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THESIS

IDENTIFICATION OF MOLECULAR MARKERS LINKED TO BROWN PLANTHOPPER RESISTANCE IN RICE (*Oryza sativa* L.)

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The brown planthopper (BPH), *Nilaparvata lugens* stål is one of the most serious insect pest in rice production worldwide. In this study, the BPH resistance genes in an indica cultivar 'Abhaya' were studied using the backcross introgressed lines of Thai Jasmine Rice 'KDML105'. KDML105 has been well known for its aromatic and excellent cooking quality. However, it is very susceptible to BPH. Abhaya is an elite lowland rice cultivar developed for gall midge resistance in India. This cultivar showed a broad spectrum of resistance to BPH populations found in Thailand. BPH resistance genes were identified using 400 BC_4F_1 individuals derived from a cross between Abhaya and KDML105. Two local BPH populations collected from Central and Northeast of Thailand were used to evaluate the BPH resistance in the 400 BC_4F_2 derived from the BC_4F_1 individual. Twenty resistance lines and twenty susceptible lines were selected based on phenotypic performance of BPH resistance to construct two DNA pools. Through bulk segregant analysis, four AFLP fragments were co-segregated with the BPH resistance. Linkage analysis revealed that these fragments were localized on rice chromosomes 6, 10 and 12. These map locations were in the same genomic regions where major BPH resistance genes or quantitative resistance were previously reported. These results indicated that multiple BPH resistance genes play a major role for BPH resistance in Abhaya. These genes may be a useful BPH resistance resource for rice breeding programs.

Student's signature

Thesis Advisor's signature

26 / 10 / 05

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Jirapong Jairin

October 2005

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INTRODUCTION

Rice (*Oryza sativa* L.) is a main staple food crop for nearly half of the world's population. It is an important source of carbohydrate for Thais. Economically, rice is among the top three export commodities in Thailand. Thailand produces 18-20 million tons of rough rice on 10.4 million hectares, annually. Rice production comes from northeast, north and central parts of Thailand. An average grain yield is below the world and Asia averages (IRRI, 1991). The widespread of insect pests is one of the main constraints limiting rice yields. Among them, brown planthoppers (BPH), *Nilaparvata lugens* (Stål), is considered to be the most devastating insect in Thailand especially in the irrigated lowland rice in the central and the lower part of northern region. BPH has caused extensive damage to rice crop in Thailand since 1974. In 1990 BPH and ragged stunt virus caused about 150,000 hectares of damage to rice crop resulting in yield reduction of about 1 million tons (Sindhusake, 1990). Again, it caused severe yield loss in some part of central and northern region in 1998 (Sep-Nov).

Four BPH biotypes are known. Biotypes 1 and 2 are widely distributed in Southeast Asia, biotype 3 is a laboratory biotype produced in Philippines and biotype 4 occurs in Indian subcontinent. Excessive utilization of insecticides and mono-culture of a single resistant variety is the main cause leading to an outbreak of BPH and a quick regeneration of new biotypes (Alam and Cohen, 1998b; Cohen et al., 1997).

Large efforts have been made to discover major of BPH resistance genes from various sources. At present, more than twenty major BPH resistance genes and 20 QTLs have been reported. Twelve major BPH resistance genes were identified and mapped in *indica* rice cultivars and wild relatives, *Oryza australiensis*, *O. officinalis, O. latifolia* and *O. eichingeri* (Guoqing et al., 2000; Hirabayashi and Ogawa, 1995;

Huang et al., 1997; Huang et al., 2001; Ikeda and Kaneda, 1981; Ishii et al., 1994; Jeon et al., 1999; Kawaguchi et al., 2001; Mei et al., 1996; Murai et al., 2001; Murata et al., 1997, 1998, 2000; Multani et al., 2003; Renganayaki et al., 2002; Wang et al., 2001; Yang et al., 2002). Although four of these BPH resistance genes, *Bph1, bph2, Bph9* and *Bph10* conferred resistance to different biotype, these genes were found to localize on rice chromosome 12 (Hirabayashi and Ogawa, 1995; Huang et al., 1997; Ishii et al., 1994; Jeon et al., 1999; Murai et al., 2001; Murata et al., 1997; Murata et al., 2000). The dominant *Bph1* gene was found to be closely linked with six DNA markers, RG463 and *Sdh-1,* RRD7, RG457, C185 and XNpb248 in three rice cultivars including IR64, Gayabyeo and IR28 (Hirabayashi and Ogawa, 1995; Ikeda and Kaneda, 1981; Jeon et al., 1999). The recessive *bph2* gene was linked to the *Bph1*. Subsequently the sequence tag site (STS) marker, which showed completed co-segregation with *bph2,* was found (Murai et al., 2001). The dominant *Bph3* gene was linked to the recessive *bph4* gene, which has been mapped on rice chromosome 6 (Kawaguchi et al., 2001). A new dominant resistance gene has been mapped on rice chromosome 9 in Sanguizhan (Mei et al., 1996).

Although improving durable and broad-spectrum resistance is necessary, little is known about the genetic control of durable or broad-spectrum BPH resistance. Quantitative trait loci (QTLs) for BPH resistance is considered an importance role to improved the durable and broad-spectrum resistance variety (Alam and Cohen, 1998a, b). Recently, several QTLs for BPH resistance were identified on the 12 rice chromosomes (Alam and Cohen, 1998a; Xu et al., 2002; Liu et al., 2001; Su et al., 2002). Improving the durable and broad-spectrum resistance variety in rice is, therefore, necessary particularly the introduction of resistance genes into the susceptible aromatic and good grain quality varieties. There is thus an urgent need to identify and introduce new genes for resistance to BPH into rice varieties from a divergent source.

OBJECTIVES

1. To identify and evaluate donors with specific as well as broad-spectrum resistance to BPH.

2. To develop molecular marker tightly linked to brown planthopper resistant gene for marker-assisted selection technology.

3. To incorporate rice brown planthopper resistant genes into Thai aromatic rice cultivar, KDML105.

LITERATURE REVIEW

Taxonomy of Brown Planthopper (BPH)

Nomenclature of the brown planthopper (BPH), *Nilaparvata lugens* Stål:

Order	Homoptera
Family	Delphacidae
Genus	*Nilaparvata*

The synonyms of the BPH were summarized (Mochida and Okada, 1979) as follows:

1854	*Delphax lugens* Stål
1863	*Delphax sordescens* Motschulsky
1903	*Liburnia sordescens* Melichar
1906	*Delphax oryzae* Matsumura
1906	*Kalpa aculeata* Distant
1906	*Nilaparvata greeni* Distant
1907	*Delphax ordovix* Kirkaldy
1907	*Delphax parysatis* Kirkaldy
1907	*Dicranotropis anderida* Kirkaldy
1907	*Delphacodes anderida* Muir
1917	*Delphacodes parysatis* Muir
1917	*Liburnia oryzae* Matsumura
1932	*Nilaparvata oryzae* Esaki et Hashimoto
1935	*Hikona formosana* Matsomura
1945	*Nilaparvata sordescens* Kuwayama

The brown planthopper is a phloem-feeding insect (Sogawa, 1982). The characteristics of BPH are yellowish brown or dark brown. Length of macropterous male 2.3-2.4 mm (3.8-4.2 mm, including fore wing), female 2.8-3.2 mm (4.4-4.8 mm, including fore wing), brachyterous male 2.0-3.1 mm, female 2.7-3.5 mm, post-tibial spur with 30-36 teeth (Okada, 1977). BPH is belonging to family Delphacidae, the

largest (more than 1,100 species) among 15 families of infra-order, Fulgoromorpha. Variations of the macropterous fore wings or teguments and the spur or calcar at the apex of the hind tibia are the genus *Nilaparvata.*

Biology of BPH

Egg Female of BPH make a slit in plant tissue with their saw-like ovipositor and then insert eggs. The eggs are usually laid as egg-groups (Figure 1), often in rows in the tissue of the lower part of the rice plant, mainly in sheaths but also in leaf blades. Eggs are covered with a dome-shaped, egg plug secreted by the female. Only the tips of eggs protrude from the plant surface (Henrichs, 1994). The number and ovipositor sites depend largely on the development of stage of the rice plant. When the adult population is high, eggs are found in the upper parts of rice plant. The egg-laying sites appear as brownish streaks. Red eye spots appear at one end of the egg before hatching (Henrichs, 1994). The egg stage is about 7 to 10 days in the tropics. It is also depend on the temperature. The duration of egg stage is found to be 26.7, 15.2, 8.2, 7.9, and 8.5 days at 15, 20, 25, 28, and 29°C constant, respectively. The shortest development time was at about 28°C (Mochida and Okada, 1979). The hatchability and survival rate are the highest around 25°C (Henrichs, 1994). The egg usually will not hatch at temperature greater than 33°C (Pathak, 1977).

Nymph The newly hatched nymph (Figure 1) is cottony white and turns purple brown within an hour (Henrichs, 1994) and the length of the nymphs around 0.6 mm upon hatching (Feakin, 1974). BPH has five nymphal stadia, which are distinguished by shape of the mesonotum and metanotum, and body size (Figure 2). The nymphal stage is about 10-15 days. The development of nymph stage is about 18.2, 13.2, 12.6, 13.1, 17.0, and 18.2 days of a constant temperature of 20, 25, 29, 31, 37, and 35°C, respectively (Mochida and Okada, 1979).

Adult BPH is dimorphic with fully winged ‘macropterous’ and truncate-winged ‘brachypterous’ forms (Figure 3). The macropterous are potentially migrants for colonizing new fields (Henrichs, 1994) when the food is limited or some other

environmental factor is unsuitable (Kisimoto, 1965; Pathak, 1968). There are many factors, which have been suggested to be responsible for wing morphism in the brown planthopper. Crowding during the nymph stage, reduction in the quality and quantity of insect food (Kisimoto, 1965; Saxena et al., 1981), short day length and low temperature (Johno, 1963) induce the production of macropterous form. Recently, there are various studies described the control of wing development and metamorphosis by juvenile hormone (Ayoade et al., 1999; Bertuso et al., 2002) The adult stage persisted for 16-17 days (Mochida and Okada, 1979).

Temperature is a critical factor in the life activities of BPH. The temperature conditions in the nymphal stage affect the longevity and oviposition of adult hoppers (Henrichs, 1994). Adult longevity is curtailed as temperature rises in a range between 20 and 33°C. The number of eggs laid by a female is highly correlated to her life span and her oviposition period. The preoviposition period in macropterous female become shorter as the temperature rises in the range between 20 and 33°C while in brachypterous females remains unchanged. The temperature range for normal behavior is 9 to 30°C in the macropterous male and 10 to 32°C in the macropterous female (Mochida and Okada, 1979).

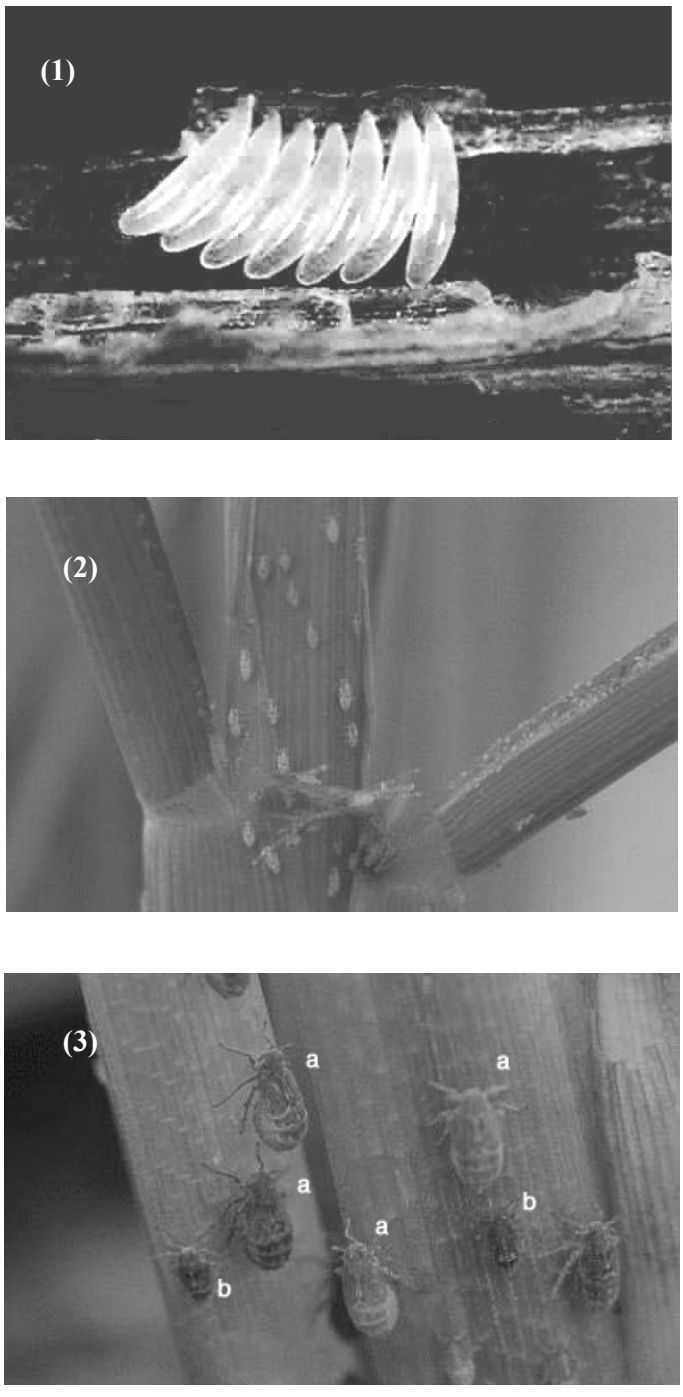

Figure 1 Eggs (1), nymphs (2) and adults (3) of brown planthopper.
a = adult female
b = adult male

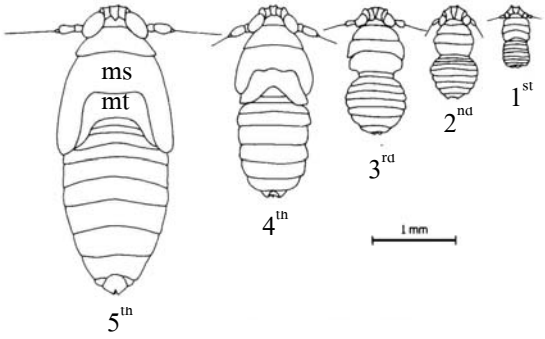

Figure 2 First to fifth instars of brown planthopper.
ms = mesonotum
mt = metanotum

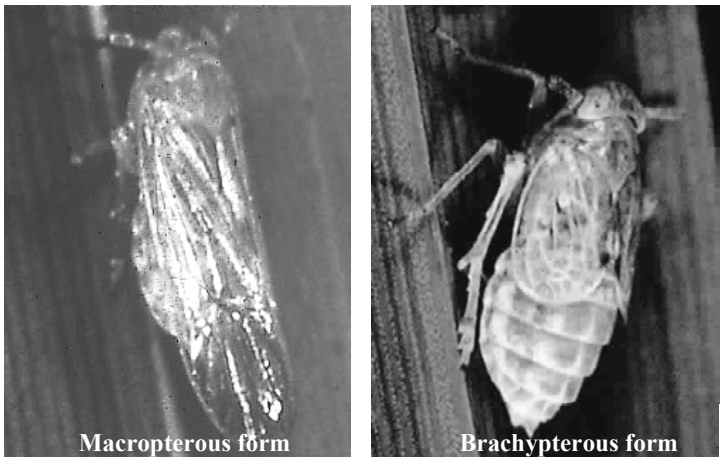

Figure 3 Two wing forms of adult female of brown planthopper, fully winged 'macropterous' and truncate-winged 'brachypterous' forms.

Distribution of BPH

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

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

Australia: Queensland

The South Pacific

Island: Fiji, Solomon Islands

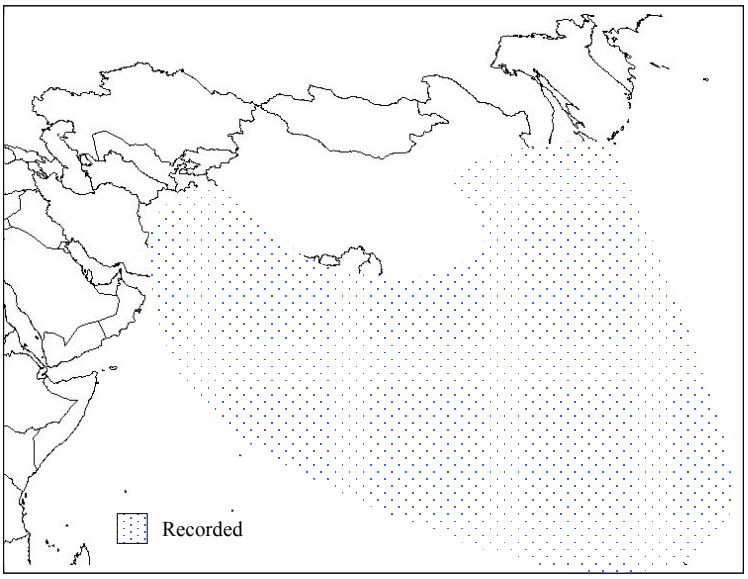

Figure 4 Distribution of brown planthopper in Asia and Australia.

Feeding Physiology of BPH

The BPH has mouth parts specialized for intake plant sap. The most conspicuous elements are stylets, which serve as a piercing and sucking organ. Stylets that are about 650-700 μm long consist of an outer pair of mandibular and an inner pair of maxillary stylets (Sogawa, 1973). The maxillary stylets are interlocked to form two canals, the dorsal and the ventral canal. The dorsal canal functions as the sucking canal and communicates with the sucking pump via the pharyngeal duct. The ventral canal is the salivary canal. Saliva is forced out by the action of the salivary pump

through this canal (Sogawa, 1982). A coagulable saliva is secreted during stylet penetration to form a stylet sheath or salivary sheath. The stylet sheaths seem to play a major role in the bundling of the stylets protruding beyond the labial tip so as to enable them to function as a piercing and sucking organ. They support for stylet penetration, by sealing them into the sucking sites of the rice plant tissues (Sogawa, 1982).

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

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

Feeding Damage

Both the nymphs and adults of BPH feed on the leaf sheaths at the basal portion of the rice plants. In most cases the BPH severely damages rice plants in the post-flowering stage. The removal of assimilates and reductions in photosynthetic rate of leaves by the BPH feeding have the greatest effect on growth and yield on rice plant (Watanabe and Kitagawa, 2000). The typical sucking damage caused by BPH is commonly referred to as hopperburn (Figure 5). The first symptom of hopperburn injury appears on rice plants as a yellowing of the older leaf blades. It extends progressively to all parts of the plants that are above the ground. In the paddy fields, hopperburn usually appears as a browning of plants in scattered patches. In severe cases the patches spread rapidly on a large scale (Sogawa, 1982). A probable cause of hopperburn damage is the reduction in the rate of translocation of photosynthates to the root system because of the drain of phloem sap and the physiological disruption of active transportation in the phloem by sustained feeding and stylet probing. Disturbance of the physiological activities of the root system enhances leaf senescence causing the accumulation of free amino acids and amides in the leaf blades (Sogawa, 1982).

Wilting symptoms can occur if the amount of energy supplied is less than that required for tissue maintenance (Watanabe and Kitagawa, 2000). The wilting symptom from the infestation is differed from those of plants under drought stress, in which the leaf blades dry up with little loss of green color. The chlorophyll content of the leaf blades of the BPH-infested plant decreased with the reduction in moisture content. The total free amino acid content of chlorotic leaf blades is conversely more than four times that of healthy ones. The concentration of aspartic acid, glutamic acid and valine decreased in the infest plants (Sogawa, 1982).

Figure 5 Wilting symptoms (Hopperburn) at flowering stage from the infestation of the brown planthoppers in the farmer's field at Ubon Ratchathani province, wet season 2001.

Biotype of BPH and Their Development on Resistant Rice Varieties

Insect populations have a wide range of genetic variability that maximizes their fitness in the presence of genetic diversity of host plants. The widespread planting of one rice variety (monocrop) that has been commonplace since the "Green Revolution" has significantly decreased the genetic diversity of rice plants. As a result, some rice insect species have overcome the resistance of certain rice varieties.

A biotype of the BPH is generally referred to as a population, which has a specific ability or inability to infest and survive on rice varieties with specific genes for resistance to BPH (Sogawa, 1981). The first BPH resistance variety was Mudgo, which identified by Pathak et al. (1969). It was found to be resistance to BPH population prevalent in the Southeast Asia but not in the South Asia. Thus, two biotypes of BPH existed before introduction of resistance varieties. Biotype 3 was

developed in the laboratory by rearing the insects on the resistance variety ASD7 that has the *bph2* gene for resistance (Panda and Khush, 1995). The rice varieties that have *bph2* gene were found to be susceptible to the South Asian biotype, called biotype 4 (Khush, 1992) but the varieties which have *Bph3* gene can resistance to this biotype. Therefore, the population that cannot infest any varieties with resistance genes is called biotype 1 while those populations infesting resistance varieties carrying *Bph1* and *bph2* genes were described as biotypes 2 and 3, respectively (Figure 6).

IR26 with the *Bph1* gene for resistance was the first brown planthopper resistant variety released by IRRI in 1974. Within the Philippines, brown planthopper outbreaks were observed in IR26 after 2 to 3 years of commercial cultivation (approximately 6 crops) as the result of a selection of a strain that could feed on IR26 (biotype 2). Resistant varieties released subsequently to IR26 have also succumbed to biotype selection within a few years after released. To cope with the brown planthopper biotype problem several gene deployments strategies have been proposed to increase the stability of insect resistant varieties.

Biotypes of the BPH in Thailand were studied since 1975 (Pongprasert and Weerapat, 1979). According to many studies, the results indicated that the BPH collected from the lower part of Northern and Northeast Thailand were different from biotype 1, 2, 3 and 4 (Phengrat, 2000; Rithmontri et al., 1998; Tripop, 1997). However until then the new biotype in Thailand was not classified and no biotype destination have been given to them.

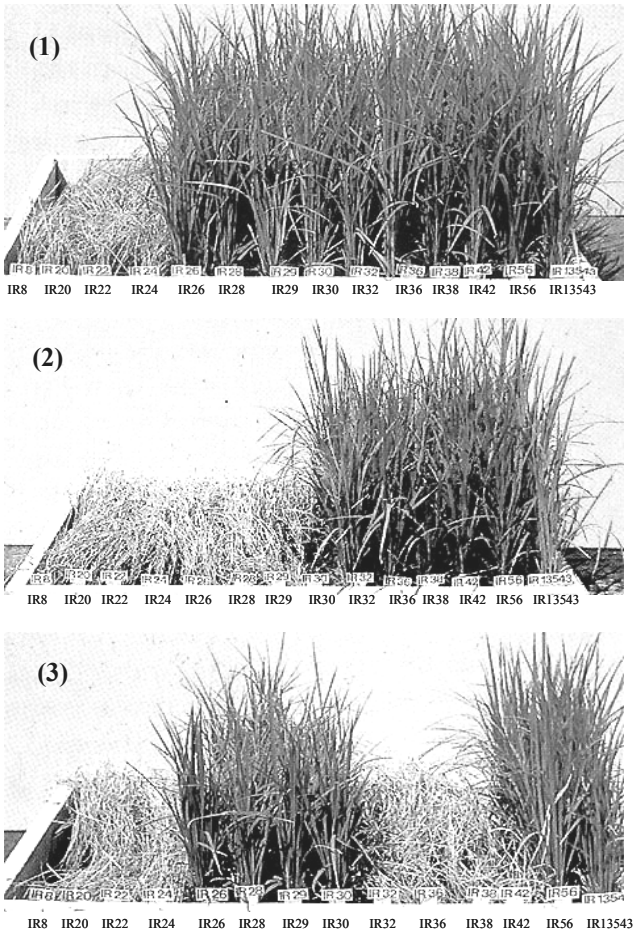

Figure 6 Resistance of rice to different biotypes of brown planthopper
(1) Biotype 1 damages varieties with no major resistance genes
(2) Biotype 2 damages varieties with the *Bph1* gene
(3) Biotype 3 damages varieties with the *bph2* gene

Varietal Resistance of Rice

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

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

Mechanisms of Resistance

The characteristics of resistance must be heritable and controlled by one or more genes, can be measured only by comparison with a susceptible cultivar, determined by analysis of the standard scoring system or insect establishment, and can be modified by the biotic and abiotic environments (Panda and Khush, 1995). The factors that determined the resistance of rice plant to BPH establishment include the presence of allelochemicals, nutritional imbalance and structural barriers.

The mechanisms of BPH resistance must be understood before the degree of resistance among plants can be determined. Painter (1951) defined the mechanism of insect resistance into three types, antixenosis, antibiosis and tolerance.

Antixenosis Antixenosis is the resistance mechanism occurs when the plant deters or reduces the colonization by insects. The plant becomes unsuitable to the insect for feed, oviposition, and shelter (Panda and Khush, 1995). The antixenotic a characteristic of the plant influences the insect's behavior and is due to biophysical or biochemical factors or a combination of both. The biophysical factors such as pubescence and tissue hardness can limit insect mobility. While selecting their hosts, insects respond to various plant stimuli such as absence of such stimuli, or presence of repellents, antifeedant or feeding deterrents are contribute to biochemical factors.

Antibiosis Antibiosis is the resistance mechanism that operates after the insects have colonized and have started utilizing the plant. The antibiotic affects the insect's growth, development, reproduction and survival. The affects may result in a decline in insect size or weight, reduced metabolic processes, increased restlessness, and greater larval or pre-adult mortality (Panda and Khush, 1995).

Tolerance Tolerance is a genetic trait of the plant that protects it against an insect population, which would damage a susceptible host variety. It dose not affect the rate of population increase but raises the threshold level and is independent of the effect an the insect (Panda and Khush, 1995)

Genetic of Resistance to BPH

Genetic Analysis for Major Gene Resistance Inheritance of resistance to BPH has been investigated since 1968 (Khush, 1979). Four resistance varieties, Mudgo, ASD7, CO22 and MTU15, were initially analyzed. F_2 populations from the crosses of susceptible TN1 with resistance varieties, Mudgo, MTU15 and CO22, segregated into a ratio 3 resistances: 1 susceptible, indicating that three varieties have a dominant gene for resistance to BPH. The F_2 population from the cross TN1 x

ASD7 segregated into 1 resistance: 3 susceptible, indicating that ASD7 has a recessive gene for resistance (Athwal et al., 1971). The single dominant gene in Mudgo, MTU15 and CO22 was at the same locus. This locus was designated as *Bph1*. The resistance in ASD7 is controlled by a single recessive gene, designated as *bph2* (Khush, 1979). No recombination between *Bph1* and *bph2* was observed. It was indicated that these two genes are closely linked (Athwal et al., 1971).

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

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

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

new gene, *Bph9*, has been found in Kaharamana, Pokkali, and Balamawee (Nemoto et al., 1989).

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

Table 1 Sources of resistance genes for brown planthopper

Gene	Variety	Reference
Bph1	Mudgo, MTU15, CO22	Athwal et al., 1971
	MGL2	Athwal and Pathak ,1972
	$IR747B_2$-6	Martinez and Khush, 1974
	Tibiriwewa, Balamawee, CO10, Heenakkulama, MTU 9, Sinnakayam, SLO12, Sudhubalawee, Sudurvi 305	Lakshminarayana and Khush, 1977
	Asdaragahawewa	Ikeda and Kaneda, 1983
	Balamawee	Ikeda and Kaneda, 1986
bph2	ASD7	Athwal et al., 1971
	PTB18	Athwal and Pathak, 1972
	H 105, IR1154-243	Martinez and Khush, 1974
	Anbaw C7, ASD9, Dilwee 328, Hathiel, Kosatawee, Madayal, Mahadikwee, Malkora, M.I.329, Murungakayan302, Ovarkaruppan, Palasithari 601, PK-1, Seruvellai, Sinna Karuppan, Vellailangayan	Lakshminarayana and Khush, 1977
	PTB21, PTB34, H5, IR9-60, Kaosen-Yu 12	Chang et al., 1971
	PTB33	Ikeda and Kaneda, 1983
	Gatabyeo	Jeon et al., 1999
Bph3	Rathu Heenati	Lakshminarayana and Khush, 1977
	PTB19, Gangala, Horana Mawee, Kuruhondarawala, Mudu Kiriyal, Hondarawala 378	Sidhu and Khush, 1978
	PTB21	Ikeda, 1985
	PTB33	Lakshminarayana and Khush, 1977; Angeles et al., 1986
bph4	Babawee	Lakshminarayana and Khush, 1977
	Gambada Samba, Heenhoranamawee, Hotel Samba, Kahata Samba, Kulukuruwee, Lekam Samba, Senawee, Sulai, Thirissa, Vellai Illankali	Sidhu and Khush, 1978

Table 1 (Continued)

Gene	Variety	Reference
bph5	ARC10550 Leb Mue Nahng, ARC15872, ARC13788, S61, ARC11367, ARC15694, ARC14342A, ARC15831 (a)	Khush et al., 1985 Kabir and Khush, 1988
Bph6	Swarnalata	Kabir and Khush, 1988
bph7	T12	Kabir and Khush, 1988
bph8	Thai Col.5, Thai Col.11, Chin Saba	Nemoto et al., 1989
Bph9	Kaharamana, Pokkali, Balamawee (70-518)	Nemoto et al., 1989
Bph10	*Oryza australiensis*	Ishii et al., 1994
bph(t)	*Oryza officinalis*	Hirabayashi et al., 2000 Huang et al., 2001
bph(t)	*Oryza officinalis*	Hirabayashi et al., 2000 Huang et al., 2001
Bph(t)	Sanguuizhan	Mei et al., 1996
Bph(t)	*Oryza eichingeri*	Guoqing et al., 2000
Bph(t)	*Oryza latifolia*	Yang et al., 2002

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

Mapping of BPH Resistance The genes for BPH resistance can be located to specific chromosome by different techniques. Ikada and Kaneda (1981) located *Bph3* and *bph4* on rice chromosome 10 through trisomic analysis. In the same way, Ikeda and Kaneda (1983) also located *Bph1* and *bph2* on chromosome 4. But recently, the result from many research studies indicated that *Bph1* and *bph2* were located on chromosome 12 and *Bph3* and *bph4* were located on chromosome 6 using DNA markers (Hirabayashi and Ogawa, 1995; Huang et al., 1997, Jeon et al., 1999; Murata et al., 1998; Kawaguchi et al., 2001).

Huang et al. (1997) was analyzed the BPH resistance gene using a doubled haploid population derived from a cross between IR64 and Azucena. IR64 has proved that has *Bph1* as well as other minor genes (Alam and Cohen, 1998a). RFLP markers were used in their study. They found that the resistance gene, *Bph1*, was located on chromosome 12 near RFLP marker, RG463 and isozyme, *Sdh-1* (Shikimate dehydrogenase). Jeon et al. (1999) have been reported the tagging of *Bph1* in rice variety Gayabyeo using RAPD and RFLP markers. The result showed that RAPD marker RRD7 cosegregated with *Bph1* locus on chromosome 12 and linked with RG457, which linked with resistance gene *Bph10* (Ishii et al., 1994). These two genes might be allelic or tightly linked and should have further study to elucidate the relationship between them (Jeon et al., 1999). Hirabayashi and Ogawa (1995) were also found *Bph1* in IR28 located on chromosome 12 near RFLP markers, C185, XNpb248 and XNpb304-1. The resistance gene *bph2* was reported to be recessive and closely linked to *Bph1*. Murata et al. (1998) reported that *bph2* was mapped at 30.5 cM from the closest RFLP marker, G2140, and was considerable distant about 30 cM from *Bph1*. Murai et al., 2001 found an AFLP marker closely linked to *bph2* (Figure

7) and the marker have been converted into a PCR-based sequence tagged site (STS) marker.

The newly discovered BPH resistance gene called *Bph(t)* derived from *O. eichingeri* was located between RM240 and RM250 on chromosome 2 with a distance of 6.1 and 5.5 cM, respectively (Guoqing et al., 2000). Jeon et al. (2001) was identified a RAPD marker, OPA16, linked to a BPH resistance gene in the introgression line IR54741-3-21-22, which derived from *O. sativa* and *O. officinalis*, using bulked segregant analysis method. This marker was mapped onto rice chromosome 11. Renganayaki et al. (2002) mapped a different resistance gene on chromosome 3 from IR54745-2-21-12-17-6, which was derived from *O. sativa* and *O. officinalis*. Beside, Hirabayashi et al. (2000), Huang et al. (2001) and Wang et al. (2001) were mapped two new recessive genes on rice chromosome 3 and 4 from introgression line IR54742-1-11-17 and B5, of which the resistance gene were derived from *O. officinalis,*. Recently, alien BPH resistance genes from *O. latifolia* and *O. eichingeri* were introgressed to *O. sativa* and were mapped on the short arm of chromosome 4 and chromosome 2, respectively (Guoqing et al., 2001; Multani et al., 2003; Yang et al., 2002) (Figure 7).

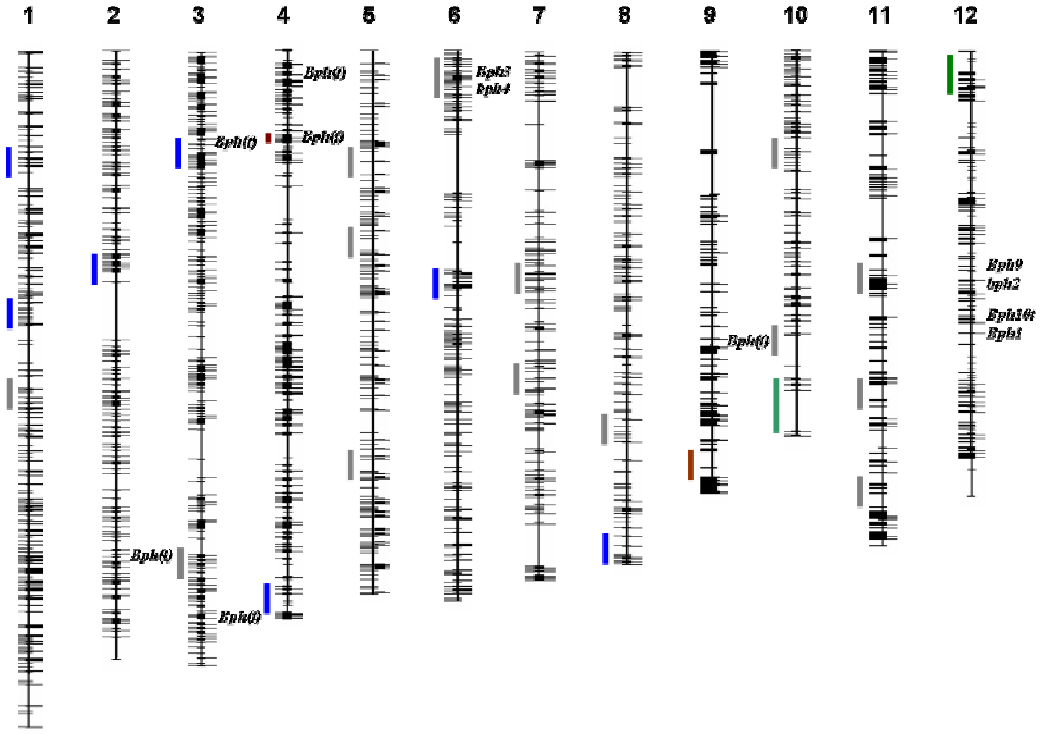

Figure 7 Linkage map of BPH resistance genes, *Bph1* (Huang et al., 1997; Jeon et al., 1999), *bph2* (Murai et al., 2001), *Bph9* (Murata et al., 2000), *Bph10* (Ishii et al., 1994) on chromosome 12, *Bph(t)* on chromosome 2 (Guoqing et al., 2000), *bph4* and *Bph3* on chromosome 6 (Kawaguchi et al., 2001), *bph(t)* on chromosome 3 and *bph(t)* on chromosome 4 (Huang et al., 2001; Hirabayashi et al., 2000; Wang et al., 2001; Yang et al., 2002), and *Bph(t)* on chromosome 9 (Mei et al., 1996). The black vertical bars indicate QTLs associated with BPH resistance (Alam and Cohen, 1998a; Xu et al., 2002; Guoqing et al., 2001; Su et al., 2002).

Table 2 Genes for resistance to the brown planthopper in rice and their reaction to different biotypes

Gene	Chromosomal location	Reaction to indicated biotype			
		1	2	3	4
Bph1	12	R	S	R	S
bph2	12	R	R	S	S
Bph3	6	R	R	R	R
bph4	6	R	R	R	R
bph5	N	S	S	S	R
Bph6	N	S	S	S	R
bph7	N	S	S	S	R
bph8	N	R	R	R	N
Bph9	12	R	R	R	N
Bph10	12	R	R	R	N
bph(t)	3	R	R	R	R
bph(t)	4	R	R	R	R

R, S, and N refer to resistance, susceptible and no information, respectively

Molecular Markers in Plant Breeding

With the advent of molecular marker (DNA markers) technology, tagging and mapping of pest resistant genes provide a unique opportunity to monitor alien gene introgression and to tag genes governing traits of economic importance.

Amplified Fragment Length Polymorphism (AFLP) AFLP is molecular marker obtained by selective PCR amplification of restriction fragments. This technique was developed by Vos et al. (1995). The technique involves three steps: 1. Restriction enzyme digestion; 2. Ligation of adaptors; 3. Selective amplification of restriction fragments. The selective amplification is based on the recognition of unique nucleotides flanking the restriction site. The principle of selective

amplification can be used to adjust the number of fragments that are amplified in single PCR reaction. This PCR-based technique permits inspection of polymorphism at a large number of loci within a very short period of time and requires very small amounts of DNA (Maheswaran et al., 1997). AFLP is a powerful, reliable, stable and rapid assay with potential application genome mapping, DNA fingerprinting, genetic distance analysis and marker-assisted breeding (Thomas et al., 1995; Vos et al., 1995). The method has been applied successfully on a wide variety of plant species (Cervera et al., 1996; Li et al., 1998; Maheswaran et al., 1997; Nandi et al., 1997; Thomas et al., 1995; Zhu et al., 1998; Zhang and Stommel, 2000).

Simple Sequence Length Polymorphism (SSLP) Microsatellites, or simple sequence repeat (SSR) length polymorphism was first used for mammalian genome mapping (Hamada et al., 1982; Love et al., 1990; Serikawa et al., 1992). Since then, it has been increasingly used in a wide range of genetic plant studies such as genetic mapping, QTL detection of agronomic traits, diversity analysis, gene isolation and marker assisted selection (Akagi et al., 1996; Becker and Heun, 1994; Brunel, 1994; McCouch et al., 1997; McCouch et al., 2000; Panaud et al., 1995; 1996; Senior and Heun, 1993; Taramino et al., 1997). The important attribute of SSR motifs is their high level of allelic diversity that makes them valuable as genetic markers. Microsatellites are made of tandemly repeated nucleotide motifs that can be as one, two, three or four nucleotides (Vegnard, 1989). They are often hypervariable within a number of repeat units and easily detected by PCR using flanking sequences as primers since the flanking sequences are usually conserved across individual or varieties. They are easily detected on high resolution agarrose or polyacrylamide gels, and generally behave as co-dominat markers (McCouch et al., 1997). Up to date, about 2240 microsatellite markers have been mapped on to the rice genome (McCouch et al., 2002).

Application of molecular markers in plant breeding

Bulked segregant analysis (BSA) Bulked segregant analysis was firstly developed by Michelmore et al. (1991). This method involves screening for differences between two pooled DNA samples derived from a segregating population. BSA is a rapid identification of molecular markers linked to any specific genes. The method was not only designed mainly for the mapping of major genes, but also was extended to the analysis of genetically complex traits if the trait is controlled by a few major genes (Chantret et al., 2000; Michelmore et al., 1991). BSA can be applied to identify markers in regions that lack markers, such as gaps in the genetic map or ends of linkage groups (Michelmore et al., 1991). This method has been extensively applied for tagging genes in several crops (Chalmers et al., 1993; Devey et al., 1995; Martin et al., 1991; Michelmore et al., 1991; Monna et al., 1995; Nair et al., 1995; Negi et al., 2000).

Mapping quantitative traits loci QTLs are used to tag resistance genes in number of different crops (Alam and Cohen, 1998a; Huang et al., 2001; Parker et al., 1998; Rouppe van der Voort et al., 1998). Most agronomically important traits are of quantitative character. The traits are typically affected by environmental as well as genetic factors. QTL analysis is based on the relationship between markers and quantitative traits. The relationship of a marker and a quantitative trait locus was analyzed using several methods including one-way ANOVA. The efficiency and reliability of QTL mapping is largely dependent on the reproducibility of the phenotypic evaluation.

MATERIALS AND METHODS

Plant Material

Eleven differential varieties which have different BPH resistance genes, Mudgo (*Bph1*), IR64 (*Bph1*), ASD7 (*bph2*), Rathu Heenati (*Bph3*), Babawee (*bph4*) ARC10550, (*bph5*), Swarnalata (*Bph6*), T12 (*bph7*), Chin Saba (*bph8*), Pokkali (*Bph9*) and IR65482-4-136-2-2 (*Bph10*) were used to identify reaction among various local BPH populations. Abhaya, Rathu Heenati and TN1 were used for feeding rate, fecundity test and BPH adaptation experiments. TN1 and Rathu Heenati were used as susceptible and resistant variety in all experiments.

Four hundred BC_4F_1 individuals were derived from a consecutive backcrossing between KDML105 and Abhaya. A Thai jasmine rice cultivar (KDML105) was used as the recipient and the BPH resistance cultivar from India (Abhaya) was used as the donor. Abhaya is moderately resistant to the BPH while KDML105 is susceptible to BPH. The BC_4F_1 DNAs were used to identify molecular markers tightly linked to BPH resistance. The BC_4F_2 and BC_4F_3, which were used for the phenotypic evaluations of BPH resistance, were derived from self-pollinated of BC_4F_1 and BC_4F_2 respectively.

BPH population

Parental screening: The BPH populations used for parental evaluation in this study were collected from various rice fields in Thailand. Four BPH populations from northeast and central of Thailand: Phisanulok population, Khon Kaen population, Ubon Ratchathani population and Pathum Thani population were collected from the rice fields in Phisanulok (PSL), Khon Kaen (KKN), Ubon Ratchathani (UBN) and Pathum Thani (PTT) provinces, respectively (Figure 8). The four populations were used for the seedbox screening test, feeding rate test and fecundity test. Two BPH populations, UBN and KKN from northeast of Thailand were selected for Abhaya adaptation.

Backcross screening: Two BPH populations, PTT and UBN, were used for evaluation of BPH resistance in the BC_4F_2 and BC_4F_3, respectively. The BPH populations were collected at the rice fields from PTT province located at the Central Thailand in 1997 and from UBN province located at the Northeast of Thailand in 1998. Greenhouse BPH colonies were established from each population and then were maintained on the TN1. Each of the BPH sample population was kept in separately labeled rearing cages (50x50x34 cm).

Evaluation of BPH resistance

Parental screening: Abhaya, KDML105 and ten differential varieties, IR64 (*Bph1*), ASD7 (*bph2*), Rathu Heenati (*Bph3*), Babawee (*bph4*), ARC10550 (*Bph5*), Swarnalata (*Bph6*), T12 (*bph7*), Chin Saba (*bph8*), Pokkali (*Bph9*) and IR65482-4-136-2-2 (*Bph10*), were used for their BPH resistance evaluation against BPH populations derived from different locations. Three biological characteristic tests including a seedbox screening, a feeding rate and an antibiosis for population growth were conducted to measure the reaction of BPH to Abhaya. Subsequently the resistance data was utilized for classification of the BPH populations and of a broad-spectrum of BPH resistance in the areas.

Greenhouse screening by the seedbox screening test (modified from Heinrichs et al., 1985) was used to determine BPH resistance against BPH populations from UBN and PTT. Twenty-five seeds of each entry were sown in a standard wooden seedbox (40x60x10 cm) in 10-cm rows, with a distance 5-cm between the rows of each entry. We used a randomized complete block design with 2 replications for each BPH colony. Six days after sowing, the seedlings were thinned to 20 per row and the seedboxes were placed on a water pan tray, which contained the 5-cm depth of water. At 7-8 days after sowing, the seedlings were infested with second- to third–instar nymphs at the rate of 8-10 per seedling. When all plants of susceptible check variety, KDML105 or TN1, were dead, approximately 7-10 days after infestation, the entries were graded on a scale of 1-9 (Table 3). The screening for BPH population from PTT

and UBN were conducted at Pathum Thani Rice Research Center (PTRC) and at Ubon Ratchathani Rice Research Center (URRC), respectively.

Antibiosis on feeding rate method was conducted to measure honeydew excretion of BPH populations from KKN, UBN, PSL, and PTT. Only one tiller from each hill was selected for a bromocresol green-treated filter paper in a plastic cup, with minor modification of Heinrichs et al. (1985). Plants were infested with 5 newly emerged adult females of BPH. The feeding rate was recorded after 24 hr, at the regulated temperature. The feeding rate was measured by honeydew production area on filter paper treated with bromocresol green solution in the plastic cup.

Fecundity of the insect was conducted as described in Cohen et al., 1997 to determine BPH resistance durability of Abhaya against BPH populations from UBN and KKN. To measure the number of progeny produced by a BPH pair, one adult male and female were placed on a potted 25-day-old plant and enclosed in a cylindrical plastic cage (15 cm diameter x 60 cm height) with a nylon mesh top and a side window. There were four replicates of each of test varieties, Rathu Heenati, TN1 and Abhaya. Twenty days after infestation, the insects from each cage were counted.

Backcross screening: The phenotyping experiments were carried out using the seedbox screening method at PTRC and URRC. This method can accommodate a large number of lines to be screened in the greenhouse.

Table 3 The Standard Evaluation System (SES) for the damage on rice by brown planthopper (IRRI, 1988)

Scale of damage	Description	Level of resistance[1/]
1	Very slight damage	R
3	First and 2nd leaves of most plants partially yellowing	MR
5	Pronounced yellowing and stunting or about 10 to 25% of the plant wilting	MS
7	More than half of the plants wilting or dead and remaining plants severely stunted or dying	S
9	All plants dead	HS

[1/] R = Resistance; MR = Moderate Resistance; MS = Moderate Susceptible; S = Susceptible and HS = Highly Susceptible

Table 4 Location of brown planthopper collection and screening sites to evaluate rice varieties for brown planthopper resistance

BPH population	Regional	Screening location
Parental screening:		
- seedbox screening test		
Pathum Thani	Central	Pathum Thani Rice Research Center
Ubon Ratchathani	Northeastern	Ubon Ratchathani Rice Research Center
Khon Kaen	Northeastern	Khon Kaen Rice Experimental Station
Phitsanulok	Northern	Phitsanulok Rice Research Center
- Feeding rate and fecundity test		
Pathum Thani	Central	Ubon Ratchathani Rice Research Center
Ubon Ratchathani	Northeastern	Ubon Ratchathani Rice Research Center
Khon Kaen	Northeastern	Ubon Ratchathani Rice Research Center
Phitsanulok	Northern	Ubon Ratchathani Rice Research Center
Backcross screening:		
- seedbox screening test		
Pathum Thani	Central	Pathum Thani Rice Research Center
Ubon Ratchathani	Northeastern	Ubon Ratchathani Rice Research Center

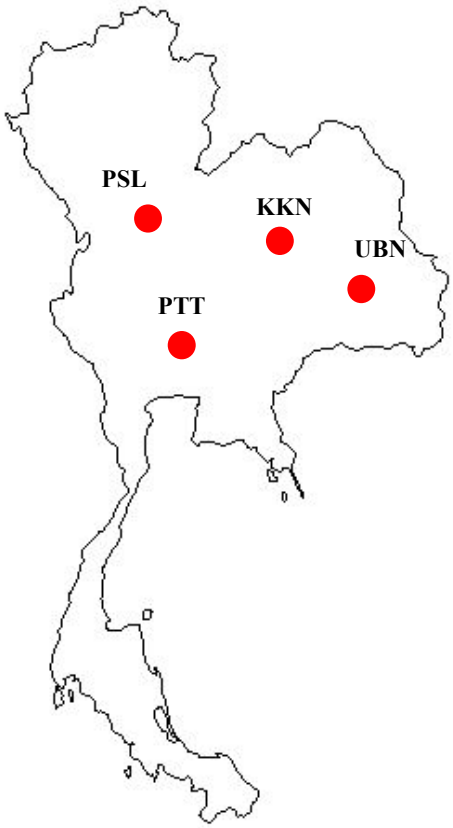

Figure 8 Location of the four areas in Thailand, where the insects were collected. Four populations of BPH: Phisanulok population, Khon Kaen population, Ubon Ratchathani population and Pathum Thani population were collected from the rice fields in Phisanulok (PSL), Khon Kaen (KKN), Ubon Ratchathani (UBN) and Pathum Thani (PTT) provinces, respectively.

DNA Extraction

DNA from each BC_4F_1 individual, along with both parents, was extracted by a modified CTAB method of Murray and Thompson (1980). Freshly collected leaf tissue was ground into fine powder using liquid nitrogen with a mortar and pestle. Twenty ml of 1.5x CTAB extraction buffer, pre-heated at 65°C was added in 40 ml tube containing the ground tissue. The buffer-tissue mixture was gently mixed to ensure even dispersal of the plant material in the buffer and was incubated at 65°C for 1 hour with occasional swirling. The mixture was cooled at room temperature and equal volume of chloroform:isoamyl alcohol (24:1) was added. The tubes were inverted repeatedly but gently and were centrifuged at 3000g for 15 min at room temperature. The upper layer was transferred into a new centrifuge tube and 1 ml of 10x CTAB was added. Equal volume of chloroform:isoamyl alcohol was again added for the second round extraction of carbohydrates and other debris. The mixture was centrifuged with the same condition and the aqueous portion was transferred to 50 ml tube. The DNA was precipitated with 1x CTAB. After precipitation the DNA was hooked and dissolved in high salt TE. Adding 95% ethanol and the DNA was transferred to 1.5-ml microfuge tubes made final precipitation. The DNA was washed with 70% ethanol and was air-dried. After complete drying, two hundred µl of TE was added to dissolve the DNA.

Microsatellite Analysis

Rice microsatellite markers (McCouch, et al., 1997) were used for genotyping the BC_4F_1 population according to the mapped location as revealed by AFLP analysis. Eleven loci on three different chromosomes were surveyed for SSR including two loci on chromosome 4 (RM303 and RM317), four loci on chromosome 10 (RM244, RM216, RM239 and RM184) and five loci on chromosome 12 (RM83, RM101, RM179, RM277 and RM313). BC_4F_2 lines were used for amplified using a hot start at 94°C for 3 min followed by 35 cycles of 94°C denaturing for 30 sec; 55°C annealing for 1 min and 72°C extension for 1 min. PCR products were analyzed on 4.5% denaturing polyacrylamide gels for 1 h at 60 W, followed by silver staining.

Table 5 The sequences of SSR primers which used to surveyed the parents

Marker	Motif	Forward	Reverse
RM303	(GT)7(ATGT)6	GCATGGCCAAATATTAAAGG	GGTTGGAAATAGAAGTTCGGT
RM317	(GC)4(GT)18	CATACTTACCAGTTCACCGCC	CTGGAGAGTGTCAGCTAGTTGA
RM83	(TCA)6(TCT)8	ACTCGATGACAAGTTGAGG	CACCTAGACACGATCGAG
RM101	(CT)37	GTGAATGGTCAAGTGACTTAGGTGGC	ACACAACATGTTCCCTCCCATGC
RM179	(TG)7	CCCCATTAGTCCACTCCACCACC	CCAATCAGCCTCATGCCTCCCC
RM313	(GT)6CA(CG)5-6-(GT)8	TGCTACAAGTGTTCTTCAGGAC	GCTCACCTTTTGTGTTCCAC
RM244	(GA)8	CCGACTGTTCGTCCTTATCA	CTGCTCTCGGGTGAACGT
RM216	(GA)18	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA
RM239	(GA)5	TACAAAATGCTGGGTACCCC	ACATATGGGACCCACCTGTC
RM184	(CA)7	ATCCCATTCGCCAAAACCGGCC	TGACACTTGGAGAGCGGTGTGG

AFLP analysis

The AFLP procedure was performed according to Vos et al. (1995) with minor modification. DNA was cut with two restriction enzymes, a 6-base cutter (*Eco*RI) and a 4-base cutter (*Tru*9I), and double-stranded (ds) adapters were ligated to the ends of the DNA fragments to create amplicons. The sequence of the adjacent restriction site and the adapters served as primer-binding sites for subsequence amplification of the restriction fragments (Vos et al., 1995). Selective nucleotides were added to the 3' ends of the PCR primers, which resulted in their priming only a subset of the restriction sites. Where the nucleotides flanking the restriction site matched the selective nucleotides, restriction fragments were amplified and separating the fragment on the denaturing polyacrylamide gels.

DNA digestion and ligation of adapters for AFLP analysis To digest the genomic DNA, two different enzymes, *Eco*RI and *Tru*9I, were chosen. Genomic DNA (200 ng) was incubated over night at 37 °C with10 microliters of a solution

containing 12 units of *Eco*RI and 10 units of *Tru*9I, 0.3 µl of T4 ligase, 2.2 µl of T4 ligase buffer (10 µ), 2.2 µl of 0.5 M NaCl, 1.1 µl of 1 mg/m BSA and water to give a 20 total sample. After incubation, the DNA templates were diluted with 180 µl of water to give a 200 µl volume.

*Eco*RI	5' CTCGTAGACTGCGTACC
	5' AATTGGTACGCAGTC
*Tru*9I	5' GACGATGAGTCCTGAG
	5' TACTCAAGGACTCAT

Amplified fragment length polymorphism Pre-amplification was carried out with two primers, *Eco*RI-primer (+1) and *Mse*I-primer (+1) (A-C, A-A, A-G, A-T, C-A, C-C, C-G, C-T, G-A, G-C, G-G, G-T, T-A, T-C, T-G, and T-T). Two hundred and forty different primer combinations were used. Each combination was consisted of one *Eco*RI primer and one *Mse*I primer. All of the primer will have two and three selective nucleotides at the 3' ends. The two and three selective nucleotide primers (Table 6), which were used for amplification including:

Primers are name "+0" when having no selective base, "+1" when having a single selective base, "+2" when having two selective bases, and "+3" for having three selective bases

*Eco*RI-primer	+0 : 5' CTCGTAGACTGCGTACC-3'
	+1 : 5' AGACTGCGTACCAATTC*N*-3'
	+2 : 5' AGACTGCGTACCAATTC*NN*-3'
	+3 : 5' AGACTGCGTACCAATTC*NNN*-3'
*Mse*I-primer	+0: 5' GACGATGAGTCCTGAG-3'
	+1: 5' GATGAGTCCTGAGTAA*N*-3'
	+3: 5' GATGAGTCCTGAGTAA*NNN*-3'

Table 6 Selective *Eco*RI and *Mse*I primers used to screen bulks of BC_4F_1 lines for polymorphisms associated with brown planthopper resistance

***Eco*RI selective primers (5'--3')**	**Primer designation**	***Mse*I selective primers (5'--3')**	**Primer designation**
AGACTGCGTACCAATTC**AAC**	E1	GATGAGTCCTGAGTAA**ACC**	M1
AGACTGCGTACCAATTC**AC**	E2	GATGAGTCCTGAGTAA**ACT**	M2
AGACTGCGTACCAATTC**ACC**	E3	GATGAGTCCTGAGTAA**AGG**	M3
AGACTGCGTACCAATTC**CAG**	E4	GATGAGTCCTGAGTAA**ATG**	M4
AGACTGCGTACCAATTC**CGT**	E5	GATGAGTCCTGAGTAA**CAA**	M5
AGACTGCGTACCAATTC**CT**	E6	GATGAGTCCTGAGTAA**CAC**	M6
AGACTGCGTACCAATTC**GCA**	E7	GATGAGTCCTGAGTAA**CAG**	M7
AGACTGCGTACCAATTC**GTA**	E8	GATGAGTCCTGAGTAA**CAT**	M8
AGACTGCGTACCAATTC**TAC**	E9	GATGAGTCCTGAGTAA**CGT**	M9
AGACTGCGTACCAATTC**TAG**	E10	GATGAGTCCTGAGTAA**CTA**	M10
AGACTGCGTACCAATTC**TCG**	E11	GATGAGTCCTGAGTAA**CTC**	M11
AGACTGCGTACCAATTC**TG**	E12	GATGAGTCCTGAGTAA**CTG**	M12
		GATGAGTCCTGAGTAA**GCC**	M13
		GATGAGTCCTGAGTAA**GCG**	M14
		GATGAGTCCTGAGTAA**GTC**	M15
		GATGAGTCCTGAGTAA**GTG**	M16
		GATGAGTCCTGAGTAA**TAC**	M17
		GATGAGTCCTGAGTAA**TAG**	M18
		GATGAGTCCTGAGTAA**TGA**	M19

Ten microliters PCR reactions were performed containing 5 µl of template-DNA, 2 µl of 1 mM dNTPs, 0.5 µl of *Eco*RI-primer (+1, 50 ng/µl), 0.5 µl of *Mse*I-primer (+1, 50 ng/µl), 0.7 µl of water, 1 µl of 10 µl PCR-buffer, and 0.3 µl of *Taq*-polymerase (1 unit/µl). The pre-amplification reactions were performed for 20 cycles with the follwing cycle profile: a 30-s DNA denaturation step of 94°C, a 1-min annealing step at 56°C, and a 1-min extension step at 72°C. After amplification, the reaction was diluted with 90 µl of water to give a 100 µl volume and stored at 4°C.

The AFLP reactions with primers having two or three selective nucleotides were performed for 34 cycles with the following cycle profile: a 30-s DNA denaturing step at 94°C, a 30-s annealing step, and a 1-min extension step at 72°C. The annealing temperature was 65 °C in the first cycle, subsequently reduced by 1°C per cycle to the next 9 cycles, finally stabilizing at 56°C for the remaining 24 cycles. All amplification reactions were carried out in a PE-9600 thermocycler (Perkin Elmer Corp., Norwalk, Conn., USA).

Denaturing polyacrylamide gel analysis and detection of AFLP band Ten microliters of AFLP reaction products were mixed with 5 µl of formamide dye (98% formamide, 10 mM EDTA pH 8.0, and with bromophenol bule and xylene cyanol as tracking dyes). The mixtures were denatured for 3-min at 94 °C, and then quickly cooled on ice. Each sample (2.5 µl) was loaded on a 5% denaturing polyacrylamide gel. The gel matrix was prepared using 5% acrylamide, methylene bisacrylamide, 7.5 M Urea in 50 mM Tris/50mM Boric acid/1mM EDTA. To 50 ml of gel solution 300 µl of 10% ammonium persulfate (APS) and 80 µl of TEMED were added. Running buffer consisted of 100 mM Tris/100mM Boric acid/2 mM EDTA. Electrophoresis was performed at a constant power, 70 W, for 2 h. after electrophoresis; gels were visualized using silver straining protocol. The silver straining was performed at room temperature. The straining solution consisted of 2L of d.i. water, 2 g silver nitrate and 3 ml of 37% formaldehyde and developer solution was consisted of 2L of chilled d.i. water, 60 g of sodium carbonate, 3 ml of 37% formaldehyde and 4 mg of sodium thisulfate.

Bulked Segregant Analysis

Bulked segregant analysis (Michelmore et al., 1991) was used in screening markers linked to BPH resistance and identifying DNA fragments co-segregated with BPH resistant phenotypes. DNA was extracted and bulked from twenty-BPH resistance (R) and twenty-BPH susceptible (S) of the BC_4F_1 lines. The BC_4 lines were selected based on the BPH screening experiments results. Two DNA pools were mixed by the equal amounts of total genomic DNA of the R and S lines. The DNA pools and parental DNAs were genotyped using 11 simple sequence repeated (SSR) markers and 138 *Eco*RI/*Tru*9I primer combinations of amplified fragment length polymorphism (AFLP). The SSR markers were assayed as described by McCouch et al. (1997). Eleven SSR primers were selected based on the BPH resistance genes linkage data on rice chromosomes 4, 10 and 12. Two markers (RM303 and RM317) were located on chromosome 4. Four makers including RM244, RM216, RM239 and RM184 were located on chromosome 10. The other five markers including RM83, RM101, RM179, RM277 and RM313 were located on chromosome 12. The AFLP procedure was performed as described by Vos et al. (1995). PCR amplified products were fractionated by electrophoresis through 4.5% denaturing polyacrylamide gels for 1 h at 60 W and stained by a sliver staining-kit. Subsequently polymorphisms detected between the parents and the pools were evaluated for the cosegregation of DNA fragments and BPH resistance phenotypes.

Confirmation of co-segregated AFLP fragments with BPH resistance

Forty individuals of BC_4F_1 were used to create pools for the AFLP marker to confirm co-segregation with BPH resistance in the linkage analysis. The AFLP fragments that clearly demonstrated the presence and absence of amplified products between resistance and susceptible pools were selected. The AFLP fragments were tested to determine how tightly linkage with the genes conferring BPH resistance by using the individuals of BC_4F_1. By using the phenotypic data conducted at URRC and PTRC, the effects and the phenotypic variance explained (PVE) by the fragments was determined and analyzed by a simple linear regression analysis.

Localization of AFLP fragments in the linkage map

AFLP fragments tightly linked with BPH resistance genes were mapped using 172 recombinant inbred lines (RIL) derived from a cross between FR13A and CT6241-17-1-5-1 as a reference population (Toojinda et al., 2003). The map location was determined using the JoinMap Version 2.0 (Stam, 1993) and MAPMAKER Version 2.0 (Lander et al., 1987). The linkage map was calculated using a maximum recombination frequency of 0.3 and LOD scores greater than 6.0. Linkage analysis of 7 markers including 3 SSR markers and 4 AFLP fragments was also performed using BC_4F_2 individuals with the JoinMap Version 2.0. The genetic linkage map was constructed based on LOD scores greater than 3.0. Map distances were calculated using Kosambi function (Kosambi, 1944).

Estimation of effects of BPH resistance genes

The SSR markers linked to BPH resistance genes were used to fingerprint the 400 BC_4F_1 individuals to determine the effects of BPH resistance genes. Then simple linear regression analysis was used to estimate the effects and the PVE.

QTL analysis

Seven markers linked to the BPH resistance genes were used to genotype the 400 BC_4F_1 individuals. A single-marker and multiple markers analysis, using the regression-based software STAT-GRAPHICS 2.1 and ANOVA, were used to determine the numbers and effects of QTL and to detect two loci interactions of QTL for BPH resistance.

RESULTS

Evaluation of the donors for Resistance to BPH

Abhaya and the set of differential rice varieties that known resistance genes: Mudgo (*Bph1*), IR64 (*Bph1*), ASD7 (*bph2*), Rathu Heenati (*Bph3*), Babawee (*bph4*) ARC10550 (*bph5*), Swarnalata (*Bph6*), T12 (*bph7*), Chin Saba (*bph8*), Pokkali (*Bph9*) and IR65482-4-136-2-2 (*Bph10*), were assessed with four BPH populations collected from four provinces of Thailand, namely Pathum Thani (PTT), Ubon Ratchathani (UBN), Khon Kaen (KKN), and Phitsanulok (PSL) using three biological characteristic tests, a seedbox screening test, a feeding rate test and an antibiosis for population growth test.

The results from the seedbox screening test showed that the different population groups of BPH vary in the amount of damage incurred upon different rice varieties. This observation indicates that genetic variation exists among BPH populations from different locations. The PTT population was showed capable of damaged virulence to *bph2, bph4, bph5, bph8, Bph9* and *Bph10* resistance genes in rice varieties ASD7, Babawee, ARC10550, Chin Saba, Pokkali, and IR65482-4-136-2-2, respectively. The UBN population was showed capable of damaged virulence to *Bph1, bph2, bph4, bph5, Bph6, bph7, bph8, Bph9* and *Bph10* resistance genes in rice varieties IR64, ASD7, Babawee, ARC10550, Swarnalata, T12, Chin Saba, Pokkali, and IR65482-4-136-2-2, respectively. The BPH population from KKN and PSL were showed capable of damaged virulence to *bph2, bph5, bph7, bph8, Bph9* and *Bph10* resistance genes in rice varieties ASD7, ARC10550, T12, Chin Saba, Pokkali, and IR65482-4-136-2-2, respectively (Table 7).

The reactions of ten resistance genes to KKN and PSL populations were showed similarly. The reactions of the rice varieties showed different from biotype 1, 2, 3, and 4, which have been reported at IRRI. Only two varieties Rathu Heenati (*Bph3*) and Abhaya (unknown resistance genes) showed resistance and moderated resistance against four BPH populations in Thailand using seedbox screening test.

Feeding rate of 11 differential resistance rice varieties to the four brown planthopper populations as indicated by area of honeydew excretion on filter papers were measured and analyzed. The areas of honeydew excretion on filter papers were varying upon different rice varieties and BPH populations. The area of honeydew from KKN population on Rathu Heenati, Babawee and Abhaya were significantly lower than the susceptible check, TN1. The area of honeydew from PSL population and PTT population on Rathu Heenati, Babawee, Sawanalata and Abhaya were significantly lower than TN1. While only on Rathu Heenati and Abhaya were significantly lower than TN1 in UBN population. On Abhaya, the feeding rates of most insect populations were significantly lower than the susceptible check. The results clearly showed that Abhaya could resistant to all BPH populations (Figure 9).

The results from the antibiosis for population growth test showed that the number of progeny produced by all BPH populations on Abhaya varieties were significantly lower than TN1. The numbers of insects were significantly lower than TN1 on Rathu Heenati, Babawee, Sawanalata and Abhaya to BPH from KKN and PSL; Rathu Heenati and Abhaya to BPH from UBN; and Rathu Heenati, Babawee, Sawanalata, T12 and Abhaya to BPH from PTT (Figure 10).

Table 7 Reaction of ten resistance genes to Pathum Thani (PTT), Ubon Ratchathani (UBN), Khon Kaen (KKN), and Phitsanulok (PSL) brown planthopper populations using seedbox screening test

Variety	Resistance gene	Reaction			
		PTT	UBN	KKN	PSL
Mudgo	*Bph1*	*	*	*	*
IR64	*Bph1*$^{+}$	MR	MS	S	*
ASD7	*bph2*	MS	S	S	HS
Rathu Heenati	*Bph3*	R	R	R	R
Babawee	*bph4*	MS	S	MR	MR
ARC10550	*bph5*	MS	S	S	S
Swarnalata	*Bph6*	R	MS	MR	MR
T12	*bph7*	MR	S	S	HS
Chin Saba	*bph8*	MS	MS	S	HS
Pokkali	*Bph9*	MS	S	S	HS
IR65482-4-136-2-2	*Bph10*	MS	MS	MS	S
Abhaya	Unknown	MR	MR	MR	MR

* not germinate
+ plus minor resistance genes
R = Resistance, MR = Moderated Resistance, MS = Moderated susceptible, S = susceptible, HS = Highly susceptible

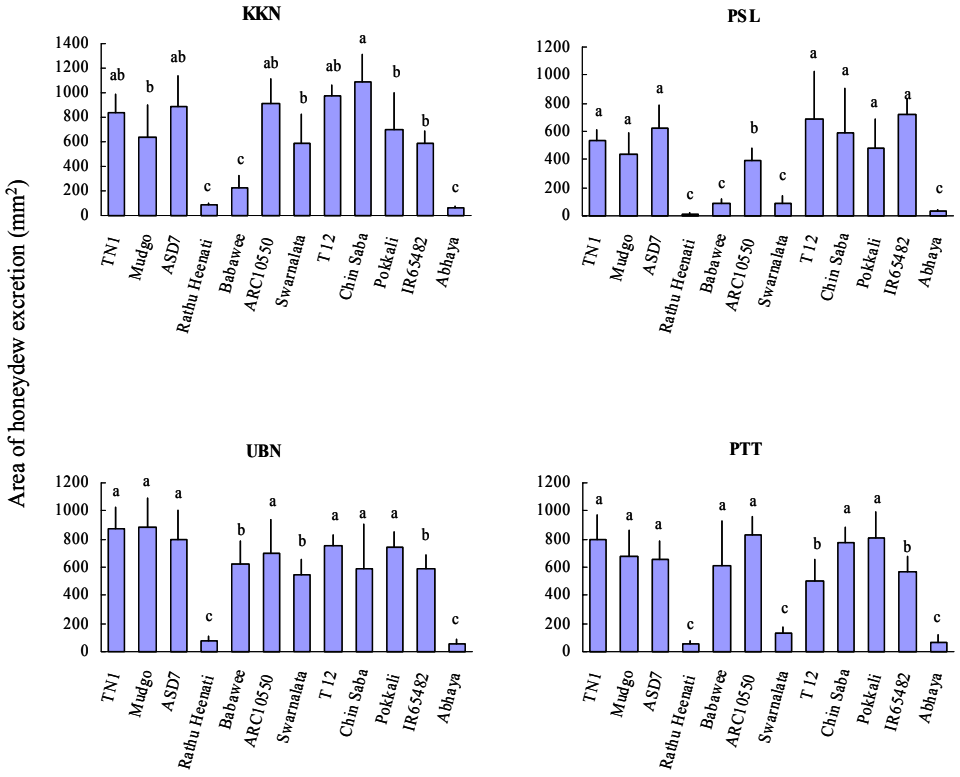

Figure 9 Area of honeydew excretion on filter paper (mm^2) of 11 resistance rice varieties to four brown planthopper populations, Khon Kaen (KKN), Pitsanulok (PSL), Ubon Ratchathani (UBN), and Patum Thani (PTT) using antibiosis on feeding rate test. Values are expressed as means ± SE. The bars with the same letter are not significantly different (P>0.05).

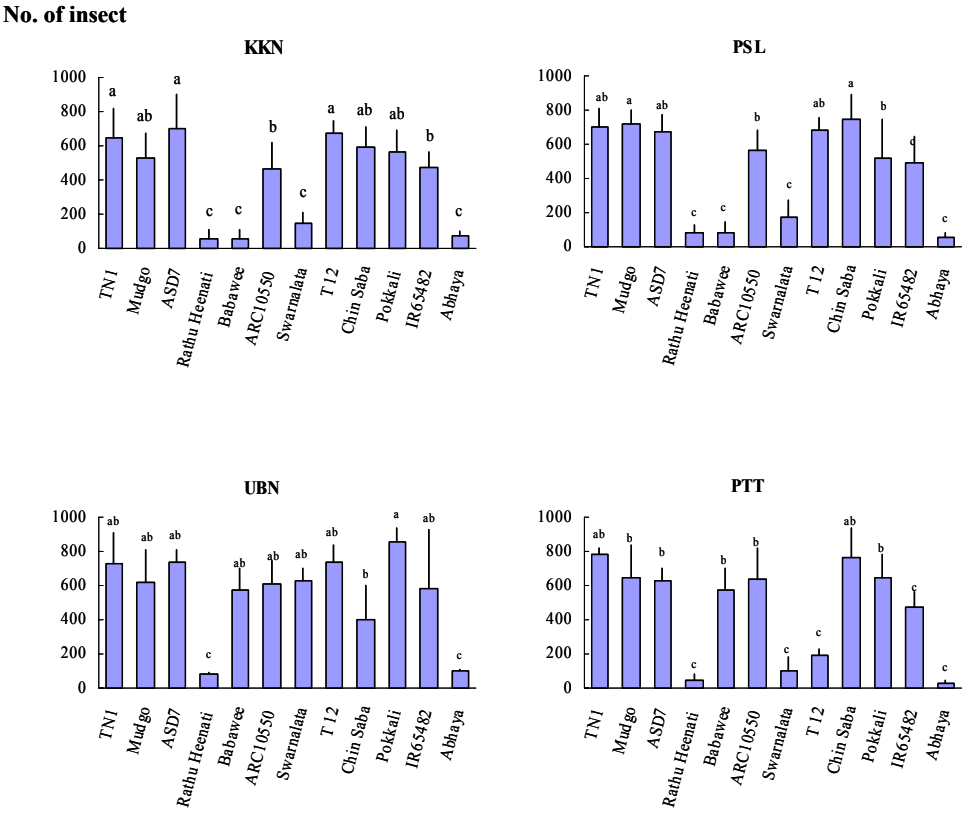

Figure 10 Fecundity of four populations of brown planthopper, Khon Kaen (KKN), Pitsanulok (PSL), Ubon Ratchathani (UBN), and Patum Thani (PTT) on 11 BPH resistance varieties. Values are expressed as means ± SE. The bars with the same letter are not significantly different (P>0.05).

Adaptation of the BPH colonies established from each of two locations, KKN and UBN, to the resistance donors were investigated using antibiosis for population growth test. Colony establishment of KKN and UBN colonies to Abhaya showed significantly lower than TN1 over 9 and 11 generations, respectively (Figure 11-12). The results indicated that Abhaya remained resistance to the BPH from KKN and UBN at least 9 and 11 generations of the selection insects.

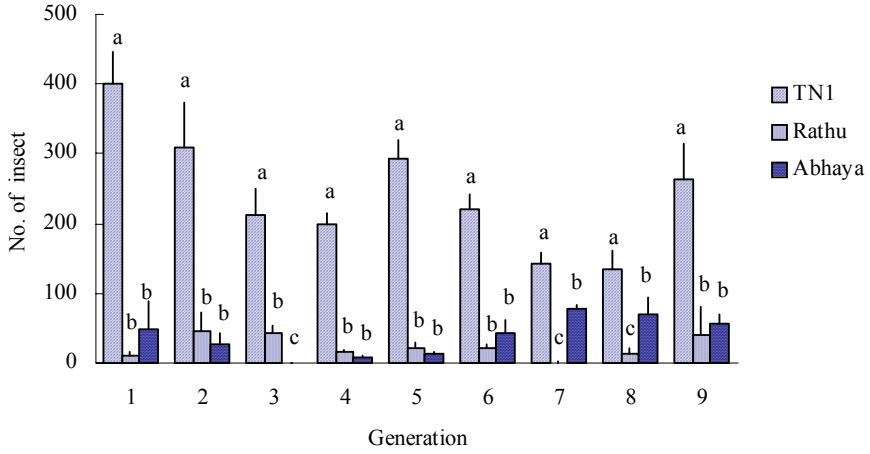

Figure 11 Fecundity of brown planthopper Khon Kaen population on rice varieties, TN1, Rathu Heenati and Abhaya. Values are expressed as means ± SE. The bars with the same letter are not significantly different (P>0.05).

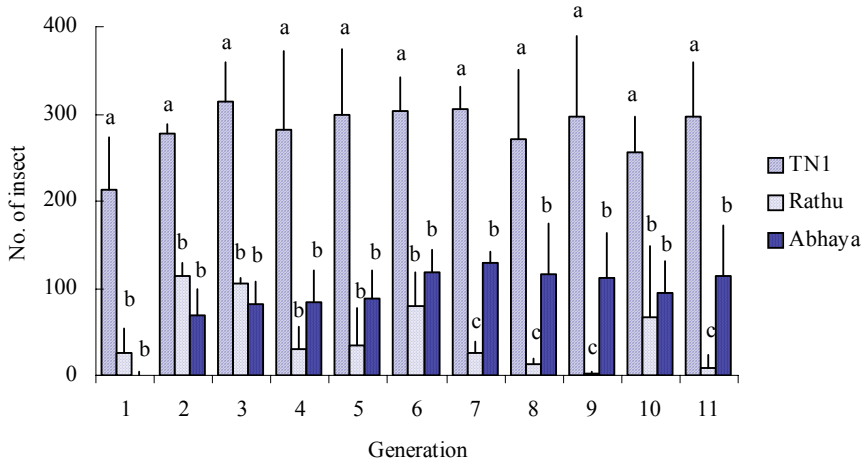

Figure 12 Fecundity of brown planthopper Ubon Ratchathani population on rice varieties, TN1, Rathu Heenati and Abhaya. Values are expressed as means ± SE. The bars with the same letter are not significantly different (P>0.05).

Phenotypic variations of damage scores in the BC_4F_2 and BC_4F_3 populations

Four hundred BC_4F_2 and BC_4F_3 populations derived from a cross between Abhaya and KDML105 were used to screen for resistance to BPH populations from UBN and PTT, respectively. The seedbox screening test with little modification was utilized. The parents differed significantly in their resistance to both BPH populations from PTT and UBN. The averaged damage score was 3 and 9 for Abhaya and KDML105, respectively. Continuous distributions of the damage score, which were skewed toward susceptibility, were observed in the BC_4F_2 and BC_4F_3 populations (Figure 13).

Table 8 Frequency distribution of insect damage scores from seedbox screening test at Pathum Thani and Ubon Ratchathani Rice Research Center

BPH population[1/]	Resistance scores[2/]				
	1	**3**	**5**	**7**	**9**
PTT	16	57	129	68	127
UBN	11	70	86	154	50

[1/] PTT = Pathum Thani Population; UBN = Ubon Ratchathani Population

[2/] Reading scale of 1 to 9; 1= highly resistant, 9=highly susceptible.

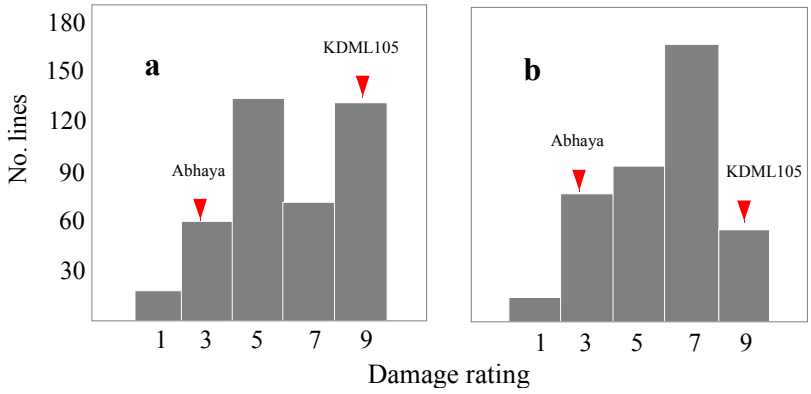

Figure 13 Frequency distributions of phenotypic values from BPH resistance tests. **a** = Seedbox screening test of 400 BC_4F_2, derived from KDML105 and Abhaya, with the Pathum Thani BPH population, **b** = Seedbox screening test of 400 BC_4F_3 with the Ubon Ratchathani BPH population. Reading scale of 1 to 9; 1= highly resistant, 9=highly susceptible.

Identification of DNA markers co-segregated with BPH resistant genes

The ultimate goal of this study is to indentify the BPH resistance genes in Abhaya using BSA and QTL approaches. Two molecular markers techniques, SSR and AFLP, were used to tagging BPH resistance genes in a cross between Ahabaya and KDML105. Microsatellite technique was firstly used to roughly survey BPH resistance genes. All the SSR markers used in this study have been mapped to rice chromosomes (McCouch et al., 1996). Ten rice microsatellite primers (Table 5) were selected base on the information of BPH resistance genes linkage on rice chromosome 4 (Murata, 1997), chromosome 10 (Ikada and Kaneda, 1981), and chromosome 12 (Jeon et al., 1999; Hirabayashi and Ogawa, 1996; Huang et al., 1997; Murata et al., 1998). BSA was conducted using the pools and parental DNA. Two markers, RM216 and RM277, showed polymorphism between individuals from the pools and parents. This is an evidence of a co-segregation of these markers with the BPH resistance in the BC_4F_2 and BC_4F_3 evaluated at URRC and PTRC respectively. The average percentage of phenotypic variance explained (PVE) by the marker RM277 were 16.8 and 10.2% of phenotypic at URRC and PTRC, respectively and marker RM216 were 12.3 and 4.1% at URRC and PTRC, respectively (Table 10). It would suggest that at least two resistance loci were detected by using SSR.

To identify more details about the BPH resistance genes in Abhaya, we used bulked segregant-AFLP analysis for tagging BPH resistance genes. A total of 138 primer combinations were generated AFLP fingerprints from four samples, the resistance and susceptible parents and two DNA pools. The total numbers of unique AFLP bands per primer combination, observed in two parents, were amplified approximately 30-60 bands. A total of about 4,000 bands were obtained with the 138 different primer combinations and revealed 4-41 % polymorphism between the parents. Thirty-nine primer combinations were omitted from further analysis because of a low quality of the fingerprint (too dense or too weak) or when PCR amplification failed in one or more of the four samples.

Twenty six primer combinations produced 36 DNA fragments presented only in a resistance parent and a pool and absented in a susceptible parent and a pool were collected. Thirteen AFLP fragments from ten primer combinations were identified. Preliminary analysis of DNA sample from 40 BC_4F_1, which used to construct the pools, demonstrated the presence and absence of amplified product (Table 9). Two DNA fragments from two AFLP primer pairs, E4/M2 and E1/M13, demonstrated the present of amplified product in resistance progenies and absent in susceptible progenies, suggesting that these two AFLP markers were tightly linked to the BPH resistance loci. Further analysis to confirm the linkage of candidate

With the primer combinations, the band at 500 bp of E4/M2-1 and the band about 185 bp of E1/M13-1 were presented in the resistance parent Abhaya and individual progenies, which were resistance to BPH (Figure 14). These bands were absented in the susceptible parent and susceptible individual lines. With the primer combination, E5/M3, E4/M1, E5M6, E10/M1, E9/M19, E3/M16, E6/M1 and E4/M13, the bands were not present in all of individual lines in the resistance pooled. Some lines were absented in the individual resistance pooled (Table 9).

Table 9 Segregation data of polymorphic and bulk specific AFLP fragments for 40 individuals (20 resistance BC_4F_1 lines and 20 susceptible BC_4F_1 lines) was amplified by 13 AFLP primer combinations

Primers	BC_4F_1 Progeny																																																
	Resistance																						Susceptible																											
	280	296	321	329	330	370	387	415	423	426	543	565	581	592	625	626	627	628	630	632	317	347	408	424	439	443	444	445	447	448	449	450	451	452	455	456	458	459	497	499
E4/M2-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E1/M13-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E5/M3-1	-	-	-	-	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E5/M3-2	-	-	-	-	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E5/M3-3	-	-	-	-	-	+		+	+	+	+	-	-	-	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E4/M1	-	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E5/M6	-	-	-	-	-	+		+	+	+	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E10/M1	-	-	-	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E9/M19	+	-	+	+	-	+	-	+	+	+	-	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E3/M16-1	-	-	-	-	-	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E3/M16-2	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E6/M1	-	-	-	-	-	+		+	+	+		+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E4/M13	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ and – denote marker presence and absence, respectively

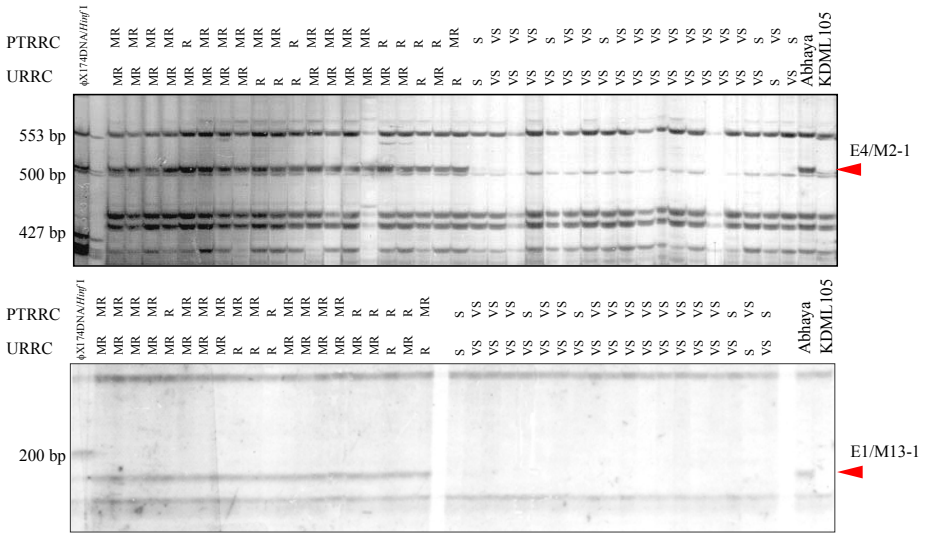

Figure 14 AFLP marker E4/M2-1 and E1/M13-1 linked to BPH resistance. The markers were identified in individual lines of the BC_4F_1 cross between susceptible KDML105 and resistant Abhaya cv., which used to construct the bulks. The arrows indicate the polymorphic fragments, E4/M2-1 and E1/M13-1. The reaction of individual lines to BPH from Pathum Thani Rice Research Center (PTRC) and Ubon Ratchathani Rice Research Center (URRC) were shown. R, MR, S and VS refer to resistance, moderate resistance, susceptible and very susceptible, respectively.

Confirmation of co-segregated AFLP fragments with BPH resistance

AFLP analysis of DNA samples from the 40 individuals of BC_4F_1 that used to make the pool clearly demonstrated the presence and absence of amplified products. Of 36 AFLPs, only two, E4/M2-1 and E1/M13-1, had shown the presence of an amplified product in all 20 resistant individuals and absence in all 20 susceptible individuals, suggesting that these two AFLP fragments were tightly linked with the BPH resistance. Figure 14 showed a 500 bp of the E4/M2-1 and a 185 bp of E1/M13-1. These two fragments were tested to determine how tightly linkage with the genes conferring BPH resistance using 140 individuals of BC_4F_1 derived from two families. The simple linear regression analysis shown that E4/M2-1 and E1/M13-1 explained 48.6 and 32.0 % of the phenotypic variance at URRC and 28.0 and 19.2 % of the phenotypic variance ($P<0.0001$) at PTRC, respectively. Another two AFLP fragments, E4/M15-1 and E5/M3-3, were also shown the presence of an amplified product in most but not all of 20 resistant individuals and absence in most but not all of 20 susceptible individuals. This indicates that these two fragments were also linked to the BPH resistance but in a greater distance than the E4/M2-1 and E1/M13-1.

Table 10 Putative molecular markers linked the BPH resistance genes detected in a BC_4F_1 population from a cross of KDML105 and Abhaya

BPH Population	Locus	Chromosomal Location	Effect	R-squared	*P*-value
UBN	E4/M2-1	12	-0.9886	48.7	0.0000
	RM277	12	-0.6892	15.4	0.0000
	E5/M3-3	10	-0.5536	12.9	0.0001
	RM216	10	-0.5962	12.3	0.0000
	RM50	6	-0.7881	21.6	0.0000
	E1/M13-1	6	-0.8748	32.0	0.0000
PTT	E4/M2-1	12	-1.3418	28.0	0.0000
	RM277	12	-0.9302	9.9	0.0000
	E5/M3-3	10	-0.6135	6.4	0.0068
	RM216	10	-0.5990	4.1	0.0001
	RM50	6	-0.7435	6.9	0.0000
	E1/M13-1	6	-1.1165	19.2	0.0000

Localization of candidate AFLP markers and linkage analysis

Four AFLP markers, E4/M2-1, E1/M13-1, E4/M15-1 and E5/M3-3, were mapped on the existing linkage map of the RIL population. This 1,310 cM-linkage map of the RIL was previously constructed using 183 markers (Toojinda et al., 2003). E4/M15-1 was mapped near RM50 on chromosomes 6. E5/M3-3 was mapped on chromosome 10 with a 3.2 cM distance from RM216. E4/M2-1 was mapped on chromosome 12 between markers *Sdh-1* and CDO344. This marker was approximately 23.5 cM distance from RM277 (Figure 15). E1/M13-1 could not be mapped using this population because there was no polymorphism between the parents. This result might indicate three possible BPH-resistant QTLs designated as *Qbph6*, *Qbph10* and *Qbph12*. Three linkage groups were also constructed based on the BC_4F_1 individuals. This result reconfirmed the linkage of these markers. Moreover, the E1/M13-1 unmapped in the RIL-linkage map was located in the same linkage group with E4/M15-1 and RM50.

Estimation of effects of BPH resistance genes

Three SSR markers, RM50, RM216 and RM277, linked to BPH resistance genes on chromosomes 6, 10 and 12 were used to fingerprint the 400 BC_4F_1 individuals to determine the effects of BPH resistance genes. Simple linear regression analysis was then used to estimate the effects and the phenotypic variance explained (PVE) by these markers using the phenotypic data conducted at URRC and PTRC. The RM50, RM216, and RM277 individually accounted for 6.8 to 21.6%, 4 to 12.3 %, and 10 to 15.4 % of PVE respectively (Table 10). The introgressed lines containing four loci from Abhaya on chromosome 6, 10, 12 and unknown chromosome were found to be resistance to moderated resistance to both insect populations. While some introgressed lines, which contain some segments from Abhaya on chromosome 6 or 10 showed moderately susceptible to susceptible to both BPH populations. Introgressed lines contain segments of chromosome 12 and unknown chromosome from Abhaya showed a slightly higher level of resistance than that contain segments of chromosome 6 or 10.

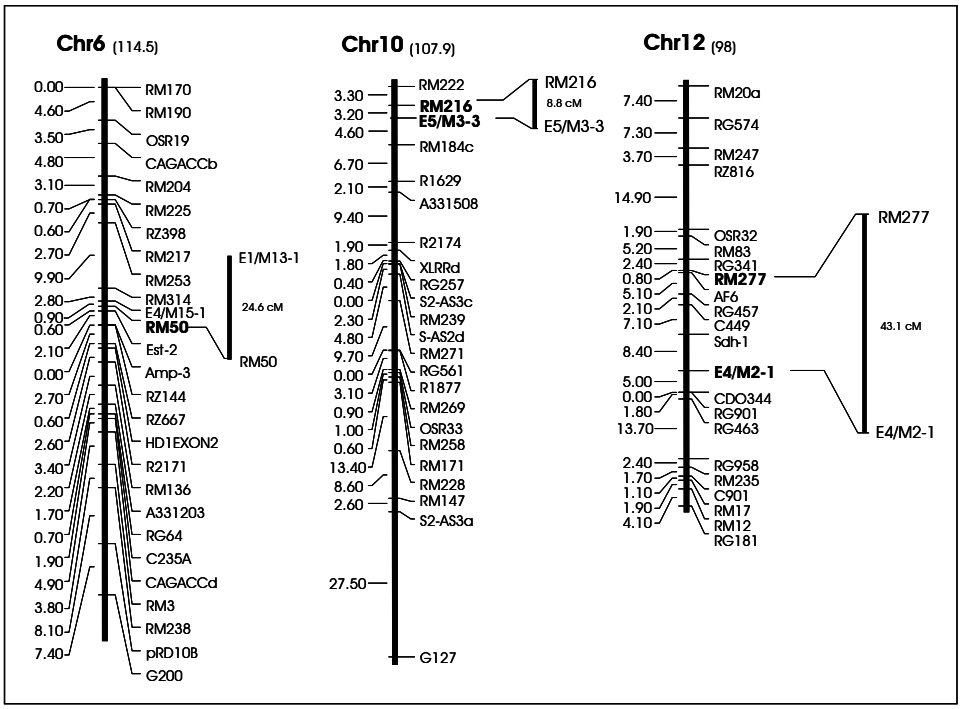

Figure 15 Linkage map of rice chromosome 6, 10 and 12, mapping done on a RILs population derived from across between FR 13 A and CT6241-17-1-2-1. The AFLP marker E4/M2-1 linked to BPH resistance is shown on chromosome 12. Other AFLP fragment, E4/M15-1, and E5/M3-3 markers derived from BSA were located on chromosome 6, and 10, respectively. The black bars on the right indicate linkage groups analyzed by JoinMap software. The distances between markers on the figure are represented as cM using the Kosambi function.

QTL analysis

Simple regression showed a significant (P<0.0001) association between 7 markers on chromosomes 6, 10 and 12, and the damage score (Table 10). When all significant markers were included in the multiple regression model, only E4/M2-1 and E1/M13-1 linked with *Qbph6* and *Qbph12* were significant at P<0.0001. The E5/M3-3 linked with *Qbph10* was not a significant explanatory genetic factor for the damage score. The *Qbph6* and *Qbph12* jointly showed a significant reduction of 2.2 and 3.3 of the DS at URRC and PTRC, respectively (Table 11).

Table 11 Comparison of QTL combinations for damage score (DS) within the BC_4F_2 and BC_4F_3 populations.

QTL combination	Damage score (DS)			
	URRC*	Reduction of DS	PTRC*	Reduction of DS
Qbph6 + *Qbph12*	4.5±0.2[a]	2.2	4.2±0.2[a]	3.3
Qbph12	5.4±0.2[b]	1.3	5.5±0.3[b]	2.0
Qbph6	6.1±0.2[c]	0.6	7.2±0.4[c]	0.3
No QTL	6.7±0.1[d]	0.0	7.5±0.2[c]	0.0

*Values are expressed as Mean ± SE. Damage score based on 1-9 scale
URRC=modified-seedbox screening test at Ubon Ratchathani Rice Research Center
PTRC= modified-seedbox screening test at Pathum Thani Rice Research Center
The means with the same letter are not significantly different (LSD, P>0.05)

DISCUSSION

We found the genetic variations occurring among the BPH populations collected from four provinces in Thailand. From the seedbox screening test, the results showed that the BPH populations found in Thailand were different from biotype 1, 2, 3 and 4 identified by IRRI. The rice cultivar "Abhaya" was chosen as the donor for BPH resistance and was assessed with four BPH populations. The result indicated that Abhaya showed moderate resistance to all BPH populations used in this study. The adaptation rate of the BPH from UBN and KKN on Abhaya retained a significant level of resistance after 9-10 generations of the selection insects. The recent studied showed that it took only 3-5 generations for BPH to adapt a new resistant variety after a continue feeding (Yu et al., 2001). Therefore, Abhaya should be a good source of BPH resistance for improving a durable and broad-spectrum resistance variety.

The frequency distributions of damage scores with the BPH by seedbox screening test were normally distributed in the recent studies (Alam and Cohen 1998a; Xu et al., 2002). The moderately resistance varieties were used in those studies as the donors to detect the QTLs associated with BPH resistance. In our study also found that the data variations of damage scores in the BC_4F_2 and BC_4F_3 from the cross Abhaya and KDML105 were not consistent with Mendelian analysis, suggesting that the BPH resistance in Abhaya may be quantitative inheritance in nature. Transgressive segregation in the BC_4F_2 and BC_4F_3 for the damage score was not observed. This indicated that all resistance genes probably came from Abhaya. The resistance genes of rice cultivar Abhaya may obtain from two donors, CR-157-392 and OR67-21. The quantitative resistance of rice to BPH usually found in rice cultivar that shows moderately resistance to BPH (Alam and Cohen 1998a; Xu et al., 2002). The moderately resistant cultivar has proven to be one of the most-efficient ways to control this pest (Alam and Cohen 1998a,b).

The BSA approach has been extensively used to identify genes or DNA markers that associate with several traits in many crops (Cho et al., 1994; Huang et

al., 2001, Li et al., 1998; Nair et al., 1996; Negi et al., 2000; Zhang et and Stommel, 2000). Although the BSA is more robust for tagging the major gene, this technique can be applied to tag the genes controlling the complex traits (Michelmore et al., 1991). A number of markers linked to the important agronomic traits have been analyzed by using the AFLP technique and BSA. However, this strategy could not detect loci with small effects (Wang and Paterson, 1994). The BSA and a single-regression analysis were utilized in 400 BC_4F_1 population. Four QTLs for BPH resistance were detected with AFLP markers. Most of alleles for resistance in introgressed lines were introgressed from Abhaya. Among four molecular markers linked with the BPH resistance, E4/M2-1 and E1/M13-1 might be the putative markers tightly linked to the BPH resistance genes in Abhaya. The AFLP marker E4/M2-1 was located in the 13.4-cM length interval between *Sdh-1* and CDO344 on chromosome 12. This location was similar to *Bph1* position as identified by Hirabayashi and Ogawa (1995), Huang et al. (1997) and Murata et al. (1997).

Two AFLP fragments, E4/M2-1 and E1/M13-1, which closely linked with BPH resistance genes, were individually explained 48.6% and 32.0% of phenotypic variance at URRC and 28% and 19.2% of phenotypic variance at PTRC, respectively. The phenotypic variance at PTRC of all loci resulted in lower than those at URRC. Because of we used the difference BPH population and deference condition to assess the BC_4F_2 and BC_4F_3. It is possible that a major allele associate with the resistance did not detect at PTRC.

The SSR marker RM277 on chromosome 12 was co-segregated with the phenotype in both URRC and PTRC. Although RM277 and E4/M2-1 loci could be co-segregated with the same BPH resistance loci on chromosome 12, the E4/M2-1 was located 23.5 cM away from RM277. Two possible explanations that have contributed to such a long genetic distance was the cross to cross variation with different recombination frequencies in different mapping population (Mohan et al. 1997) or there may be two resistance-associated loci in this region. However, the relationships between them should be further investigated.

Two minor resistance QTLs were detected with E4/M15-1 and E5/M3-3 on chromosome 6 and 10, respectively. Two microsatellite markers, RM50 and RM216, which closely linked to the AFLP markers on chromosome 6 and 10, were used to determine the potential co-segregation with the resistance QTLs in 400 individuals of BC_4F_1 population. The QTL on chromosome 6 could explain higher percentage of the observed phenotypic variation at URRC (21.6%) than PTRC (6.8%). The QTL on chromosome 6 confirmed Alam and Cohen (1998a) that detected one QTL near RM50. This QTL was detected by four measuring antixenosis as well as the seedbox screening tests. The other QTL on chromosome 10 explained 12.2 and 4.1% of PVE at URRC and PTRC, respectively. This QTL was located in the same region that detected in Teqing cultivar by seedbox screening test (Xu et al., 2002)

Two out of four AFLP fragments, E4/M1-1 and E5/M3-1 were localized on chromosome 2 and 4 where minor resistance genes for BPH resistance had been previously mapped using seedbox screening test (Su et al., 2002; Xu et al., 2002). Nevertheless, the co-segregation of these two markers with resistance QTLs could not determine by the franking markers.

Abhaya alleles of all loci resulted in lower damage score. Only *Qbph6* and *Qbph12* significantly decreased the damage scores at URRC and PTRC. The *Qbph10* was not a significant explanatory genetic factor for the damage score. This lends some support to the concept of two major QTL with one minor QTL underlying BPH resistance in Abhaya. Because of the *Qbph10* contributed to such a low proportion of the genetic variation, its effect might be obscured by the presence of the *Qbph6* or *Qbph12*. The *Qbph6* and *Qbph12* jointly showed a significant reduction of 2.2 and 3.3 of the damage score at URRC and PTRC, respectively. We found some introgressed lines carry *Qbph6* and *Qbph12* could resistance to BPH from UBN and PTT in the same level as the donor, Abhaya. This provides clear evidence that Abhaya has at least two BPH resistance genes.

The quantitative resistance to BPH may result from different mechanisms. Three mechanisms, antixenosis or non-preference, antibiosis, and tolerance, are generally recognized to function in quantitative resistance to BPH. From the previous studies the quantitative resistance to BPH may result from different mechanisms, such as non-preference or antixenosis, antibiosis and tolerance (Alam and Cohen 1998a; Su et al., 2002; Xu et al., 2002). Some BPH resistance genes or QTL confer resistance principally attributable to a particular mechanism. Since the damage score measured in this experiment (seedbox screening test) was designed to provide an overall evaluation on different resistance mechanisms, we found that *Qbph6* was located in a genomic location similar to where QTL for antixenosis, feeding rate and damage scores were mapped in the doubled haploid population of IR64 x Azucena (Alam and Cohen 1998a). In our data, Abhaya retained a significant level of resistance in more than 9-10 generations of selection BPH. It's possible that antibiosis is one of the resistance mechanisms of the *Qbph6* in reducing insect survival, growth rate, or reproduction following the ingestion of host tissue. Because of the coincidental location of *Qbph6* and the QTL for antixenosis, *Qbph6* may also confer antixenosis by repelling or disturbing insects, causing a reduction in colonization or oviposition. However, further independent experiments for a specific mechanism need to be carried out to address its function.

CONCLUSION

The main finding of this study is the identification of molecular markers closely linked to the BPH resistance genes for improving BPH resistance in rice. Additional advantage of this study is to find rice cultivars with broad-spectrum and with durability of resistance against BPH biotypes in Thailand. It may be used as donors in breeding and introgressed the resistance genes into new genetic background

According to this study, BPH resistance in Abhaya appeared to be controlled by multiple QTLs. Similar to gene utilization in disease resistance, several strategies of manipulating resistance genes have been developed for fighting the insect pest. It has been suggested that the introgressed lines that contained resistance QTLs from Abhaya should provide more durable resistance than varieties that have single major gene, as it is unlikely that the insect would be able to simultaneously overcome multiple resistance genes. For more effective protection, the markers identification linked to major and minor loci associated with BPH resistance genes have the potential to accelerate selection for resistance in the breeding program. In this regard, closely linked markers associated with the resistance QTL segments that introgressed from Abhaya remains to be determined in future studies. Thus, it will be useful in marker assisted selection and the positional cloning of these resistance genes in rice.

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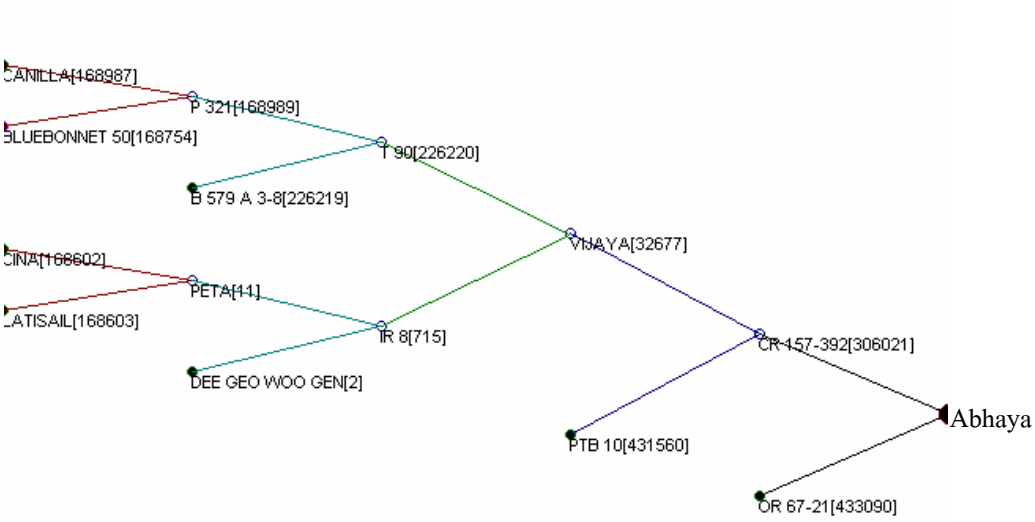
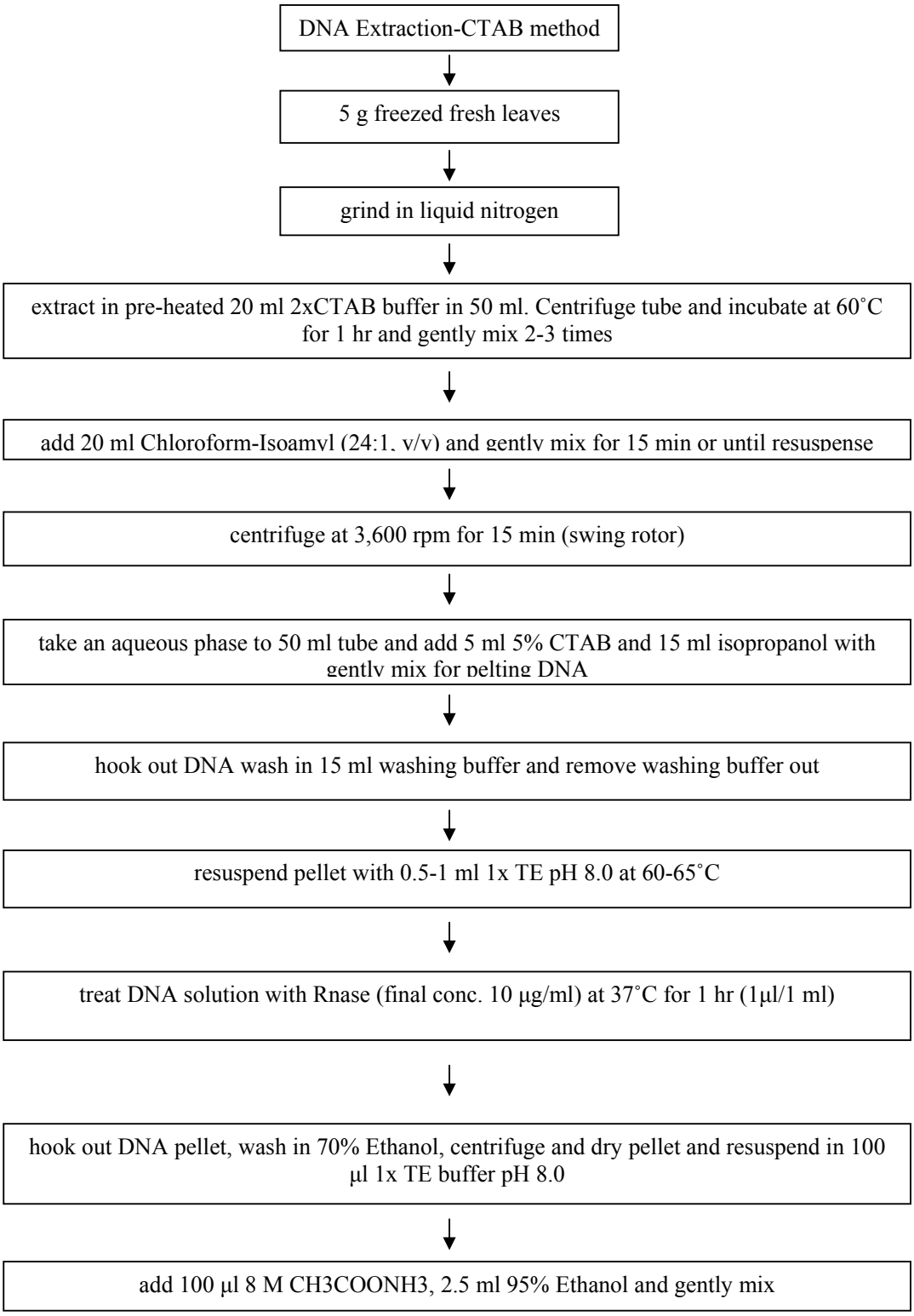
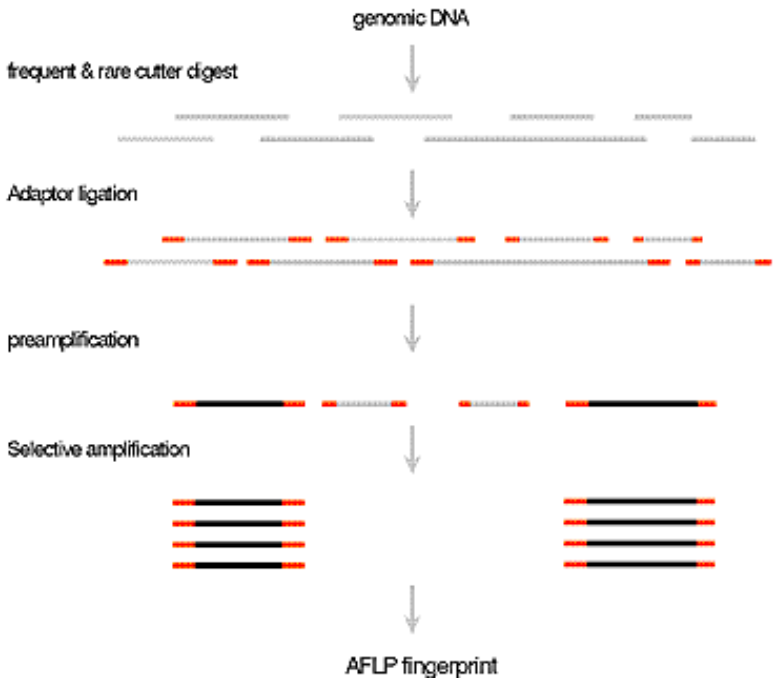
APPENDIX



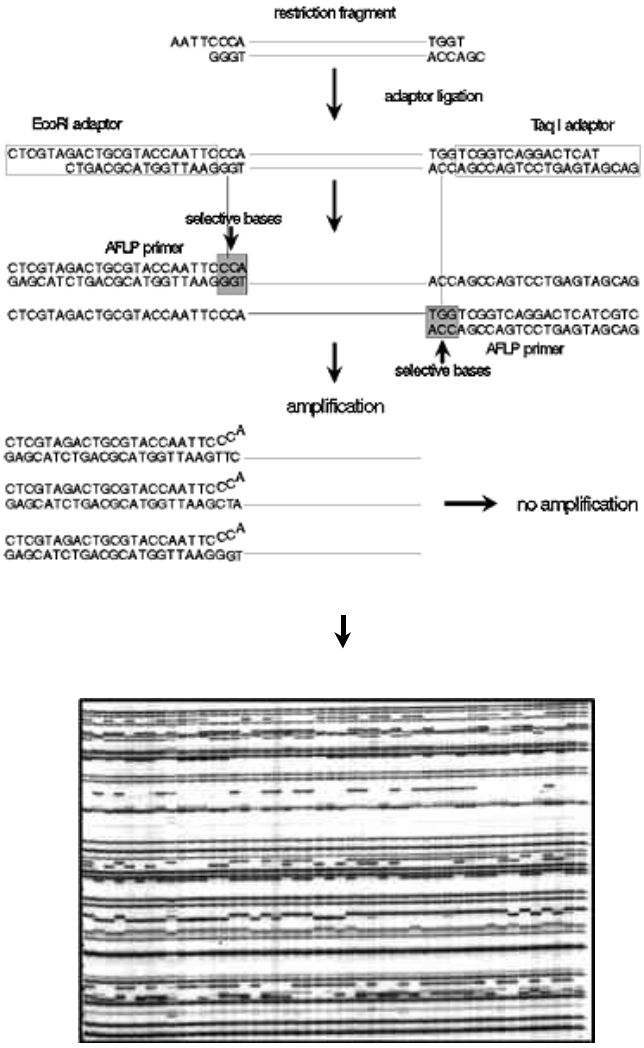
Appendix Figure 1 Diagram of pedigree genotype of Abhaya.



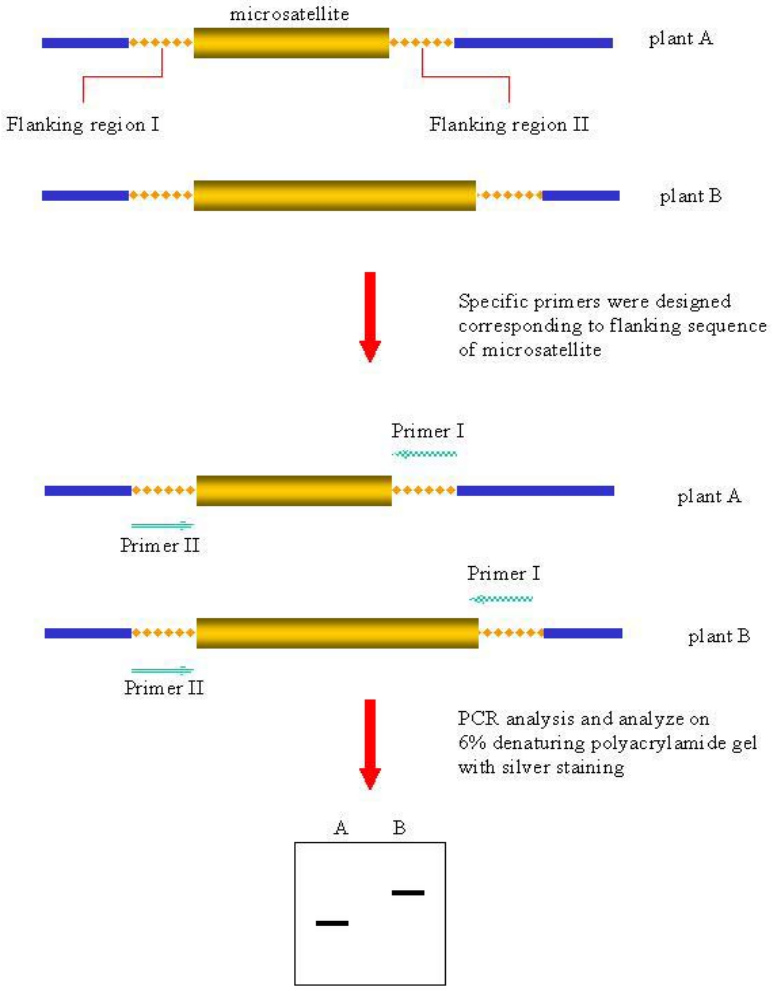
Appendix Figure 2 Standard genomic DNA extraction protocol (CTAB method)



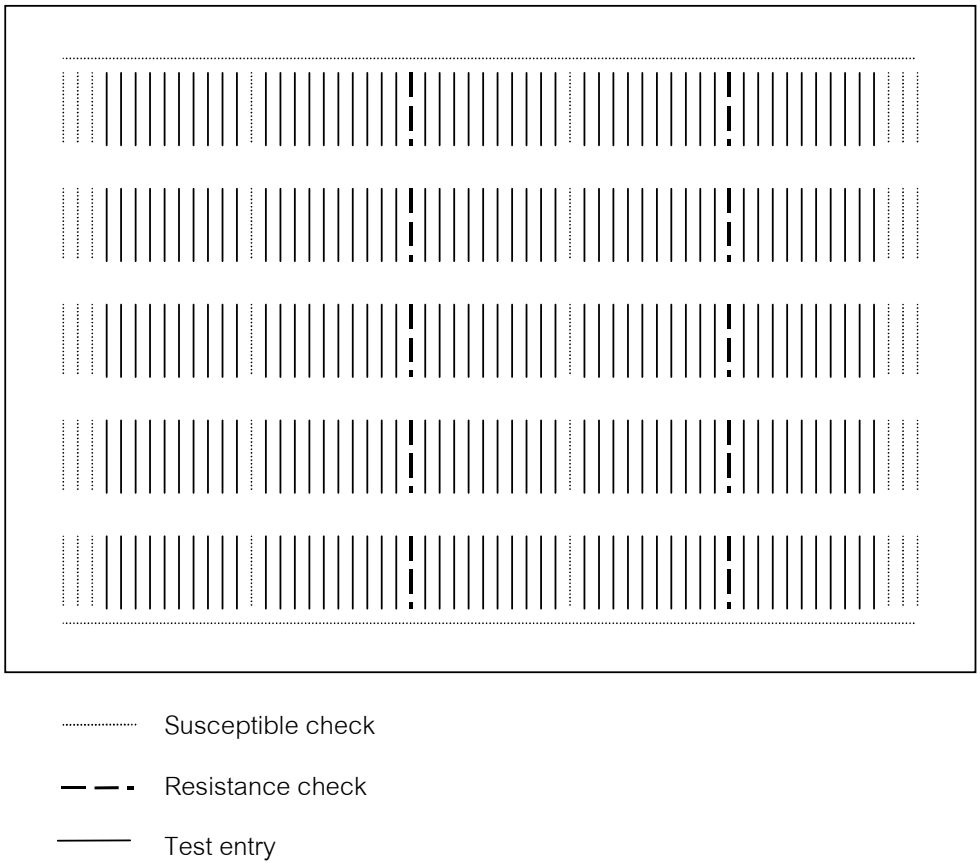
Appendix Figure 3 The schematic of AFLP assays.



Appendix Figure 4 The AFLP technique usually consists of 5 steps: the restriction of the DNA with 2 restriction enzymes, a hexa-cutter and a tetra-cutter; the ligation of double-stranded (ds) adapters to the ends of the restriction fragments; the amplification of a subset of the restriction fragments using 2 primers complementary to the adapter and restriction site sequences, and extended at their 3' ends by 1 to 3 "selective" nucleotides; gel electrophoresis of the amplified restriction fragments on denaturing polyacrylamide gels; the visualization of the DNA fingerprints by means of silver staining method.



Appendix Figure 5 The schematic of SSLP assays.



Appendix Figure 6 Modification of standard seedbox screening technique for rice brown planthopper resistance in greenhouse.

Appendix Table 1 Damage rating of 400 BC_4F_2 and BC_4F_3 infested by BPH population from UBN and PTT at 7 days with 2nd and 3rd instar nymphs of brown planthopper.

Entry No.	Pedigree	Reaction to BPH		Entry No.	Pedigree	Reaction to BPH	
		UBN	PTT			UBN	PTT
264	F1IR71279///KDML105-28-9-11-2	MS	MS	330	F1IR71279///KDML105-28-9-11-4		R
265	F1IR71279///KDML105-28-9-11-2	MR	MS	331	F1IR71279///KDML105-28-9-11-4	MR	MR
266	F1IR71279///KDML105-28-9-11-2	MS	MS	332	F1IR71279///KDML105-28-9-11-4	MR	MS
267	F1IR71279///KDML105-28-9-11-2	MR	MS	333	F1IR71279///KDML105-28-9-11-4	MR	MS
268	F1IR71279///KDML105-28-9-11-2	S	S	334	F1IR71279///KDML105-28-9-11-4	MS	MS
269	F1IR71279///KDML105-28-9-11-2	MS	MS	335	F1IR71279///KDML105-28-9-11-5	MS	MS
270	F1IR71279///KDML105-28-9-11-2	MR	S	336	F1IR71279///KDML105-28-9-11-5	MS	MS
271	F1IR71279///KDML105-28-9-11-2	MS	S	338	F1IR71279///KDML105-28-9-11-5	MS	MS
272	F1IR71279///KDML105-28-9-11-2	MR	MS	339	F1IR71279///KDML105-28-9-11-5	MS	MS
273	F1IR71279///KDML105-28-9-11-2	S	MS	340	F1IR71279///KDML105-44-8-2-1	S	MS
274	F1IR71279///KDML105-28-9-11-2	MS	MS	344	F1IR71279///KDML105-44-8-2-1	MS	MR
275	F1IR71279///KDML105-28-9-11-2	MR	MS	346	F1IR71279///KDML105-44-10-11-4	MR	S
276	F1IR71279///KDML105-28-9-11-2	MR	MS	347	F1IR71279///KDML105-44-10-11-4	S	S
278	F1IR71279///KDML105-28-9-11-2	MR	MS	348	F1IR71279///KDML105-44-10-11-4	MS	S
280	F1IR71279///KDML105-28-9-11-2	MR	MS	349	F1IR71279///KDML105-44-10-11-4	S	S
281	F1IR71279///KDML105-28-9-11-2	MR	S	350	F1IR71279///KDML105-44-10-11-4	MS	S
282	F1IR71279///KDML105-28-9-11-2	MS	S	351	F1IR71279///KDML105-44-10-11-4	S	S
283	F1IR71279///KDML105-28-9-11-2	MS	MS	352	F1IR71279///KDML105-44-10-11-4	MS	S
284	F1IR71279///KDML105-28-9-11-2	MR	MR	353	F1IR71279///KDML105-44-10-11-4	MS	S
285	F1IR71279///KDML105-28-9-11-2	MS	MS	354	F1IR71279///KDML105-44-10-11-4	MR	-
286	F1IR71279///KDML105-28-9-11-2	S	MS	355	F1IR71279///KDML105-44-10-11-4	MR	S
287	F1IR71279///KDML105-28-9-11-2	MR	MS	356	F1IR71279///KDML105-44-10-11-4	MR	MS
288	F1IR71279///KDML105-28-9-11-2	MR	MS	357	F1IR71279///KDML105-44-10-11-4	MR	S
290	F1IR71279///KDML105-28-9-11-2	MR	R	358	F1IR71279///KDML105-44-10-11-4	MS	S
292	F1IR71279///KDML105-28-9-11-2	S	MS	361	F1IR71279///KDML105-44-10-11-6	MR	S
293	F1IR71279///KDML105-28-9-11-2	MS	MR	362	F1IR71279///KDML105-44-10-11-6	MR	S
294	F1IR71279///KDML105-28-9-11-3	MS	MS	363	F1IR71279///KDML105-44-10-11-6	MR	S
295	F1IR71279///KDML105-28-9-11-3	S	MR	364	F1IR71279///KDML105-44-10-11-6	MR	S
296	F1IR71279///KDML105-28-9-11-3	MR	MR	365	F1IR71279///KDML105-44-12-1-3	MR	S
297	F1IR71279///KDML105-28-9-11-3	MS	S	366	F1IR71279///KDML105-44-12-1-3	MS	MS
298	F1IR71279///KDML105-28-9-11-3	MS	S	367	F1IR71279///KDML105-44-12-1-3	MR	MS
300	F1IR71279///KDML105-28-9-11-3	S	S	368	F1IR71279///KDML105-44-12-1-3	MS	MS
301	F1IR71279///KDML105-28-9-11-3	MS	S	369	F1IR71279///KDML105-44-12-1-3	MR	MS
302	F1IR71279///KDML105-28-9-11-3	S	S	370	F1IR71279///KDML105-44-12-1-3	MS	MR
303	F1IR71279///KDML105-28-9-11-3	MS	MS	371	F1IR71279///KDML105-44-12-1-3	MR	MS
304	F1IR71279///KDML105-28-9-11-3	MS	MS	372	F1IR71279///KDML105-44-12-1-3	MR	MS
305	F1IR71279///KDML105-28-9-11-3	MS	S	373	F1IR71279///KDML105-44-12-1-3	MR	MR
306	F1IR71279///KDML105-28-9-11-3	S	S	374	F1IR71279///KDML105-44-12-1-3	MR	R

Appendix Table 1 (Continued)

Entry No.	Pedigree	Reaction to BPH		Entry No.	Pedigree	Reaction to BPH	
		UBN	PTT			UBN	PTT
307	F1IR71279///KDML105-28-9-11-3	MS	S	375	F1IR71279///KDML105-44-12-1-3	MR	MS
308	F1IR71279///KDML105-28-9-11-3	MS	S	376	F1IR71279///KDML105-44-12-1-3	MR	S
309	F1IR71279///KDML105-28-9-11-3	MS	S	377	F1IR71279///KDML105-44-12-1-3	MR	S
310	F1IR71279///KDML105-28-9-11-3	MS	S	378	F1IR71279///KDML105-44-12-1-3	MS	MS
311	F1IR71279///KDML105-28-9-11-3	MS	S	379	F1IR71279///KDML105-44-12-1-3	MS	S
312	F1IR71279///KDML105-28-9-11-3	MS	S	380	F1IR71279///KDML105-44-12-1-3	MS	S
313	F1IR71279///KDML105-28-9-11-3	S	MS	381	F1IR71279///KDML105-44-12-1-3	MS	S
314	F1IR71279///KDML105-28-9-11-3	MS	MS	382	F1IR71279///KDML105-44-12-1-3	MS	MS
315	F1IR71279///KDML105-28-9-11-4	MR	MS	383	F1IR71279///KDML105-44-12-1-3	S	MS
316	F1IR71279///KDML105-28-9-11-4	MR	MS	384	F1IR71279///KDML105-44-12-1-3	MS	MS
317	F1IR71279///KDML105-28-9-11-4	S	S	386	F1IR71279///KDML105-44-12-1-3	MS	MR
318	F1IR71279///KDML105-28-9-11-4	S	MS	387	F1IR71279///KDML105-44-12-1-5	MR	MR
319	F1IR71279///KDML105-28-9-11-4	S	MS	389	F1IR71279///KDML105-44-12-1-5	S	MR
320	F1IR71279///KDML105-28-9-11-4	MR	MS	390	F1IR71279///KDML105-44-12-1-5	MS	MR
321	F1IR71279///KDML105-28-9-11-4	MR	MR	391	F1IR71279///KDML105-9-9-9-4	MS	MR
322	F1IR71279///KDML105-28-9-11-4	S	S	394	F1IR71279///KDML105-9-9-9-4	MS	MS
323	F1IR71279///KDML105-28-9-11-4	S	S	396	F1IR71279///KDML105-9-9-9-4	S	MS
324	F1IR71279///KDML105-28-9-11-4	MS	MS	397	F1IR71279///KDML105-9-9-9-4	S	MS
325	F1IR71279///KDML105-28-9-11-4	R	MS	398	F1IR71279///KDML105-9-9-9-4	MS	S
326	F1IR71279///KDML105-28-9-11-4	MR	MS	400	F1IR71279///KDML105-10-1-2-4	MS	MS
327	F1IR71279///KDML105-28-9-11-4	MS	S	401	F1IR71279///KDML105-10-1-2-4	MR	MS
328	F1IR71279///KDML105-28-9-11-4	MS	S	402	F1IR71279///KDML105-10-1-2-5	S	S
329	F1IR71279///KDML105-28-9-11-4		MR	403	F1IR71279///KDML105-10-1-2-5	S	MR
404	F1IR71279///KDML105-10-1-2-5	MR	MS	469	F1IR71279///KDML105-10-7-4-1	MR	MS
406	F1IR71279///KDML105-10-1-2-5	MS	MS	470	F1IR71279///KDML105-10-7-4-1	MR	MS
407	F1IR71279///KDML105-10-1-2-5	MS	MR	471	F1IR71279///KDML105-10-7-4-1	MR	S
408	F1IR71279///KDML105-10-2-4-3	S	S	472	F1IR71279///KDML105-10-7-4-1	MR	S
409	F1IR71279///KDML105-10-2-4-3	MS	MS	473	F1IR71279///KDML105-10-7-4-1	MS	S
410	F1IR71279///KDML105-10-2-4-3	MS	MS	474	F1IR71279///KDML105-10-7-4-1	MS	S
411	F1IR71279///KDML105-10-2-4-3	S	MS	475	F1IR71279///KDML105-10-7-4-1	MS	S
412	F1IR71279///KDML105-10-2-4-3	MR	S	477	F1IR71279///KDML105-10-7-4-1	MR	S
413	F1IR71279///KDML105-10-2-4-3	S	S	478	F1IR71279///KDML105-10-7-4-2	MS	S
414	F1IR71279///KDML105-10-2-4-3	MR	S	479	F1IR71279///KDML105-10-7-4-2	MR	MS
415	F1IR71279///KDML105-10-2-4-3	MR	MR	480	F1IR71279///KDML105-10-7-4-2	MS	S
416	F1IR71279///KDML105-10-2-4-3	MR	MS	481	F1IR71279///KDML105-10-7-4-2	MR	S
417	F1IR71279///KDML105-10-2-4-3	MS	MR	482	F1IR71279///KDML105-10-7-4-2	S	S
418	F1IR71279///KDML105-10-2-4-3	MS	MS	483	F1IR71279///KDML105-10-7-4-2	S	S
419	F1IR71279///KDML105-10-2-4-3	MS	MS	484	F1IR71279///KDML105-10-7-4-6	MR	MS
420	F1IR71279///KDML105-10-2-4-3	MR	S	485	F1IR71279///KDML105-10-7-4-6	MS	S
421	F1IR71279///KDML105-10-2-4-3	MR	MS	486	F1IR71279///KDML105-10-7-4-6	MS	S

Appendix Table 1 (Continued)

Entry No.	Pedigree	Reaction to BPH		Entry No.	Pedigree	Reaction to BPH	
		UBN	PTT			UBN	PTT
422	F1IR71279///KDML105-10-2-4-3	MR	MR	487	F1IR71279///KDML105-10-7-4-6	MS	S
423	F1IR71279///KDML105-10-2-4-3	MR	MR	488	F1IR71279///KDML105-10-7-4-6	S	S
424	F1IR71279///KDML105-10-2-4-6	S	S	489	F1IR71279///KDML105-10-7-4-6	MS	S
425	F1IR71279///KDML105-10-2-4-6	S	MS	490	F1IR71279///KDML105-10-7-4-6	MR	MR
426	F1IR71279///KDML105-10-2-4-6	MR	MR	491	F1IR71279///KDML105-10-7-4-6	MS	MS
427	F1IR71279///KDML105-10-2-4-6	MS	MS	492	F1IR71279///KDML105-10-7-4-6	S	MR
428	F1IR71279///KDML105-10-2-4-6	MR	MS	493	F1IR71279///KDML105-10-7-4-6	S	MS
429	F1IR71279///KDML105-10-2-4-6	MS	S	494	F1IR71279///KDML105-10-8-9-2	MR	MS
430	F1IR71279///KDML105-10-2-4-6	MR	-	495	F1IR71279///KDML105-10-8-9-2	MR	S
431	F1IR71279///KDML105-10-2-4-6	MR	MS	496	F1IR71279///KDML105-10-8-9-2	S	S
432	F1IR71279///KDML105-10-2-4-6	MR	MS	497	F1IR71279///KDML105-10-8-9-2	S	S
433	F1IR71279///KDML105-10-2-4-6	MS	MS	498	F1IR71279///KDML105-10-8-9-2	S	MS
434	F1IR71279///KDML105-10-2-4-6	MS	MS	499	F1IR71279///KDML105-10-8-9-4	S	S
435	F1IR71279///KDML105-10-2-4-6	S	S	500	F1IR71279///KDML105-10-8-9-4	MS	S
436	F1IR71279///KDML105-10-2-4-6	S	S	501	F1IR71279///KDML105-10-8-9-4	MS	MR
437	F1IR71279///KDML105-10-2-4-6	S	S	502	F1IR71279///KDML105-10-8-9-4	MR	MR
438	F1IR71279///KDML105-10-2-4-6	S	S	503	F1IR71279///KDML105-10-8-9-6	S	S
439	F1IR71279///KDML105-10-2-4-6	S	S	504	F1IR71279///KDML105-10-8-9-6	MS	MR
440	F1IR71279///KDML105-10-3-1-2	S	S	505	F1IR71279///KDML105-10-8-9-6	S	MS
441	F1IR71279///KDML105-10-3-1-2	MS	S	506	F1IR71279///KDML105-10-8-9-6	S	S
442	F1IR71279///KDML105-10-3-1-2	S	-	507	F1IR71279///KDML105-10-8-9-6	MS	S
443	F1IR71279///KDML105-10-3-1-2	S	S	508	F1IR71279///KDML105-10-8-9-6	S	S
444	F1IR71279///KDML105-10-3-1-2	S	S	509	F1IR71279///KDML105-10-8-9-6	S	S
445	F1IR71279///KDML105-10-3-1-5	S	S	510	F1IR71279///KDML105-10-8-9-6	MR	MS
446	F1IR71279///KDML105-10-3-1-5	S	S	511	F1IR71279///KDML105-10-8-9-6	MS	MS
447	F1IR71279///KDML105-10-3-1-5	S	S	512	F1IR71279///KDML105-10-8-9-6	MS	S
448	F1IR71279///KDML105-10-3-1-5	S	S	513	F1IR71279///KDML105-10-8-9-6	MR	S
449	F1IR71279///KDML105-10-3-1-5	S	S	514	F1IR71279///KDML105-10-8-9-6	MR	S
450	F1IR71279///KDML105-10-3-1-5	S	S	515	F1IR71279///KDML105-10-8-9-6	S	S
452	F1IR71279///KDML105-10-3-1-5	S	S	516	F1IR71279///KDML105-10-8-9-6	MS	S
453	F1IR71279///KDML105-10-3-1-5	MS	S	517	F1IR71279///KDML105-10-8-9-6	S	S
454	F1IR71279///KDML105-10-3-1-5	S	S	518	F1IR71279///KDML105-10-8-9-6	S	S
455	F1IR71279///KDML105-10-3-1-5	S	S	519	F1IR71279///KDML105-10-8-9-6	MS	S
456	F1IR71279///KDML105-10-7-4-1	S	S	520	F1IR71279///KDML105-10-8-9-6	MS	S
457	F1IR71279///KDML105-10-7-4-1	S	S	521	F1IR71279///KDML105-10-8-9-6	S	S
458	F1IR71279///KDML105-10-7-4-1	S	S	524	F1IR71279///KDML105-13-2-9-1	S	S
459	F1IR71279///KDML105-10-7-4-1	S	S	527	F1IR71279///KDML105-13-2-9-1	S	S
460	F1IR71279///KDML105-10-7-4-1	S	S	528	F1IR71279///KDML105-13-2-9-1	S	S
461	F1IR71279///KDML105-10-7-4-1	S	S	530	F1IR71279///KDML105-13-5-9-2	MS	S
462	F1IR71279///KDML105-10-7-4-1	MS	MS	533	F1IR71279///KDML105-13-5-9-2	S	S

Appendix Table 1 (Continued)

Entry No.	Pedigree	Reaction to BPH		Entry No.	Pedigree	Reaction to BPH	
		UBN	PTT			UBN	PTT
463	F1IR71279///KDML105-10-7-4-1	MS	S	534	F1IR71279///KDML105-13-5-9-2	S	S
464	F1IR71279///KDML105-10-7-4-1	MR	S	535	F1IR71279///KDML105-13-5-9-2	S	MS
465	F1IR71279///KDML105-10-7-4-1	MS	MS	536	F1IR71279///KDML105-13-5-9-2	MS	S
466	F1IR71279///KDML105-10-7-4-1	MS	MS	537	F1IR71279///KDML105-13-5-9-2	MR	S
467	F1IR71279///KDML105-10-7-4-1	MR	MR	538	F1IR71279///KDML105-13-5-9-2	MS	MS
468	F1IR71279///KDML105-10-7-4-1	MR	MR	539	F1IR71279///KDML105-13-5-9-2	MS	MR
540	F1IR71279///KDML105-13-5-9-3	MS	R	607	F1IR71279///KDML105-15-10-2-3	S	S
541	F1IR71279///KDML105-13-5-9-3	S	MS	608	F1IR71279///KDML105-15-10-2-3	MR	S
542	F1IR71279///KDML105-13-5-9-3	MR	R	609	F1IR71279///KDML105-15-10-2-7	MS	MS
543	F1IR71279///KDML105-13-5-9-3	R	R	610	F1IR71279///KDML105-15-10-2-7	S	S
544	F1IR71279///KDML105-13-5-9-4	MR	MR	611	F1IR71279///KDML105-15-10-2-7	MS	S
545	F1IR71279///KDML105-13-5-9-4	MS	MS	612	F1IR71279///KDML105-15-10-2-7	MR	MR
546	F1IR71279///KDML105-13-5-9-4	MS	MS	613	F1IR71279///KDML105-15-11-7-4	S	S
547	F1IR71279///KDML105-13-5-9-4	MR	MR	614	F1IR71279///KDML105-15-11-7-4	MS	MS
548	F1IR71279///KDML105-13-5-9-4	MR	MR	615	F1IR71279///KDML105-15-11-7-4	S	S
549	F1IR71279///KDML105-13-5-9-4	MS	MR	616	F1IR71279///KDML105-15-11-7-4	S	MS
550	F1IR71279///KDML105-13-5-9-4	MS	MR	617	F1IR71279///KDML105-15-11-7-4	S	S
551	F1IR71279///KDML105-13-5-9-4	MR	MS	618	F1IR71279///KDML105-15-11-7-6	S	S
552	F1IR71279///KDML105-13-5-9-4	S	S	619	F1IR71279///KDML105-15-11-7-6	MS	S
553	F1IR71279///KDML105-13-5-9-4	MR	R	620	F1IR71279///KDML105-15-11-7-6	MS	S
554	F1IR71279///KDML105-13-5-9-4	MS	MR	621	F1IR71279///KDML105-15-11-7-6	S	S
555	F1IR71279///KDML105-13-5-9-4	S	S	622	F1IR71279///KDML105-15-11-7-6	MR	S
556	F1IR71279///KDML105-13-5-9-4	S	S	623	F1IR71279///KDML105-15-11-7-6	MR	S
557	F1IR71279///KDML105-13-5-9-4	MS	S	624	F1IR71279///KDML105-16-2-7-4	MR	MS
558	F1IR71279///KDML105-13-5-9-4	S	S	625	F1IR71279///KDML105-16-2-7-4	MR	MS
559	F1IR71279///KDML105-13-5-9-4	S	S	626	F1IR71279///KDML105-16-2-7-4	MR	MR
560	F1IR71279///KDML105-13-5-9-4	S	S	627	F1IR71279///KDML105-16-2-7-4	MR	R
561	F1IR71279///KDML105-13-6-3-4	MS	S	628	F1IR71279///KDML105-16-2-7-4	MR	R
562	F1IR71279///KDML105-13-6-3-4	MR	S	629	F1IR71279///KDML105-18-10-8-2	MR	R
563	F1IR71279///KDML105-13-6-3-4	S	S	630	F1IR71279///KDML105-18-10-8-2	MR	R
564	F1IR71279///KDML105-13-6-3-4	MR	MS	631	F1IR71279///KDML105-18-10-8-2	MR	MR
565	F1IR71279///KDML105-13-6-3-4	MR	MR	632	F1IR71279///KDML105-18-10-8-2	MR	R
566	F1IR71279///KDML105-13-6-3-4	MS	MS	633	F1IR71279///KDML105-18-10-8-2	MR	MR
567	F1IR71279///KDML105-13-6-3-4	MS	MS	634	F1IR71279///KDML105-18-10-8-2	MS	MR
568	F1IR71279///KDML105-13-6-3-5	MS	S	635	F1IR71279///KDML105-18-10-8-2	S	MS
569	F1IR71279///KDML105-13-6-3-5	MS	MS	636	F1IR71279///KDML105-18-10-8-2	MR	MR
570	F1IR71279///KDML105-13-6-3-5	MR	S	637	F1IR71279///KDML105-18-10-8-2	MS	S
571	F1IR71279///KDML105-13-6-3-5	MR	MS	638	F1IR71279///KDML105-18-10-8-2	MS	MS
572	F1IR71279///KDML105-13-6-3-5	S	S	639	F1IR71279///KDML105-18-10-8-2	S	MS
573	F1IR71279///KDML105-13-6-3-5	MR	S	640	F1IR71279///KDML105-19-6-3-4	S	S

Appendix Table 1 (Continued)

Entry No.	Pedigree	Reaction to BPH		Entry No.	Pedigree	Reaction to BPH	
		UBN	PTT			UBN	PTT
575	F1IR71279///KDML105-13-6-3-5	S	S	641	F1IR71279///KDML105-19-6-3-4	S	S
576	F1IR71279///KDML105-13-6-3-5	MS	S	642	F1IR71279///KDML105-19-6-3-4	S	S
579	F1IR71279///KDML105-14-6-1-3	MR	S	643	F1IR71279///KDML105-19-6-3-4	S	S
580	F1IR71279///KDML105-14-6-1-3	S	S	644	F1IR71279///KDML105-19-16-11-7	MS	MS
581	F1IR71279///KDML105-14-6-1-3	MR	MR	645	F1IR71279///KDML105-19-16-11-7	S	S
582	F1IR71279///KDML105-14-6-1-3	MS	S	646	F1IR71279///KDML105-19-16-11-7	S	S
583	F1IR71279///KDML105-14-6-1-3	S	S	647	F1IR71279///KDML105-33-4-4-2	MS	MS
584	F1IR71279///KDML105-14-6-1-3	MS	MS	648	F1IR71279///KDML105-33-4-4-2	MR	MR
585	F1IR71279///KDML105-14-6-1-3	MR	MR	649	F1IR71279///KDML105-33-4-4-2	MS	MR
587	F1IR71279///KDML105-14-6-1-3	S	S	650	F1IR71279///KDML105-33-4-4-2	MS	S
588	F1IR71279///KDML105-15-3-7-2	MR	R	652	F1IR71279///KDML105-35-5-4-2	MS	S
589	F1IR71279///KDML105-15-3-7-2	MS	R	653	F1IR71279///KDML105-35-5-4-2	MS	S
590	F1IR71279///KDML105-15-3-7-2	MR	R	654	F1IR71279///KDML105-35-5-4-2	MS	S
591	F1IR71279///KDML105-15-3-7-2	MS	MS	655	F1IR71279///KDML105-35-5-4-2	MS	MR
592	F1IR71279///KDML105-15-3-7-4	MR	MR	656	F1IR71279///KDML105-35-5-4-2	S	S
593	F1IR71279///KDML105-15-3-7-4	MR	MR	657	F1IR71279///KDML105-35-5-4-2	S	MR
594	F1IR71279///KDML105-15-3-7-4	S	S	658	F1IR71279///KDML105-10-3-1-4	MS	MR
595	F1IR71279///KDML105-15-3-7-4	MR	S	659	F1IR71279///KDML105-10-3-1-4	MS	S
596	F1IR71279///KDML105-15-3-7-4	S	S	660	F1IR71279///KDML105-10-3-1-4	MS	S
597	F1IR71279///KDML105-15-3-7-4	S	S	662	F1IR71279///KDML105-10-4-7-5	MR	MR
598	F1IR71279///KDML105-15-3-7-4	MS	S	664	F1IR71279///KDML105-16-3-6-5	MR	MR
599	F1IR71279///KDML105-15-3-7-4	S	S	665	F1IR71279///KDML105-19-6-3-5	MR	MR
600	F1IR71279///KDML105-15-3-7-4	S	S	666	F1IR71279///KDML105-19-6-3-5	S	MR
601	F1IR71279///KDML105-15-3-7-4	MS	S	667	F1IR71279///KDML105-19-11-5-8	S	S
602	F1IR71279///KDML105-15-10-2-3	S	S	668	F1IR71279///KDML105-19-11-5-8	S	S
603	F1IR71279///KDML105-15-10-2-3	MS	MS	669	F1IR71279///KDML105-19-15-15-6	S	S
604	F1IR71279///KDML105-15-10-2-3	S	MR	670	F1IR71279///KDML105-19-15-15-6	MS	S
605	F1IR71279///KDML105-15-10-2-3	MS	MS	671	F1IR71279///KDML105-33-15-4-3	S	S
606	F1IR71279///KDML105-15-10-2-3	S	S				