode
			1	2	3	4	5		
UP-52	CNT86095-42-2-3	1	5	7	9	9	9	9	7
		2	5	7	7	7	9	7	
		3	7	7	7	9	9	7	
UP-53	UNKNOWN UP-53	1	9	7	7	7	7	7	9
		2	7	9	9	9	9	9	
		3	7	7	9	9	9	9	
UP-54	IR13240-108-2-2-3	1	7	7	7	9	9	7	7
		2	5	5	5	7	9	5	
		3	7	7	7	9	9	7	
UP-55	IR5675-81-2-3	1	7	7	9	9	9	9	9
		2	9	9	9			9	
		3	7	7	9	9	9	9	
UP-58	Bue Wa	1	9	5	5	9	9	9	9
		2	9	9	9	9	9	9	
		3	7	9	9	9	9	9	
UP-59	Khao Ta Chee Long2	1	9	9	9	5	5	9	9
		2	9	9	9			9	
		3	9	9	9	9	9	9	
UP-70	Khao Rai Ka Reang	1	5	5	5	9	9	5	5
		2	7	7	9	9	9	9	
		3	5	5	5	5	5	5	
UP-108	2R-40	1	5	5	9	9	9	9	9
		2	7	7	7	9	9	7	
		3	5	9	9			9	
UP-110	2R-43	1	7	9	9	9	9	9	9
		2	5	9	9			9	
		3	9	9	9			9	
UP-135	Khao Pang Kam Noi	1	9	9	9			9	9
		2	9	9	9			9	
		3	9	9	9			9	
UP-140	Ja Su	1	9	7	9			9	5
		2	5	5	5	7	7	5	
		3	5	5	5	5	5	5	
UP-143	Lai San	1	9	9	9			9	9
		2	9	9	9	9	9	9	
		3	9	9	9			9	

Table B1 (continued)

Accession Number	Variety	Replication	Score of a seedling					Mode	Final mode
			1	2	3	4	5		
UP-145	La Ngae	1	9	9	9	9	9	9	9
		2	9	9	9			9	
		3	9	9	9			9	
UP-148	Bue Ko	1	9	9	9	9	9	9	9
		2	7	7	7	7	7	7	
		3	7	7	9	9	9	9	
UP-151	Bue Sor Mee	1	9	9	9	9	9	9	9
		2	9	9	9	9	9	9	
		3	9	9	9			9	
UP-160	Lai Chan	1	9	9	9	9	9	9	9
		2	9	9	9	9	9	9	
		3	7	9	9	9	9	9	
UP-163	Ka Reang Kao	1	7	9	9	9	9	9	9
		2	9	9	9			9	
		3	7	7	9	9	9	9	
UP-208	Bue Por Lor Lay Ko	1	7	9	9	9	9	9	9
		2	9	9	9	9	9	9	
		3	9	9	9	9	9	9	
UP-215	Khao Lai Pang Kwam Noi	1	7	7	7	9	9	7	9
		2	7	7	9	9	9	9	
		3	5	7	9	9	9	9	
UP-244	Khao Maled Yao Hnong Keaw	1	9	9	9	9	9	9	9
		2	9	9	9	9	9	9	
		3	9	9	9	9	9	9	
UP-247	Khao Mun Huey San Nai	1	9	9	9			9	9
		2	9	9	9			9	
		3	7	9	9			9	
UP-248	Ja Nor Toy Huey San Nai	1	9	9	9	9	9	9	9
		2	9	9	9			9	
		3	9	9	5			9	
UP-251	Leuang Rai Pui Ngam	1	9	9	9	9	9	9	9
		2	9	9	9			9	
		3	9	9	9			9	
UP-252	Lai San Pung Yam	1	9	9	9			9	9
		2	9	9	9			9	
		3	9	9	9	7	7	9	

Table B1 (continued)

Accession Number	Variety	Replication	Score of a seedling					Mode	Final mode
			1	2	3	4	5		
UP-258	Khao Lang Hua Lan	1	9	9	9	7	7	9	9
		2	9	9	9	9	9	9	
		3	9	9	9	9	9	9	
UP-271	Khao Fueng Kham	1	9	9	9	9	9	9	9
		2	9	9	9	9	9	9	
		3	9	9	9	9	9	9	
UP-290	Pi Ai Par Ngo Mae Rad Noi	1	7	9	9			9	9
		2	9	9	9	9	9	9	
		3	9	9	9	9	7	9	
UP-291	Ja No Mei	1	9	9	9	9	9	9	7
		2	5	5	7	7	7	7	
		3	5	7	7	7	9	7	
UP-292	Nai Jong Hwa	1	7	9	9	9	9	9	9
		2	9	9	9			9	
		3	9	9	9			9	
UP-293	Beu Chi	1	7	7	7	7	7	7	7
		2	5	5	5	9	9	5	
		3	7	7	7	9	9	7	
UP-295	Jao Ho	1	9	9	9			9	9
		2	9	9	9			9	
		3	9	9	9			9	
UP-298	IR78875-15-B-3-B	1	5	5	5	5	5	5	5
		2	5	5	5	7	7	5	
		3	5	5	5	5	5	5	
UP-299	IR81063-B-94-U3-4	1	5	5	5	5	9	5	5
		2	5	5	5	5	5	5	
		3	5	5	5			5	
UP-302	Heng Wa Ra Nong	1	7	9	9	9	9	9	9
		2	7	7	9	9	9	9	
		3	7	9	9	9	9	9	
UP-305	Kaset Poo	1	7	7	5	5	5	5	9
		2	9	9	9	9	9	9	
		3	9	9	9	9	9	9	
UP-308	2R-10	1	7	7	7	9	9	7	9
		2	5	9	9	9	9	9	
		3	7	9	9	9	9	9	

Table B1 (continued)

Accession Number	Variety	Replication	Score of a seedling					Mode	Final mode
			1	2	3	4	5		
UP-313	Tee Hmee Jar	1	9	9	9	9	9	9	9
		2	9	9	9	9	9	9	
		3	9	9	9	9	9	9	
UP-314	Lai Nok Khum	1	9	9	9			9	9
		2	7	9	9			9	
		3	7	9	9			9	
UP-315	Huey Lang	1	5	9	9	9	9	9	9
		2	7	7	9	9	9	9	
		3	9	9	9	9	9	9	
UP-317	Beu Hmeu Mae La	1	9	9	9			9	9
		2	7	9	9	9	9	9	
		3	7	9	9			9	
UP-318	Beu Su Mae Rad Par Kae	1	9	9	9			9	9
		2	9	9	9			9	
		3	9	9	9			9	
UP-319	Fueng Kaum Pang Kwam Noi	1	9	9	9	9	9	9	9
		2	9	9	9	9	9	9	
		3	9	9	9	9	9	9	
UP-320	Beu Hmeu Po Mae Kwang Noi	1	7	9	9	9	9	9	9
		2	5	7	9	9	9	9	
		3	9	9	9	9	9	9	
UP-321	Beu Por Mae Pang	1	9	9	9			9	9
		2	9	9	9			9	
		3	9	9	9			9	
UP-322	Beu Nor Pae Sob Kong	1	9	7	9	9	9	9	7
		2	5	7	7	7	7	7	
		3	7	7	7	7	9	7	
UP-323	Khao Jao Doi Mae Ou Kor	1	7	9	9	9	9	9	9
		2	9	9	9	9	9	9	
		3	7	7	7	7	7	7	
UP-324	Nieow Jak Pa Mar	1	5	7	9	9	9	9	9
		2	9	9	9	9	9	9	
		3	7	7	7	9	9	7	
UP-325	Ja Nor Mei Ae Ko	1	7	7	7	7	7	7	9
		2	9	9	9			9	
		3	7	9	9	9	9	9	

Table B1 (continued)

Accession Number	Variety	Replication	Score of a seedling					Mode	Final mode
			1	2	3	4	5		
UP-326	Beu Ku Pa Tong	1	5	5	5	7	9	5	9
		2	9	9	9	9	9	9	
		3	9	9	9	9	9	9	
UP-327	Kang Mo Ba	1	7	9	9	9	9	9	9
		2	7	7	9	9	9	9	
		3	9	9	9	9	9	9	
UP-331	Leuang Tum	1	7	7	7	7	9	7	7
		2	7	7	7	9	9	7	
		3	7	7	9	9	9	9	
UP-334	Prae Kao Chue	1	7	7	9	9	9	9	9
		2	9	9	9	9	9	9	
		3	9	9	9			9	
UP-335	Khao Nieow Hom Lee Sor	1	5	9	9			9	9
		2	9	9	9	9	9	9	
		3	9	9	9			9	
UP-340	CPAC08016	1	7	7	9	9	9	9	9
		2	9	9	9	9	9	9	
		3	5	7	7	7	9	7	
UP-342	CPAC08020	1	7	9	9	9	9	9	9
		2	9	9	9	9	9	9	
		3	9	9	9	9	9	9	
UP-349	CPAC1008	1	5	7	7	7	7	7	9
		2	9	9	9	9	9	9	
		3	7	9	9	9	9	9	
UP-353	Kor Dang	1	3	3	3			3	7
		2	7	7	7			7	
		3	7	7	7			7	
UP-354	Sew Dang	1	3	7	7			7	9
		2	5	9	9			9	
		3	7	9	9	9	9	9	
UP-357	Ber Sue Ha	1	5	5	7	7	7	7	7
		2	5	7	7	7	7	7	
		3	7	7	7	3	3	7	
UP-364	Beu So	1	5	5	5	5	5	5	9
		2	7	7	9	9	9	9	
		3	7	9	9	9	9	9	

Table B1 (continued)

Accession Number	Variety	Replication	Score of a seedling					Mode	Final mode
			1	2	3	4	5		
UP-367	Khao Man	1	5	5	5			5	5
		2	5	5	7	7	7	7	
		3	7	5	5	5	5	5	
UP-371	Beu Sor La So Som Kong	1	9	9	9	9	9	9	9
		2	9	9	9	9	9	9	
		3	9	9	9			9	
UP-376	Bee Ai Kor Kae	1	3	3	3			3	7
		2	7	7	7	7	7	7	
		3	7	7	7	9	9	7	
UP-389	Ba Dai Koi Ngam	1	3	3	3	3	3	3	3
		2	5	5	5	5	5	5	
		3	5	3	3	3	3	3	
UP-415	Pi Ai Su Mae Pang1	1	9	9	9	9	9	9	9
		2	9	9	9			9	
		3	3	9	9			9	
UP-422	Khao Sang Loi	1	9	9	9			9	9
		2	5	5	7	7	7	7	
		3	5	9	9	9	9	9	
UP-424	Sue No Na	1	9	9	9			9	9
		2	5	9	9			9	
		3	9	9	9			9	
UP-429	Or Sae Nai Huey San Nai	1	5	5	5			5	5
		2	5	5	5	5	5	5	
		3	3	3	3	3	3	3	
UP-436	Khaokeo-4	1	5	9	9	9	9	9	9
		2	7	9	9			9	
		3	9	9	9			9	
UP-463	Khao Hwaw	1	5	7	7	7	7	7	7
		2	7	9	9	9	5	9	
		3	7	7	7	9	9	7	
UP-465	Beu Po Lo Hnong Kang	1	5	7	7			7	7
		2	3	3	3	5	5	3	
		3	3	7	7	7	7	7	
UP-467	Pi Ai Kor	1	3	3	3	3	3	3	3
		2	3	3	3	3	3	3	
		3	5	5	5	5	5	5	

Table B1 (continued)

Accession Number	Variety	Replication	Score of a seedling					Mode	Final mode
			1	2	3	4	5		
UP-469	Khao Nieow Kla	1	3	3	3			3	9
		2	9	9	9			9	
		3	9	9	9			9	
UP-471	Seu No Nu	1	3	3	3	3	3	3	3
		2	3	3	3	3	3	3	
		3	5	3	3	3	3	3	
UP-492	Khao Rai Mae Hong Son	1	5	5	5	7	7	5	5
		2	5	9	9	9	9	9	
		3	5	5	5	5	5	5	