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THESIS

GENETIC DIVERSITY IN RICE BASED ON SSR MARKERS, MORPHO-AGRONOMIC CHARACTERS AND RESISTANCE TO BROWN PLANTHOPPER (*Nilaparvata lugens* Stål)

FISSEHA WOREDE KIRKOS

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The availability of and access to diverse genetic materials is important to be successful in any plant breeding effort. Three investigations were undertaken to assess genetic diversity of rice genotypes based on SSR markers, morpho-agronomic traits and resistance to brown planthopper.

In the SSR analysis, a total of 144 alleles were detected at the 29 SSR primer pairs evaluated in the 24 cultivars. The mean expected hetrozygosity (H_e) was 0.659. Cluster analysis divided the cultivars into two major groups. The SSR diversity analysis showed the presence of higher allelic diversity in the cultivars analyzed. The morpho-agronomic traits diversity showed that days to flowering, plant height, culm length, panicle length, biomass, seed length and shape were the important traits in differentiating the genotypes. The result of the brown planthopper screen experiment showed that out of the 22 genotypes tested, only Ptb33 and Rathu Heenati were resistant to all the brown planthopper populations used. Two genotypes, IR64 and RD31, were moderately resistant. However, NERICA3, NERICA4, Azucena, CH1 and TN1 were susceptible to all the six populations.

Generally, the hierarchical cluster and PC analyses were in agreement in grouping the genotypes in all experiments. Based on the results, crossing of KOH1 with Azucena, CH1, KDML105 and SPR1 may result in heterotic expression in the F_1 hybrids. NERICA cultivars, which showed greater SSR diversity, could be used as parents in future breeding programs. The resistant genotypes, Rathu Heenati and Ptb33 could be used as sources of resistance for BPH in Central Thailand.

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Fisseha Worede Kirkos
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LIST OF ABBREVIATIONS

AFLP = Amplified Fragment Length Polymorphism
AIC = Akaike’s Information Criterion
BPH = Brown planthopper
df = degree freedom
DNA = Deoxyribo neucleic acid
dNTPs = Deoxynucleotide Triphosphates
F_2 = Second filial generation
FAO = Food and agriculture organization
GSV = Grassy stunt virus
H_e = Expected heterozygosity
IRRI = International Rice Research Institute
MS = Mean Square
Ne = Number of effective alleles
NERICA = New Rice for Africa
PAST = PAlaeontological STatistics
PC = Principal Component
PCA = Principal Component Analysis
PCoA = Principal Coordinate Analysis
PCR = Polymerase chain reaction
PIC = Polymorphism information content
QTL = Quantitative trait locus
RAPD = Random Amplified Polymorphic DNA
RSV = Rugged stunt virus
RFLP = Restriction Fragment Length Polymorphism
SE = Standard Error
SNP Single Nucleotide Polymorphism
SSLP = Simple Sequence Length Polymorphism
SSR = Simple Sequence Repeat
UHe = Unbiased expected heterozygosity
UPGMA = Unweighted Pair Group Method with Arithmetic Averages

GENETIC DIVERSITY IN RICE BASED ON SSR MARKERS, MORPHO-AGRONOMIC CHARACTERS AND RESISTANCE TO BROWN PLANTHOPPER (*Nilaparvata lugens* Stål)

INTRODUCTION

Rice is the world's most important food crop and a primary source of food for more than half of the world's population. More than 90% of the world's rice is grown and consumed in Asia. It accounts for 35-75% of the calories consumed by more than 3 billion Asians. The crop is planted on about 11% of the world's cultivated land (Khush, 2005). In the year 2012 about 163.46 million ha of land was used for rice production worldwide. In the same year 10.58 and 12.6 million ha of land was allocated for rice in the content of Africa and in Thailand, respectively (FAO, 2013). However, rice productivity is generally constrained by uncertain water supply, low soil fertility, pest infestations and poverty (Dawe, 2007; Wade *et al*., 1999). Planthoppers are one of the major insects which cause yield loss by inflicting direct damage to rice (Heinrichs, 1986). Of the six kinds of planthoppers, brown planthopper (BPH; *Nilaparvata lugens* Stal) is the most damaging insect pest of rice in Asia. It feeds by phloem abstraction and causes hopper burn, and transmits viral diseases such as grassy- and ragged stunt-viruses (Jena and Kim, 2010).

Genetic diversity has a multitude of importance. The amount of genetic diversity within species is essential for the survival of species and their adaptation to changing environments (Gao, 2003; Rao and Hodgkin, 2002). This knowledge is valuable for germplasm conservation; individual, population and variety identification; and for the improvement of crop plants and for studying the evolutionary ecology of populations (Duran *et al*., 2009). Moreover, the information helps to study heterosis as genetic diversity between parents is generally related to the magnitude of heterosis (Virk *et al*., 2003). This shows the necessity of divergent parents in any crossing program in order to be benefited from the hybrid vigor in the F_1, and from the variability created in the subsequent segregating population. Plant breeding involves the continuous development and release of improved cultivars of crop plants and the success of this endeavor depends on availability of and access to a pool of diverse genetic materials (Hoisington *et al*., 1999; Maxted *et al*., 2002).

Although major advances has been made in rice production, a significant number of people in developing countries could not satisfy their daily needs. Any attempt towards alleviating the problem of food shortage by improving productivity will always be challenging. To meet the challenges of producing more rice from the existing lands, varieties with higher yield potential and greater stability are needed (Khush, 2005).

Among the management options, host-plant resistance is a better way to control rice pests as it is free to the farmer, environmentally friendly and compatible with other control measures (Heinrichs, 1986). The search for resistant varieties of rice and incorporation of resistance factors into modern varieties has been the major strategy to control BPH (Claridge and den Hollander, 1983). Numerous sources of genetic resistance to the BPH are available in the rice germplasm and that resistance appears to be compatible with other important traits. However, the development of biotypes capable of surviving on resistant plants is a major threat to the stability of varietal resistance and complicated breeding for resistance to this pest (Heinrichs, 1986; Pathak and Khush, 1979).

Evaluation of genetic resources for various agronomic traits and the assessment of the amount of genetic variation within them is useful to allow more efficient genetic improvement (Haussmann *et al*., 2004). In order to further improve the already adapted crop varieties for traits of agronomic importance, crossing with other parents with proved agronomic superiority is necessary. This calls for the assessment of genetic diversity and identification of parental lines for hybridization or crossing.

Different molecular markers, Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR), have been used to study genetic diversity in rice. These days a number of rice SSR markers are available (Chen *et al*., 1997; McCouch *et al*., 1997, 2002; Temnykh *et al*., 2000, 2001), and these markers have been extensively utilized to study genetic diversity both in weedy and cultivated rice (Cao *et al*., 2006; Faivre-Rampant *et al*., 2010; Giarrocco *et al*., 2007; Lapitan *et al*., 2007; Saini *et al*., 2004; Shivrain *et al*., 2010; Thomson *et al*., 2007; Tu *et al*., 2007 and Zhao *et al*., 2009).

It is, therefore, imperative to assess genetic diversity of rice genotypes by using molecular markers and morpho-agronomic traits and to identify important characters in differentiating the genotypes. There is also a need to continuously evaluate rice genotypes for their resistance to different BPH populations, and monitor the shift or development of new biotypes.

OBJECTIVES

Overall objectives:

To study the extent of genetic diversity and relationships within rice cultivars using Simple Sequence Repeat (SSR) DNA markers, and morphological and agronomic characters and investigate whether this variation is good enough to design further breeding program; and to study the resistance of rice genotypes to six population of BPH from central Thailand.

Specific objectives:

1. To study the genetic diversity and relationships of rice cultivars using Simple Sequence Repeat (SSR) markers.

2. To study the genetic diversity and relationships of rice cultivars using morphological and agronomic traits, and to identify the most important traits in differentiating the cultivars.

3. To study the resistance of rice genotypes to six population of BPH from central Thailand.

LITERATURE REVIEW

Genetic diversity

Genetic diversity, variation in genes and genotypes (Rao and Hodgkin, 2002) or sequence variation within species (Duran *et al*., 2009), is of paramount importance for the success of any plant breeding program; and the conservation of plant diversity is of critical importance, because of the direct benefits that can arise from its exploitation in improved agricultural crops (Maxted *et al*., 2002).

Plant breeding depends on the correct combination of specific alleles at the genetic loci present in a plants' genome (Hoisington *et al*., 1999). The amount of genetic diversity within species is essential for the survival of species and their adaptation to changing environments (Gao, 2003). This knowledge is valuable for germplasm conservation; individual, population and variety identification (Duran *et al*., 2009). The large-scale cultivation of genetically uniform cultivars has increased the genetic vulnerability of many crops, often with disastrous consequences (Rao and Hodgkin, 2002). The 1840s Irish potato famine, due to the susceptibility of the varieties to leaf blight, is a case in point. In addition to being a means of adaptation to precarious environments and conservation, information on genetic diversity and relationships among and between individuals, populations, plant varieties and species is of importance for the improvement of crop plants and for studying the evolutionary ecology of populations (Duran *et al*., 2009).

Assessment of genetic diversity

The analysis of genetic diversity and relatedness between or within different populations, species, and individuals is a central task (Weising *et al*., 2005). Different markers, morphological, isozyme and molecular (Karp *et al*., 1997) methods could be used to assess or measure genetic diversity in crop plants. Marker technology based on polymorphisms in proteins or DNA has catalyzed research in a variety of disciplines including plant breeding (Weising *et al*., 2005).

Morphological markers

Morphological characters, the traditional approach to measure diversity (Karp *et al*., 1996), have long been used to evaluate systematic relationships; and to discriminate cultivars and breeding lines (Weising *et al*., 2005). Morphological markers are manifested on the outside of the organism as a product of the interaction of genes and the environment (Acquaah, 2007). Therefore, they are strongly influenced by the environment, and consequently, special breeding programs and experimental designs are needed to distinguish genotypic from phenotypic variation (Weising *et al*., 2005). Breeding effort with these markers is time consuming, labor intensive and the large populations of plants required need large space in which to be grown (Stuber *et al*., 1999).

However, these markers are still important in genetic diversity studies. A number of works have been reported by using different morphological and agronomic traits in rice. By evaluating 434 landraces for various morphological characters (traits) and employing Principal Component (PC) analysis, Sanni *et al*. (2012) reported the importance of days to heading in the first; tiller number, filled tillers, grain length and 100-seed weight in the second; and grain width and shape in the third PC axes. In an attempt to study the diversity of 78 improved varieties released from 1965-1995 by using 33 qualitative and quantitative traits, Caldo *et al*. (1996a) estimated a range of Euclidean distance varying between 2.23 to 16.71 with a mean of 7.55. In their result, 7 components accounted for 75% of the total variation; plant height and culm length in the first; productive tillers and culm number in the second; and maturity and heading in the third PC axes were some of the important traits.

The diversity analysis of 51 landraces for 18 morphological characters reported by Chakravorty *et al*. (2013) explained the total variability by six PC axes. In their study, leaf length, plant height, culm diameter and number, and panicle length contributed most to the first PC; leaf length and grains per panicle to the second PC; grain breadth, grain length/ breadth ratio to the third; flag-leaf angle, maturity and sterile-lemma lenght in the fourth; grain length in the fifth; and culm length in the sixth PC axes. In a study of 81 ancestral lines of Philippines modern rice varieties, Caldo *et al*. (1996b) used 41 traits to explain the total variability. The pairwise Euclidean distance estimates ranged from 3.97 to 17.389 with a mean of 8.80. In their work, the first 10 principal components contributed to 67% the total variation; in which plant height, heading and maturity, culm and leaf lenght were importan in the first PC axis, however, productive tillers and culm number; and seed coat colour, culm strength, lemma- and palea-colour were important in the second and the third PC axes, respectively.

Biochemical markers

Isozymes are the most widely used protein (biochemical) markers in plant breeding and applied genetics. They are multiple forms of an enzyme that differ from each other by the substrate they act on, their maximum activity, or their regulatory properties. Isozyme methods are fairly simple and inexpensive. However, their use is limited by the insufficient number of assays available, their uneven distribution in the genome and their sensitivity to tissue type and age (Acquaah, 2007; Tanksley, 1983).

Molecular markers

The detection of polymorphisms in restricted genomic DNA of plants has paved the way to the development of molecular markers for plant breeding. Molecular markers are detected at the subcellular level and can be assayed before the adult stage in the life cycle of the organism. They are also used to connect the gene action underlying a specific phenotype with the distinct regions of the genome in which the gene resides (Acquaah, 2007; Winter and Kahl, 1995). Molecular techniques vary in the way that they resolve genetic differences, in the type of data they generate and in the taxonomic levels at which they can be most suitably applied (Karp *et al*., 1996). DNA based markers have many advantages over phenotypic (morphological) markers in that they are highly heritable, relatively easy to assay, and are not affected by the environment. Several molecular marker technologies have been developed and applied for genome analysis, mainly assessing the differences between individuals within a species (Duran *et al*., 2009).

Ideal DNA markers should have the following desirable properties: Easily availability, easy and rapid assay, high polymorphism and reproducibility, codominant inheritance and recurrent occurrence in the genome, selective neutrality to environmental conditions or management practices, and easy data exchange between different laboratories (Sharma *et al*., 2008). DNA markers are classified as hybridization- and polymerase chain reaction (PCR)-based depending upon how the polymorphism is revealed. In hybridization based markers, DNA profiles are visualized by hybridizing restriction endonuclease digested DNA fragment to a labeled probe while PCR based markers involve *in vitro* amplification of particular DNA sequences with the help of primers and a DNA polymerase enzyme (Karp *et al*., 1997; Kumar, 1999; Sharma *et al*., 2008).

RFLP employs the use of restriction endonucleases to cut genomic DNA at specific recognition sequences. These markers are co-dominant, and are very reliable, since they can distinguish all the three morphs. However, the generation of RFLP markers and their application is time-consuming, expensive and also requires large amounts of DNA (Jones *et al*., 1997; Winter and Kahl, 1995). Kojima *et al*. (2005) studied genetic diversity in cultivated rice by using RFLP marker.

RAPDs are dominant markers due to their presence or absence at particular loci, and they will segregate from a heterozygous diploid as Mendelian alleles. RAPDs are much simpler and less expensive to work with than RFLPs because no prior knowledge of sequences is required and there is no need for radioactive probes (Jones *et al*., 1997). However, the difficulty of achieving robust profiles makes its reliability for ‘typing’ questionable (Karp *et al*., 1996). RAPD has been used to study genetic diversity in both weedy (Yu *et al*., 2005) and cultivated (Ravi *et al*., 2003) rice species.

AFLP DNA fingerprinting technique involves restriction of the DNA and ligation of oligonucleotide adapters, and selective PCR amplification of restriction fragments. Fingerprints are produced without prior sequence knowledge using a limited set of generic primers. The restriction fragments are generated by rare- and frequent-cutter restriction enzymes. The technique detects genomic restriction fragments and resembles in that respect the RFLP. The technique displays presence or absence of restriction fragments rather than length differences (Vos *et al*., 1995). The AFLP system is technically difficult and expensive to set up, but it detects a large number of loci, reveals a great deal of polymorphism and produces high complexity DNA fingerprints which can be used for identification and for high resolution mapping and marker assisted cloning (Jones *et al*., 1997). AFLPs appear to be as reproducible as RFLPs, but they require more DNA than RAPDs. Because of their large genome coverage, AFLPs are particularly good for mapping and fingerprinting. They do, however, share many of the limitations, with respect to band homologies and identities as that of RAPDs (Karp *et al*., 1996). Genetic diversity study in rice by using AFLP has been reported (Hashimoto *et al*., 2004; Saini *et al*., 2004).

Single Nucleotide Polymorphism (SNP) are single-base pair positions in the genomes, at which different sequence alternatives (alleles) exist. Although SNP locus can have 2-4 alleles in a population, biallelic SNPs are common. SNPs are highly abundant, but their density differs in different regions of a genome and from genome to genome, and from species to species (Weising *et al*., 2005). Because of their abundance and codominance, the use of SNPs as a marker system has the potential for providing the highest map resolution (Nasu *et al*., 2002). SNP analysis is essential to identify specific point mutations (Shirasawa *et al*., 2004). SNPs are applicable to studies of genetic diversity, marker-assisted breeding, positional cloning, cultivar discrimination for quality control (Nasu *et al*., 2002) and in linkage studies for many purposes such as determination of loss of heterozygosity. They are more prevalent than microsatellites and that many SNPs are located within the genes, directly affecting the gene product or protein (Ahmadian *et al*., 2000).

Microsatellites or SSRs are one of the predominant and very abundant sequence based marker systems (Duran *et al*., 2009; Winter and Kahl, 1995). SSRs are random tandem repeats of 2-5 nucleotides that occur in microsatellites and they detect Simple Sequence Length Polymorphism (SSLP). The copy number of these repeats varies among individuals and is a source of polymorphism in plants (Acquaah, 2007; Chen *et al*., 1997). The highly mutable nature of SSR loci increases the possibility of having many allelic variants at each locus (Karp *et al*., 1996). PCR-amplified microsatellite or SSR markers are rapidly displacing RFLPs for many kinds of genetic studies, largely because of their technical simplicity, the small amount of starting DNA required, the relatively low cost for users, rapid turn-around time, multiallelic nature, codominant transmission, relative abundance, extensive genome coverage, hypervariability (high information content), reproducibility and high power of genetic resolution (Chen *et al*., 1997; Powell *et al*., 1996; Weising *et al*., 2005).

Different workers studied genetic diversity using microsatellite or SSR markers. By using 20 SSR primer pairs, Cao *et al*. (2006) reported a relatively high (expected heterozygosity; H_e= 0.313) overall genetic diversity of weedy rice (*Oryza sativa* f. spontanea) populations from Liaoning, with about 35% of the genetic variation found among regions. The result also showed that the weedy rice populations were closely related to rice varieties from Liaoning and *japonica* varieties from other regions but distantly related to *indica* rice varieties and wild *O. rufipogon*.

In a study of 69 Argentine rice cultivars using 26 SSR markers, Giarrocco *et al*. (2007) reported the detection of 219 polymorphic bands. Cluster analysis based on pairwise genetic similarity matrices resolved the *O. sativa* accessions into *indica* and *Japonica* groups; and the *japonica* group into *tropical* and *temperate* subgroups. They also reported the agreement of the clusters with the pedigree information. Genetic diversity analysis of 246 landrace and 63 improved Indonesian rice (*Oryza sativa* L.) germplasm by using 30 evenly-spaced SSR markers, as reported by Thomson *et al*. (2007), showed the detection of 394 alleles at 30 SSR loci, with an average number of 13 alleles per locus across all accessions, and an average polymorphism information content (PIC) value of 0.66. The analysis characterized the landraces as 68% *indica* and 32% *tropical japonica*, with an *indica* gene diversity of 0.53 and a *tropical japonica* gene diversity of 0.56.

Faivre-Rampant *et al*. (2010) evaluated genetic diversity of 172 Italian rice varieties and 47 introduced accessions on the basis of a total of 218 alleles detected at 24 microsatellite loci. On average, they detected 9.1 SSR alleles per locus. Out of the 218 alleles, 154 were present in the Italian and 180 in the foreign accessions. PIC values ranged from 0.04 to 0.81, with an average of 0.56 across the whole collection. The major allele frequency in the whole collection ranged from 0.21 to 0.98. For the Italian accessions, the occurrence of the total number specific alleles was 38, while for the foreign ones it was 64. The two sets of accessions shared the 54% of detected alleles. Lapitan *et al*. (2010) evaluated genetic diversity of 24 rice cultivars carrying good quality traits, and 890 alleles were detected by 151 polymorphic SSR markers with an average of 5.89 per locus. Out of these markers 89 generated a total of 147 rare alleles. Based on Shannon’s diversity index, an overall diversity of 0.71 was revealed. PIC values of the markers ranged from 0.18 to 0.91 with an average of 0.68 per marker. Cluster analysis identified three groups at 40% level of similarity with additional subclusters within each group. From the study they concluded that SSR markers classified the cultivars according to their subspecies where *indica* gave more alleles than *japonica*, and genetic diversity of *indica* was high on chromosome 11, while that of *japonica* was high on chromosome 2.

In a study of assessing the extent of genetic variation in 39 traditional and 11 improved varieties widely planted in Cuba, Alvarez *et al*. (2007) estimated number of alleles per microsatellite loci ranging from 4 to 8, averaging 6.6 alleles per locus using 10 microsatellite primer pairs. Higher heterozygosity was reported for the traditional varieties ($H_{TV} = 0.72$) than the improved varieties ($H_{IV} = 0.42$), and 68% of the total microsatellite alleles were found exclusively in the traditional varieties. In their study, genetic relationship estimates based on the proportion of microsatellite loci with shared alleles indicated that the majority of traditional varieties were poorly related to the improved varieties.

Brown planthopper (BPH)

Biology and life cycle

Most of the world's rice production is from irrigated and rainfed lowland fields where insect pests are severe constraints. The major insect pests, which contribute to yield losses, are leafhoppers and planthoppers (Heinrichs, 1986). The genus *Nilaparvata* reported to have 14 determined and 2 undetermined members of species (Mochida and Okada, 1979). Among the six kinds of planthoppers, brown plant hopper (*Nilaparvata lugens* Stål) is the most important insect pest in Asia (Jena and Kim, 2010).

The eggs of BPH are usually laid as egg-groups in the tissue of the lower part of the rice plant, mainly in sheaths but also in leaf blades. But the sizes and sites of egg-groups depend upon the stages of the rice plants. When the adult population is high, eggs are found in the upper parts of the plants. The egg stage is about 7 to 11 days in the tropics. Although, the duration of each stage depends on temperature and cultivars, the nymphal stage is 10 to 15 days, and the preoviposition period averages 3 to 4 days (Mochida and Okada, 1979). Adults usually live for 10-20 days in summer and 30-50 days during autumn (Pathak and Khan, 1994).

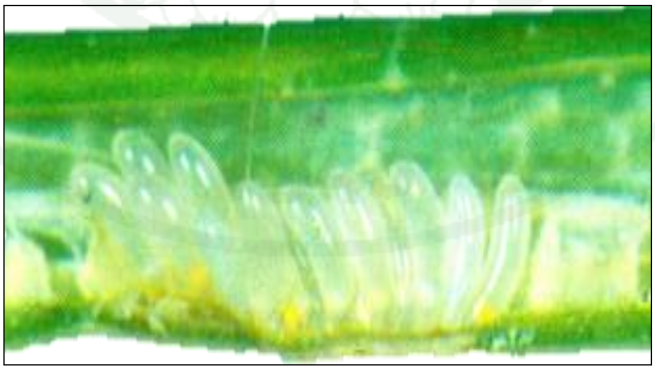


Figure 1 Eggs of BPH laid in groups on the leaf of rice.

Source: Pathak and Khan (1994)

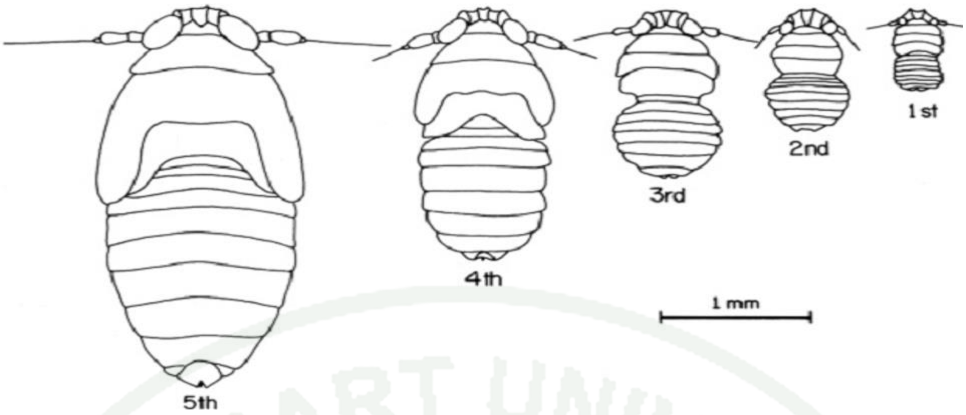


Figure 2 The five instars of brown planthopper (*Nilaparvata lugens* Stal).

Source: Mochida and Okada (1979)

The time required for the completion of embryonic and postembryonic development depends to a considerable extent upon temperature. The oviposition rate rises with temperature. The preoviposition period in macropterous females becomes shorter as the temperature rises from 20 to 30 ^{o}C; however, in brachypterous females it remains unchanged (Mochida and Okada, 1979). Two major generations develop before the crop matures. Insect density is highest when nymphs dominate the population. Most dispersion of adults from the field takes place near crop maturity (Dyck *et al*., 1979). BPH is characterized by its high migratory ability and its high reproductivity on modern, susceptible rice varieties (Kisimoto, 1979).

Nature of damage and distribution

It is widely distributed in tropical, subtropical and temperate regions of Asia, the South Pacific Islands and Australia (Dyck and Thomas, 1979; Kuno, 1979). The ecology of BPH in tropical and temperate areas is distinct (Kuno, 1979). Infestations of this species in temperate eastern Asia are initiated every year by wind-assisted long-distance migration from over-wintering areas in the tropics and subtropics (Holt *et al*., 1996). In the tropics population remain in the paddy field throughout the year. Such a difference results in different patterns of pest damage and correspondingly different strategies for efficient control (Kuno, 1979).

Since its populations are favored by some of the factors which contribute to the high yields of modern rice varieties, epidemics of the BPH have occurred frequently in several tropical countries with the spread of high-yielding varieties and intensive cultivation of rice since 1970s (Claridge and den Hollander, 1983; Heinrichs, 1986). The BPH has become destructive of rice pests in Asia primarily because of the unpredictability of the infestation and because of the severe direct damage it causes (Dyck and Thomas, 1979). It also transmits viral diseases such as ragged stunt virus (RSV) and grassy stunt virus (GSV) into rice cultivars (Khush and Brar, 1991; Pathak and Khan, 1994).

A number of reports are available about the economic importance of the insect in Asia. For instance, in china, Indonesia and Vietnam a BPH damage of rice on 9.4 million, 28421 and 348927 hectares of land was observed in 2006, respectively. A study of relative abundance of BPH for ten years (1998-2007) showed an increasing trend of general damage in China and Vietnam (Catindig *et al*., 2009).

Figure 3 The damage (hopperburn) on rice caused by brown planthopper.

Source: IRRI (2009)

Plants infested by the BPH before maximum tillering usually have fewer panicles per unit area and fewer grains per panicle; while an attack after the heading stage affects the percentage of ripened grain and grain weight (Sogawa and Cheng, 1979).

Biotypes

The natural insect populations are generally believed to include small proportions of individuals that can survive on resistant varieties. When resistant varieties are intensively planted, a population of insect that can survive on them builds up, and the general population may shift to a new insect biotype (Pathak and Khush, 1979). So far four BPH biotypes are reported: biotype 1, the type that generally exists at IRRI; biotype 2, capable of surviving on plants which carry the *Bph1* gene for resistance and biotype 3, which survives on rice varieties carrying the *bph2* gene (Pathak and Khush, 1979). The most destructive biotype is biotype 4 which occurs in the Indian subcontinent and it is also called South Asian biotype (Jena and Kim, 2010). Cultivars with *Bph1* gene confer resistance to biotypes 1 and 3 but are susceptible to biotype 2. The *bph2* gene confers resistance to biotypes 1 and 2 but not to biotype 3, and the *Bph3*, *bph4*, *bph8* and *Bph9* genes confer resistance to all the four biotypes. Genes such as *bph5*, *Bph6*, and *bph7* confer resistance to biotype 4 only (Khush and Brar 1991).

Table 1 The relationship between biotypes of BPH and resistance genes from diverse sources.

Variety/source	**Gene**	**Reaction to biotypes***			
		1	**2**	**3**	**4**
Mudgo	*Bph1*	R	S	R	S
ASD7	*bph2*	R	R	S	S
Rathu Heenati	*Bph3*	R	R	R	R
Babawee	*bph4*	R	R	R	R
ARC10550	*bph5*	S	S	S	R
Swarnalata	*Bph6*	S	S	S	R
T12	*bph7*	S	S	S	R
Chin Saba	*bph8*	R	R	R	-
Balamawee	*Bph9*	R	R	R	-
TN1	*none*	S	S	S	S
O. officinalis (acc.100896)	*Bph6, Bph13*	R	R	R	S
O. minuta (acc.101141)	*Bph20, Bph21*	R	ND	ND	ND
O. latifolia (B14)	*Bph12*	ND	R	ND	ND
O. australiensis (acc.100882)	*Bph18*	R	R	R	R

*R = resistant, S = susceptible, ND = no data

Source: Jena and Kim (2010)

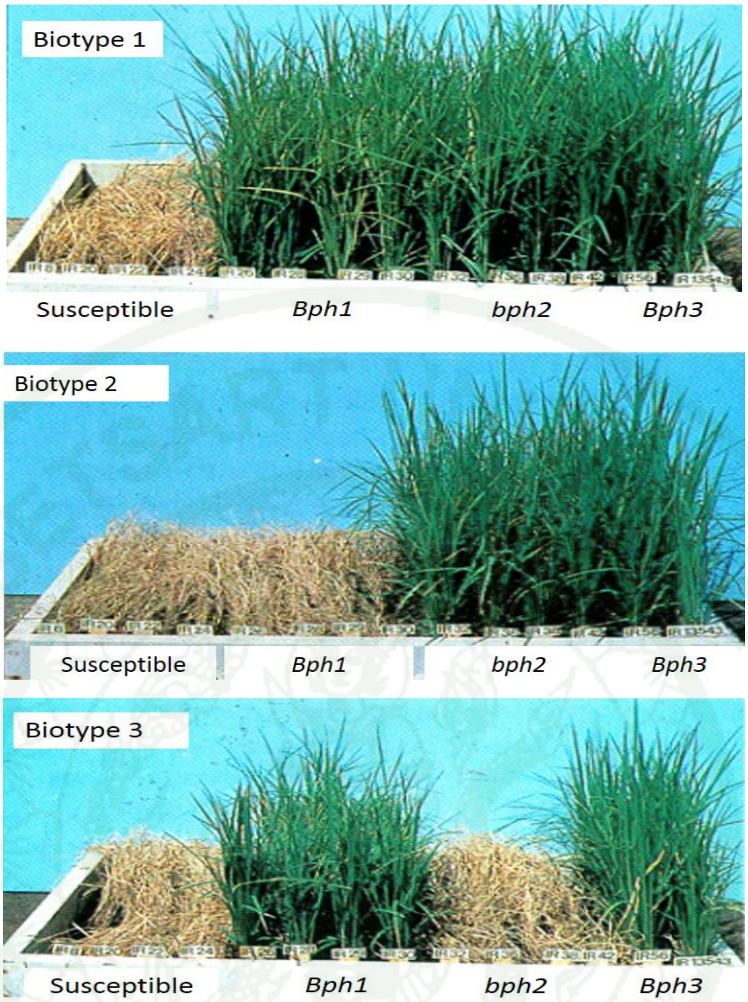


Figure 4 Reactions of different rice varieties with *Bph1*, *bph2* and *Bph3* genes to the three BPH biotypes.

Source: Pathak and Khan (1994)

Factors affecting BPH outbreaks

The BPH population responds positively to a greater number of tillers per square meter, which may be achieved through close spacing or a high number of tillers per hill. In a critical experiment at IRRI, it appears to multiply best on plants in the 20-days period before flowering. It was also concluded that the insect changes during a crop's life are influenced by crop age and the pattern of insect generations, which is rather distinct in a given field (Dyck *et al*., 1979). Besides killing natural enemies of BPH, insecticides, when used at sub-lethal doses, increase reproduction, fecundity and migratory capacity of BPH (Chelliah *et al*., 1980; Wu *et al*., 2001; Yin *et al*., 2008). Moreover, it causes resurgence and secondary outbreaks of BPH (Bottrell and Schoenly, 2012). Increased plant nitrogen, as a result of higher doses of nitrogen fertilizer, increase survival of nymphs, fecundity, and population growth and the tendency for BPH outbreaks (Lu and Heong, 2009; Visarto *et al*., 2001).

Brown planthopper in Thailand

The incidence of BPH in Thailand has been reported since 1973 with the introduction of double cropping and high-yielding varieties. Outbreaks were increasing with the expansion of irrigated rice production and the continuous cultivation of high-yielding varieties (Pongprasert and Weerapat, 1979). A damage on 535,190 ha of rice field was recorded in 1998 (Catindig *et al*., 2009). Rice varieties have been screened for resistance to the BPH since 1972 (Pongprasert and Weerapat, 1979) and many resistant varieties have been released.

Varietal resistance to brown planthopper

Host plant resistance is a better way to control rice pests as it is essentially free to the farmer, environmentally friendly and compatible with other control measures (Heinrichs, 1986). The search for resistant varieties of rice and incorporation of resistance factors into modern varieties has been the major strategy for the control of BPH (Claridge and den Hollander, 1983). A wide variety of sources of genetic resistance to the BPH is available in the rice germplasm and that resistance appears to be compatible with other desirable plant characters. However, the development of biotypes capable of surviving on resistance plants is a major threat to the stability of varietal resistance and complicated breeding for resistance to this pest (Heinrichs, 1986; Pathak and Khush, 1979). Screening for resistance to brown planthopper started in 1967 at IRRI and a number of varieties with high level of resistance were identified; IR26 being the first high-yielding resistant variety released (Heinrichs, 1986).

Table 2 Genes and their sources of resistance to BPH in rice varieties developed by IRRI.

Variety	BPH resistance gene	Genetic source
IR26	*Bph1*	Mudgo
IR28	*Bph1*	Mudgo
IR29	*Bph1*	Mudgo
IR30	*Bph1*	Mudgo
IR32	*bph2*	ASD7
IR34	*Bph1*	Mudgo
IR36	*bph2*	ASD7
IR38	*bph2*	ASD7
IR40	*bph2*	ASD7
IR42	*bph2*	ASD7
IR44	*Bph1*	Mudgo
IR45	*Bph1*	Mudgo
IR46	*Bph1*	Mudgo
IR48	*bph2*	ASD7
IR50	*bph2*	ASD7
IR52	*bph2*	ASD7
IR54	*bph2*	ASD7
IR56	*Bph3*	Rathu Heenati
IR58	*Bph3*	Rathu Heenati
IR60	*Bph3*	Rathu Heenati
IR62	*Bph3*	Rathu Heenati
IR64	*Bph1*	Mudgo
IR65	*bph2*	ASD7
IR66	*Bph4*	Babawee
IR68	*Bph3*	Rathu Heenati
IR70	*Bph3*	Rathu Heenati
IR72	*Bph3*	Rathu Heenati
IR74	*Bph3*	Rathu Heenati

Source: Jena and Kim (2010)

Genetics of resistance to brown planthopper

Genetic analysis of resistance to brown planthopper in rice has been executed by different researchers. Athwal *et al*. (1971) reported that BPH resistance in an Indian local variety (Mudgo) and two other breeding lines (CO22 and MTU15) was controlled by *Bph1*, a dominant gene. A recessive gene, *bph2*, which was reported to be either allelic or closely linked to *Bph1*, was also identified in a breeding line ASD7 by the same workers. Two other BPH resistance genes, *Bph3* and *bph4* were identified in Sri Lankan local varieties Rathu Heenati and Babawee, respectively (Lakshminarayana and Kush, 1977). Latter, resistance genes *bph5* in ARC10550, *Bph6* in Swarnalata and *bph7* in T12 were identified by Kabir and Kush (1988) by using BPH biotypes of Bangladesh. Additional resistance genes, *bph8* in Thai Col. 5, Thai Col. 11 and Chin Saba; *Bph9* in local varieties of Sri Lanka, Pokkali, Balamawee and Kaharamana were identified (Ikeda and Kaneda, 1986; Nemoto *et al*., 1989).

Ishii *et al*. (1994) reported the introgression of a dominant gene *Bph10(t)*, which was identified in *Oryza australiensis* and introgressde into *indica* breeding line. Moreover, Yang *et al*. (2002) identified a BPH resistance gene carried by 'B14' derived from *Oryza latifolia* and determined its chromosomal location by SSR and RFLP analysis. They reported that a single dominant gene, which they tentatively assigned it as *Bph12(t)* and mapped on the short arm of chromosome 4 was responsible for the resistance. Liu *et al*. (2001) identified a BPH resistance gene carried by an accession of *Oryza eichingeri* (acc 105159) from Uganda and determined its chromosomal location by using SSR and RFLP analysis. Their report showed that a single dominant gene which was mapped on the long arm of chromosome 2 is responsible for BPH resistance, and they tentatively assigned it as *Bph13(t)*. Huang *et al*. (2001) reported the existence of two QTLs, *Qbp1* and *Qbp2*, for BPH resistance in a population derived from a BPH resistant line B5 on the long arm of chromosome 3 and on the short arm of chromosome 4, respectively by employing linkage and QTL analyses with RFLP markers. Later, *Qbp1* and *Qbp2* were named as *Bph14* and *Bph15*, respectively (Yang *et al*., 2004). In the attempt to construct linkage map and QTL analysis by using SSR marker with an F_2 population derived from Rathu Heenati and a susceptible cultivar 02428, Sun *et al*. (2005) reported the presence of a major resistance gene in Rathu Heenati which is located in the short arm of chromosome 4, and they tentatively designated as *Bph17*.

In other work, Li *et al*. (2006) reported the existence of two recessive resistance genes in Guangxi common wild rice (*Oryza rufipogon* Griff.) entry 2183, which they tentatively designated as *bph18(t)* and *bph19(t)*. Very recently, by using the introgression line IR71033-121-15 (derived from an interspecific cross between IR31917-45-3-2 and a wild species *O. minuta*, Acc. No. 101141) and Junambyeo (a Korean *Japonica* cultivar susceptible to BPH), Rahman *et al*. (2009) identified two BPH resistance genes carried by IR71033-121-15, introgressed from *O. minuta*. The two loci which were located on chromosome 4 and 12 were designated as *Bph20(t)* and *Bph21(t)*, respectively. By using an F_2 population derived from the cross of WB01, an introgression line resistant to brown planthopper originated from *Oryza rufipogon* Griff. and a susceptible *indica* variety 9311, Hou *et al*. (2011) reported two genes resistant to BPH, temporarily named as *bph22(t)* and *bph23(t)*, which were located on chromosomes 4 and 8, respectively. Although *bph22(t)* was reported to be physically distant from other BPH resistant genes located on chromosome 4, *bph23(t)* however was located at the same chromosomal region with the one reported by Wu *et al*. (2005).

By screening an accession of *O. rufipogon* (Acc 106412) and its derived line IR73678-6-9-B along with IR64 (recurrent parent), known gene donors and resistant (PTB33) and susceptible checks (TN1 and BPT5204) from 2005 to 2007 in glass house, Deen *et al*. (2010) reported that the *O. rufipogon* accession and introgression line (IR73678-6-9-B) showed resistance reaction similar to the resistant check PTB33. While IR64 found to be moderately resistant. Further to know whether the resistance gene in IR73678-6-9-B was introgressed from *O. rufipogon* or not, and its allelic relation with *bph4* present in Babawee, IR73678-6-9-B was crossed with IR64, Babawee and BPT5204 (susceptible variety). After analyzing the F_2 they concluded that the gene is a new gene for BPH resistance introgressed from *O. rufipogon* and, hence they tentatively designated the gene present in IR73678-6-9-B as *bph24*(t).

Myint *et al*. (2012) produced an F_2 population derived from a cross between an Indian BPH resistanct rice cultivar, ADR52 and a susceptible cultivar, Taichung 65 (T65) for QTL analysis. Antibiosis testing of the F_2 population showed that multiple loci controlled the high level of BPH resistance. Further linkage analysis using backcross populations resulted in the identification of two BPH resistance gene loci, BPH25 and BPH26, from ADR52. They reported that BPH25 co-segregated with marker S00310 on the distal end of the short arm of chromosome 6, and BPH26 co-segregated with marker RM5479 on the long arm of chromosome 12.

In order to identify the BPH resistance gene in Balamawee, He *et al*. (2013) produced an F_2 population derived by crossing Balamawee and susceptible *japonica* cultivar 02428. They identified a single major resistance gene and mapped to the long arm of chromosome 4. Further recombination analysis showed that the gene was located in an interval of about 63 kb between InDel markers Q52 and Q20. This new BPH resistance locus was designated as *Bph27(t)*.

Table 3 Chromosomal location of BPH resistance genes on molecular map of rice.

Gene	Chromosome*	Markers	References
Bph1	12	G148 (RFLP)	(Hirabayashi *et al*. 1998; Sun *et al*. 2007)
	12L	em5814N (AFLP)	Sharma *et al*. 2004
	12	BpE18-3 (STS)	Kim and Sohn 2005
	12L	XNpb248, XNpb336 (RFLP)	Hirabayashi *et al*. 1998
	12L	AFLP em5814N	Sharma *et al*. 2002
	12	OPD-7 RD7 (RAPD), RG869, RG457 (RFLP), RM247 (SSR)	Jeon *et al*. 1999
Qbp1	3L	R1925, R2443 (RFLP)	Huang *et al*. 2001
Qbp1 (Bph14t)	3	R1925, G1318 (RFLP)	Ren *et al*. 2004
bph2	12	G2140 (RFLP)	Murata *et al*. 2001
	12	RM463, RM7102 (SSR)	Sun *et al*. 2007
	12	KAM4 (STS)	Murai *et al*. 2001; Sharma *et al*. 2002
Qbph2	2L	RM6843, RM3355 (SSR)	Sun *et al*. 2006
	4S	C820, R288 (RFLP)	Huang *et al*. 2001
	2L	RFLP, SSR	Liu *et al*. 2001
	2	5529-1358 (SSR)	Liu *et al*. 2009
Bph3	6S	RM589 (SSR)	Jairin *et al*. 2007a
Qbph3	3	RM313, RM7 (SSR)	Sun *et al*. (2005)
bph4	6S	RM190 (SSR), C76A (RFLP)	Kawaguchi *et al*. (2001)
Qbph4 (Bph17)	4	RM8213, RM5953 (SSR)	Sun *et al*. (2005)
	4S	RM401, RM335 (SSR)	Liu *et al*. 2009; Yang *et al*. 2004

Table 3 (Continued)

Gene	Chromosome*	Markers	References
Bph6	11	$OPA16_{938}$ (RAPD)	Jena *et al*. 2002
Qbph6	6S	RM510, RM314 (SSR)	Sun *et al*. (2006)
Qbph7	7	RM542, RM500 (SSR)	Liu *et al*. (2009)
Bph9	12L	RM463, RM5341 (SSR)	Su *et al*. (2006)
	12L	OPR04 (RFLP), S2545 (RAPD)	Murata *et al*. (2001)
Qbph9	9L	RM3533, RM242 (SSR)	Liu *et al*. (2009)
Bph10(t)	12L	RG457 (RFLP)	Ishii *et al*. (1994)
Qbph10	10	RM484, RM496 (SSR)	Sun *et al*. (2005)
bph11(t)	3L	G1318 (RFLP)	Hirabayashi and Ogawa (1995)
Bph12(t)	4S	RM261 (SSR)	Yang *et al*. (2002)
Bph13(t)	2L	RM250, RM240 (SSR)	Liu *et al*. (2001)
Bph13(t)	3S	$AJ09b_{230}$ (RAPD), AJ09c (STS)	Renganayaki *et al*. (2002)
Bph14 (Qbp1)	3L	R1925, G1318 (RFLP)	Yang *et al*. (2004)
Bph15 (Qbp2)	4S	C820, S11182 (RFLP)	Yang *et al*. (2004)
Qbp2 (Bph15)	4S	C820, R288 (RFLP)	Ren *et al*. (2004)
Bph17(t)	4S	RM8213, RM5953 (SSR)	Sun *et al*. (2005)
Bph18	12L	RM463, S15552, 7312.T4A (STS)	Jena *et al*. (2006)
bph19(t)	3S	RM6308, RM3134 (SSR)	Chen *et al*. (2006)
Bph20(t)	4	MS10, RM5953	Rahman *et al*. (2009
Bph21(t)	12	RM3726, RM5479	Rahman *et al*. (2009)
bph22(t)	4	RM8212, RM261 (SSR)	Hou *et al*. (2011)
bph23(t)	8	RM2655, RM3572 (SSR)	Hou *et al*. (2011)
bph24(t)	-	-	Deen *et al*. (2010)
BPH25(t)	6S	S00310 (SSR)	Myint *et al*. (2012)

Table 3 (Continued)

Gene	Chromosome*	Markers	References
BPH26(t)	12L	RM5479 (SSR)	Myint *et al*. (2012)
Bph27(t)	4L	Q52, Q20 (InDel)	He *et al*. (2013)

*L=long arm, S=short arm

MATERAIALS AND METHODS

Place of study

The research work was composed of three experiments: Genetic diversity analyses in rice using Simple Sequence Repeats (SSR) markers and morpho-agronomic characters, and evaluation of BPH resistance in rice. All of the three experiments were executed in the laboratory and nursery of the Department of Agronomy, Faculty of agriculture, Kasetsart University Bangkhen campus, located at 13.8486° N, 100.568° E coordinates.

I. Genetic diversity analysis in rice using SSR markers

Plant materials

For the SSR marker diversity study, a total of 24 rice cultivars from diverse sources were used. The materials include *indica*, *japonica*, tropical *japonica*, new plant types and NERICA (New Rice for Africa; varieties developed by interspecific hybridization between *O. glaberrima* and *O. sativa*) varieties (Table 4).

DNA extraction and primer selection

DNA was extracted from fresh leaves of young rice plants, using the method described by Doyle and Doyle (1990). Agarose gel electrophoresis was used to estimate DNA concentration.

Sixty five microsatellite primer pairs covering all the twelve chromosomes selected on the basis of published rice microsatellite framework map was used (Table 5). Primer sets in the study were from the panel of 50 standard SSR markers recommended for rice diversity analysis by the Generation Challenge Program and random selections from McCouch *et al*. (2002). The original source and chromosomal positions for these markers can be found in the rice genome database (http: // www. gramene. org).

Table 4 Descriptions of the rice cultivars used for SSR and morpho-agronomic diversity studies.

Sr. No.	Variety name	Sub-species/type	Origin
1.	NERICA 3	Interspecific cross	Côte d'Ivoire
2.	NERICA 4	Interspecific cross	Côte d'Ivoire
3.	KOH1	Japonica	Japan
4.	TW1	Japonica	Taiwan
5.	TW2	Japonica	Taiwan
6.	NI	Japonica	Japan
7.	NPT4	New plant type	Philippines
8.	NPT8	New plant type	Philippines
9.	NPT13	New plant type	Philippines
10.	NPT18	New plant type	Philippines
11.	CH1	Indica	China
12.	CH2	Indica	China
13.	CH3	Indica	China
14.	PTT1	Indica	Thailand
15.	CNT1	Indica	Thailand
16.	SPR1	Indica	Thailand
17.	PSL60-2	Indica	Thailand
18.	KDML105	Indica	Thailand
19.	IR64	Indica	Philippines
20.	AZUCENA	Tropical japonica	Philippines
21.	RD31	Indica	Thailand
22.	TDK1	Indica	Laos
23.	TDK5	Indica	Laos
24.	CO39	Indica	India

Polymerase Chain Reaction (PCR) profile

Amplification reactions were carried out in 10 µl reaction mixture containing 20 ng of template DNA, 0.5 µM of each primers, 0.2 µl Phire Hot Start DNA Polymerase and 5 µl of 2x Phire Plant PCR Buffer (FINNZYMES; containing dNTPs and 1.5 mM $MgCl_2$). Reactions were performed using a MULTIGENE thermal cycler (Labnet International, Inc.) programmed as 98°C for 5 min, followed by 40 cycles of 5 sec at 98°C, 5 sec at 57°C, 30 sec at 72°C with a final extension of 1 min at 72°C. Amplification products were separated in 6% denaturing polyacrylamide gel electrophoresis and visualized by silver staining method (Benbouza *et al*., 2006). A 10 bp DNA ladder was used as a size marker to compare the molecular weights of amplified products.

Data collection and analyses

Multi-state scoring was employed to score clear SSR polymorphic bands. The common diversity parameters estimated for each SSR marker include average number of alleles, effective number of alleles (Ne), allele frequencies, gene diversity or expected heterozygosity (Nei, 1973), unbiased expected heterozygosity (Nei, 1978), Shannon's information index (I), and genetic distances (Nei, 1978). Genetic distance values were used for cluster analysis of the rice cultivars using the UPGMA (Unweighted Pair Group Method with Arithmetic Averages) algorithm. POPGENE 1.31 (Yeh *et al*., 1997) software was used for data analysis. GenAlEx 6.41 (Peakall and Smouse, 2006) software was used for principal coordinate analysis by using Nei (1978) unbiased minimum distance coefficients as input, and Tools for Population Genetic Analysis (TFPGA) 1.3 (Miller, 1997) was used for dendrogram construction.

Table 5 The description of the 65 rice SSR markers used for the diversity analysis.

Chromosome	Primer name	Total Number
1	RM495, RM1, RM283, RM259, RM312, RM129, RM5, RM237, RM543, RM431	10
2	RM154, RM452, RM6, RM208, RM535	5
3	RM489, OSR13, RM338, RM55, RM514	5
4	RM307, RM551, RM124, RM280, RM559	5
5	RM507, RM413, RM161, RM178, RM334	5
6	RM133, RM510, RM454, RM162, RM141	5
7	RM125, RM11, RM455, RM118, RM428	5
8	RM408, RM152, RM25, RM44, RM284, RM433, RM447	7
9	RM316, RM105, RM201, RM215, RM205	5
10	RM474, RM271, RM484, RM590, RM171	5
11	RM286, RM552, RM536, RM287, RM144	5
12	RM415, RM19, RM277, RM270, RM235	5
	Total	65

II. Genetic diversity analysis in rice using morpho-agronomic characters

Plant materials

The set of materials used for the SSR marker diversity study were also used in the morphological and agronomic diversity analysis (Table 4).

Planting

Seeds of the rice cultivars were germinated by using wet tissue paper on petri dish on 12 May 2012 and transplanted on 13 June 2012, just a month after germination. A spacing of 20 cm between genotypes and between plants was left. Fertilization with urea (46-0-0; N-P_2O_5-K_2O) and compound fertilizer (16-16-16;

N-P_2O_5-K_2O), and the broad spectrum insecticide Fipronil application were done uniformly to all plots. The plots were kept clean from weeds throughout the growing period. The plants were protected from the possible damage of birds and rats by wire mesh.

Experimental design, data collection and analyses

The treatments were arranged in a randomized complete block design with three replications. Data for 17 morphological and agronomic traits were collected from each plot using the Standard Evaluation System (SES) for rice (IRRI, 2002). Rough-rice seed width and length were measured as averages of 15 seeds by using digital Rice Grain Analyzer. The traits studied include days to flowering, number of total tillers, number of productive tillers, plant height (cm), culm length (cm), flag-leaf length (cm), panicle length (cm), number of total spikelets, number of fertile spikelets, 100-seed weight (gm), biomass yield per plant (gm), grain yield per plant, harvest index (%), seed width (mm), seed length (mm), seed shape (length:width ratio) and spikelet fertility (%).

Univariate ANOVA was computed by using CropStat 7.2 and the mean values of traits were used for further analysis. The mean values were then standardized to a mean of zero and variance of unity before cluster analysis to remove the biases due to differences in the scale of measurement. PAST 1.93 (Palaeontological Statistics; Hammer *et al*., 2001) computer software was used for hierarchical cluster and principal component analyses. Phenotypic correlation and two-step cluster analysis were worked out in SPSS 19. Two-step cluster analysis was executed by selecting Log-likelihood as a distance measure and using Akaike's information criterion (AIC) to automatically determine the number of clusters.

III. Evaluation of BPH resistance in rice

Plant materials

A total of 22 genotypes, including the checks were used for the study. The genotypes Rathu heenati and PtB33 were included as resistant checks, while Taichung native1 (TN1) was used as a susceptible check. Mudgo, a source of the resistance gene *Bph1* (Athwal *et al*., 1971) was also included, although the resistance is believed to be broken down. The detailed description of the genotypes is presented on Table 6.

Insect populations

Six populations of brown planthopper were used in the study. The populations were from three provinces namely, Bangkok, Chachoengsao and Pathum Thani, of central Thailand. Each province was represented by a BPH population both from a research center/station and farmer's field. Therefore, the populations were from Pathum Thani rice research center and farmer's field, Chachoengsao rice research center and farmer's field, and Kasetsart University-Bangkhen station and farmer's field. The provinces and sites of collection of the populations are depicted on Table 7.

Six trays of BPH-susceptible rice seedlings were kept in each location for seven to 10 days to catch the BPH population of each location. Then, each tray of rice seedling was covered by plastic bag in order to prevent mixing up of the BPH population while bringing back to Bangkhen. The six populations were kept in nylon screening net separately to maintain the desired number of insect population.

Table 6 Description of the rice genotypes used in the BPH resistance screening.

Sr. No.	Genotypes	Sub-species/type	Origin
1	NERICA 3	Interspecific cross	Côte d'Ivoire
2	NERICA 4	Interspecific cross	Côte d'Ivoire
3	NPT4	New plant type	Philippines
4	NPT8	New plant type	Philippines
5	NPT13	New plant type	Philippines
6	NPT18	New plant type	Philippines
7	CH1	Indica	China
8	CH2	Indica	China
9	PTT1	Indica	Thailand
10	CNT1	Indica	Thailand
11	SPR1	Indica	Thailand
12	KDML105	Indica	Thailand
13	IR64	Indica	Philippines
14	AZUCENA	Tropical japonica	Philippines
15	RD31	Indica	Thailand
16	TDK1	Indica	Laos
17	TDK5	Indica	Laos
18	CO39	Indica	India
19	Rathu Heenati	Indica	Sri Lanka
20	Mudgo	Indica	India
21	TN1	Indica	Taiwan
22	Ptb33	Indica	India

Table 7 Brown planthopper populations used in the varietal resistance study.

Province	Site of collection	Type of population	Abbreviation
Bangkok	Kasetsart University	Research station	BKK_R
	Khlong Sam Wa district	Farmer’s field	BKK_F
Chachoengsao	Chachoengsao Rice Research Center	Research station	CCS_R
	Bang Nam Priao district	Farmer’s field	CCS_F
Pathum Thani	Pathum Thani Rice Research Center	Research station	PTT_R
	Thanyaburi district	Farmer’s field	PTT_F

Standard seed-box screening test (SSST) was used to determine the reaction of the genotypes to the BPH populations. To ensure that all the seedlings are at the same growth stage for BPH infestation, each genotype was germinated on a moist tissue paper in petri dish and then seedlings of each genotype were transplanted. After inspecting each and every plant for predators and other insects, approximately 10 nymphs (2nd to 3rd instar) per seedling were used to infest the plants. Scoring was started when the susceptible check was dead. The reaction of the rice genotypes to BPH was scored as per the standard evaluation system for rice (IRRI, 2002) as indicated in Table 8. The whole process of the experiment, planting susceptible rice variety, collection of BPH populations, maintaining the populations, planting the test genotypes, infestation and scoring the reaction of the test genotypes was started on the second week of November 2012 and ended on the first week of February 2013. The trial was conducted under natural light condition.

Table 8 The criteria for brown planthopper resistance-scoring.

Resistance score	Plant state	Resistance level
0	No damage	R
1	Very slight damage	R
3	The first and the second leaf of most plants partially yellowing	R
5	Pronounced yellowing and stunting or about 10 to 25% of the plants wilting or dead and remaining plants severely stunted or dying	MR
7	More than half of the plants died	S
9	All plants died	S

R = resistant, MR = moderately resistant, S = susceptible

Experimental design, data collection and analyses

Randomized complete block design was used. Each test cultivar was replicated three times. Cluster analysis was done by using NTSYSpc 2.01 software. The SIMINT module of NTSYSpc 2.01 was selected to compute Canberra distance coefficients by using unweighted paired group method with arithmetic average (UPGMA), and SAHN module was used to execute hierarchical clustering. Principal component analysis was implemented by using PAST (Palaeontological Statistics; Hammer *et al*., 2001) 1.93 software. Variance-covariance matrix was used during PC analyses.

RESULTS AND DISCUSSION

Results

In order to achieve the objectives of the study, three experiments were conducted. The results of each experiment are presented separately.

I. Genetic diversity analysis in rice using SSR markers

Allele frequency and hetrozygosity

Out of the 65 rice SSR markers screened, 29 (21 standard and 8 additional from McCouch *et al*. (2002)) markers were used for the diversity analysis. The rest which were found either nonspecific or monomorphic, were excluded.

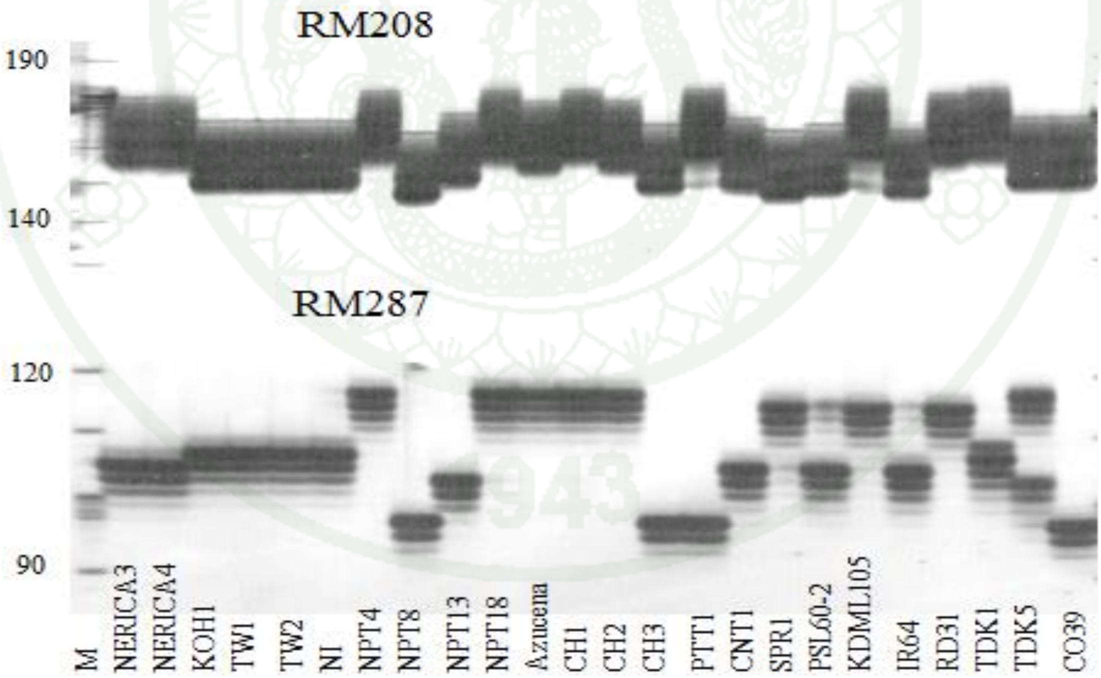


Figure 5 Silver stained polyacrylamide gel of amplification products obtained with SSR markers RM208 and RM287 among the 24 rice cultivars (M= size marker).

A total of 144 alleles were detected at the 29 SSR primer pairs evaluated in the 24 rice cultivars. The number of alleles per SSR loci ranged from 3 (RM162, RM284, RM312, RM338 and RM507; all standard markers) to 8 (RM19; standard marker) with a mean of 4.966 alleles per locus. The size of PCR products ranged between 80 - 200 bp depending on the SSR marker and the cultivar. Unbiased expected hetrozygosity (UH_e) over loci was 0.674 and it ranged from 0.334 (RM162) to 0.841 (RM552). Similarly, the mean expected hetrozygosity (H_e) was 0.659 and it ranged from 0.327 (RM162) to 0.823 (RM280) (Table 9).

Table 9 Summary of genetic diversity parameters over all loci estimated for the 24 rice cultivars.

Chromosome	Locus	Na	Ne	I	He	UHe	Repeat motif
1	RM237	5	4.174	1.507	0.760	0.777	(CT)18
1	RM259	5	2.439	1.175	0.590	0.605	(CT)17
1	RM312	3	1.882	0.762	0.469	0.479	(ATTT)4(GT)9
1	RM5	6	3.000	1.328	0.667	0.681	(GA)14
2	RM208	6	4.114	1.574	0.757	0.773	(CT)17
2	RM452	4	3.612	1.331	0.723	0.740	(GTC)9
2	RM6	5	4.431	1.538	0.774	0.791	(AG)16
3	RM338	3	2.597	1.010	0.615	0.631	(CTT)6
4	RM280	7	5.647	1.830	0.823	0.840	(GA)16
4	RM559	6	3.369	1.409	0.703	0.719	(AACA)6
5	RM161	4	2.880	1.199	0.653	0.667	(AG)20
5	RM178	4	1.869	0.857	0.465	0.475	(GA)5(AG)8
5	RM413	5	3.374	1.365	0.704	0.723	(AG)11
5	RM507	3	2.651	1.028	0.623	0.642	(AAGA)7
6	RM133	4	3.023	1.235	0.669	0.684	(CT)8
6	RM162	3	1.485	0.619	0.327	0.334	(AC)20
6	RM510	4	2.642	1.105	0.622	0.635	(GA)15
8	RM284	3	1.716	0.709	0.417	0.427	(GA)8

Table 9 (Conitnued)

Chromosome	Locus	Na	Ne	I	He	UHe	Repeat motif
9	RM105	5	4.500	1.545	0.778	0.794	(CCT)6
9	RM201	5	2.851	1.253	0.649	0.663	(CT)17
10	RM590	6	4.881	1.679	0.795	0.812	(TCT)10
11	RM144	7	3.310	1.531	0.698	0.713	(ATT)11
11	RM286	5	3.600	1.411	0.722	0.738	(GA)16
11	RM287	6	5.143	1.691	0.806	0.823	(GA)21
11	RM536	7	2.160	1.234	0.537	0.552	(CT)16
11	RM552	6	5.628	1.756	0.822	0.841	(TAT)13
12	RM19	8	5.538	1.871	0.819	0.837	(ATC)10
12	RM270	4	1.882	0.918	0.469	0.479	(GA)13
12	RM277	5	2.909	1.257	0.656	0.670	(GA)11
	Mean	4.966	3.356	1.301	0.659	0.674	
	SE	0.255	0.228	0.062	0.024	0.025	

Na = number of different allales, Ne = number of effective alleles,
I = Shannon's information index, He = expected hetrozygosity,
UHe = Unbiased expected hetrozygosity

Although the number of loci sampled from the rice chromosomes were not equal and chromosome 7 was not represented by any polymorphic SSR marker in this study, higher number of alleles were detected on chromosome 4 (6.5), chromosome 11 (6.2) and chromosome 10 (6); the lowest number of allele (3), however, was estimated for chromosomes 3 and 8. The genetic diversity, as measured by the expected hetrozygosity, was also higher for chromosome 10 (0.795), chromosome 4 (0.763), chromosome 2 (0.751); however, that of chromosome 8 (0.417) was the lowest (Appendix Table 1).

Genetic distance

A total of 276 pairwise unbiased minimum distance values were estimated using the possible pairwise combination of the 24 cultivars (Table 10). When these pairwise distance values were compared, the two NERICA cultivars showed the smallest (0.04) distance between them. Other smaller values 0.115 and 0.154 were for TW1-TW2 and TW1-NI (Nipponbare) paired cultivars; both of them are japonica pairs. The two NERICA cultivars, however, showed the highest (1) genetic distance from the cultivar TDK5 followed by TW2-NPT13 (0.964), NI-TDK5 (0.963), NI-RD31 (0.962) and Azucena-CH3 (0.96) pairwise genetic distances.

Table 10 Pairwise Nei (1978) unbiased minimum distance coefficients for all possible combinations of the 24 cultivars using allele frequencies of 29 SSR markers.

	NERICA3	NERICA4	KOH1	TW1	TW2	NI	NPT4	NPT8	NPT13	NPT18	AZUCENA
NERICA4	0.040										
KOH1	0.680	0.690									
TW1	0.708	0.654	0.269								
TW2	0.680	0.690	0.379	0.115							
NI	0.680	0.655	0.207	0.154	0.207						
NPT4	0.833	0.741	0.926	0.880	0.926	0.926					
NPT8	0.880	0.828	0.828	0.808	0.793	0.793	0.407				
NPT13	0.880	0.857	0.821	0.846	0.964	0.929	0.556	0.679			
NPT18	0.917	0.929	0.821	0.885	0.929	0.929	0.539	0.607	0.593		
AZUCENA	0.667	0.714	0.714	0.760	0.786	0.714	0.885	0.857	0.852	0.852	
CH1	0.880	0.893	0.857	0.808	0.857	0.857	0.500	0.536	0.630	0.519	0.889
CH2	0.917	0.889	0.889	0.885	0.889	0.926	0.280	0.444	0.615	0.407	0.923
CH3	0.818	0.769	0.923	0.833	0.846	0.846	0.417	0.539	0.600	0.615	0.960
PTT1	0.957	0.852	0.889	0.792	0.815	0.852	0.440	0.407	0.692	0.577	0.923
CNT1	0.870	0.889	0.852	0.880	0.926	0.926	0.539	0.333	0.577	0.519	0.885
SPR1	0.917	0.857	0.893	0.846	0.821	0.857	0.577	0.464	0.741	0.607	0.852
PSL602	0.905	0.880	0.960	0.913	0.960	0.960	0.652	0.480	0.708	0.560	0.880
KDML105	0.875	0.857	0.893	0.880	0.857	0.857	0.615	0.571	0.778	0.667	0.821
IR64	0.913	0.926	0.815	0.833	0.852	0.889	0.600	0.556	0.692	0.500	0.885
RD31	0.864	0.885	0.923	0.913	0.923	0.962	0.760	0.654	0.600	0.640	0.840
TDK1	0.957	0.926	0.926	0.875	0.889	0.926	0.615	0.593	0.731	0.654	0.923
TDK5	1.000	1.000	0.926	0.917	0.926	0.963	0.680	0.704	0.731	0.654	0.923
CO39	0.875	0.929	0.857	0.840	0.857	0.893	0.667	0.536	0.593	0.519	0.852

Table 10 (Coninued)

	CH1	CH2	CH3	PTT1	CNT1	SPR1	PSL602	KDML105	IR64	RD31	TDK1	TDK5
NERICA4												
KOH1												
TW1												
TW2												
NI												
NPT4												
NPT8												
NPT13												
NPT18												
AZUCENA												
CH1												
CH2	0.556											
CH3	0.400	0.440										
PTT1	0.577	0.440	0.500									
CNT1	0.462	0.500	0.480	0.440								
SPR1	0.593	0.519	0.500	0.346	0.407							
PSL602	0.625	0.583	0.667	0.500	0.292	0.560						
KDML105	0.630	0.539	0.480	0.308	0.423	0.370	0.440					
IR64	0.615	0.480	0.600	0.654	0.360	0.539	0.458	0.577				
RD31	0.680	0.625	0.652	0.640	0.480	0.560	0.609	0.680	0.480			
TDK1	0.654	0.560	0.542	0.462	0.500	0.423	0.625	0.539	0.539	0.346		
TDK5	0.615	0.640	0.708	0.654	0.680	0.654	0.625	0.692	0.615	0.731	0.615	
CO39	0.556	0.577	0.600	0.539	0.482	0.519	0.708	0.630	0.539	0.346	0.407	0.577

Cluster and principal coordinate analyses

The unbiased minimum genetic distance (Nei, 1978) values were used for clustering the 24 cultivars and to construct the dendrogram depicted in Figure 6. The diagram showed two main groups with additional subclusters within group two. Group I contained NERICA and *japonica* (both tropical and temperate) types, while group II was made up of NPT (New Plant Type) and *indica* types.

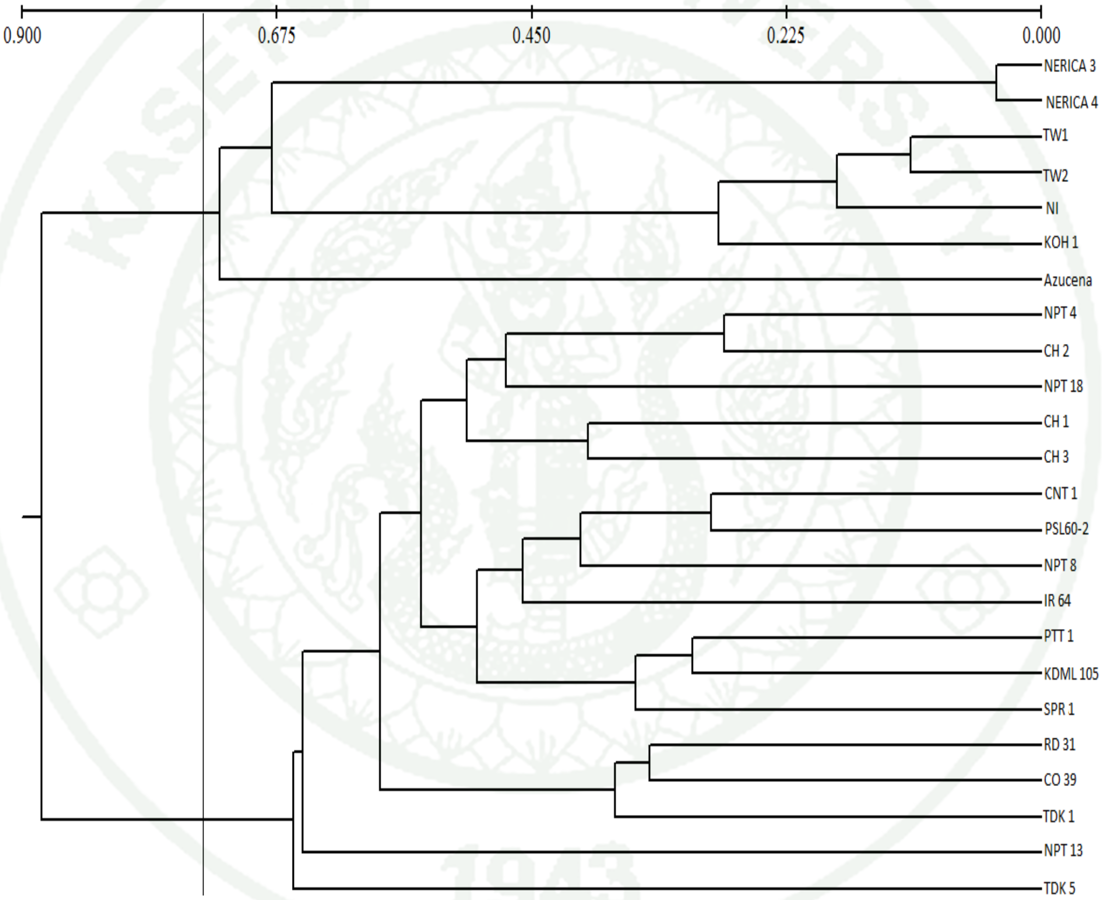


Figure 6 Dendrogram of the 24 rice cultivars based on Nei (1978) unbiased minimum distance and constructed by using UPGMA algorithm.

Group I was comprised of seven cultivars. This group is further divided into 3 subgroups. Subgroup 1 contained NERICA 3 and NERICA 4. Subgroup 2 is composed of four japonica cultivars: TW1, TW2, NI (Nipponbare) and KOH1. However, the third subgroup contained the distinct, aromatic, non-glutinous and traditional tropical *japonica* cultivar Azucena. Group II, a subset of 17 cultivars, was composed of 13 *indica* and four NPT type cultivars.

Principal coordinate analysis (PCoA) was executed to complement the cluster analysis, where the first three principal coordinate axes explained 40.13, 16.37 and 13.15 % of the variance, respectively; and which summed up to 69.65 %. The scattered distribution of the rice cultivars against the first two axes is depicted on Figure 7.

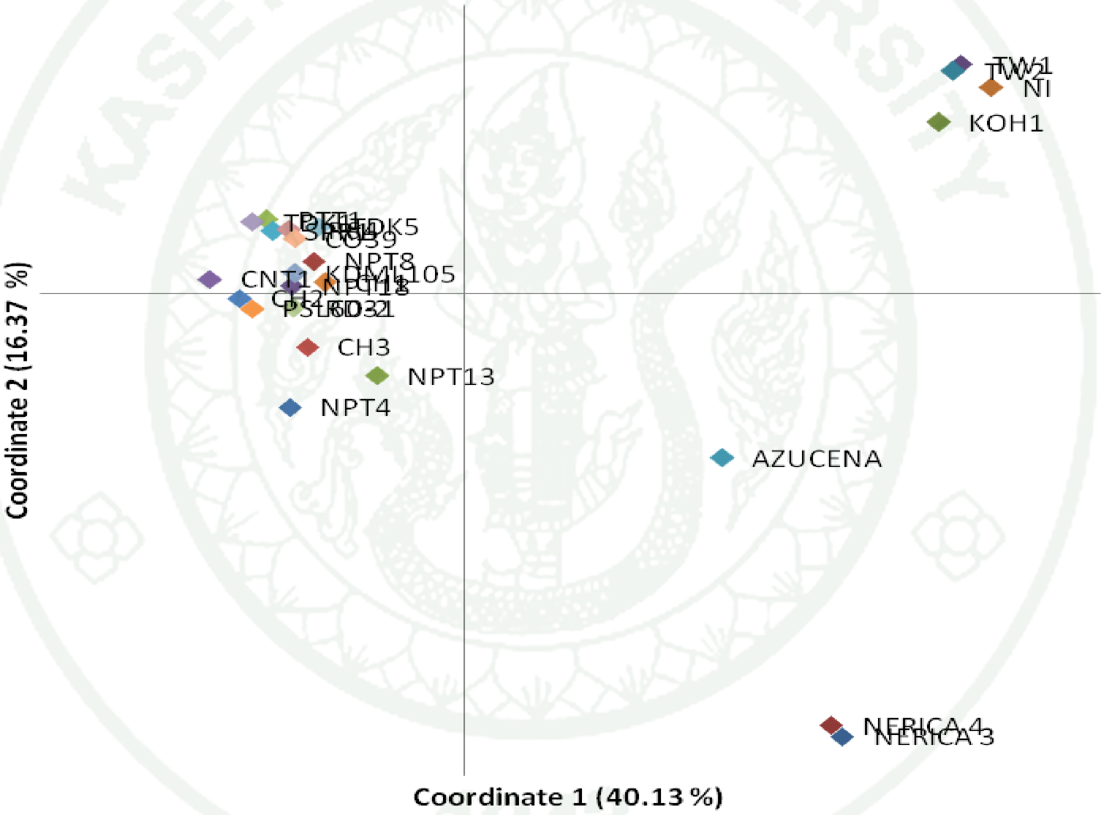


Figure 7 Two-dimensional representation of the 24 cultivars analyzed by using 29 polymorphic SSR markers.

II. Genetic diversity analysis in rice using morpho-agronomic characters

Analysis of variance

The univariate analyses of variance showed significant (*P<0.01*) variation among the rice genotypes for all the morpho-agronomic traits considered (Table 11). The significance signifies the possibility of using all traits for further analyses. From the result it was observed that CO39 was the earliest while KDML105 was the latest genotypes to flower. Similarly, CO39 and Azucena were the shortest and the tallest genotypes, respectively. The analysis also showed that KOH1 was the lowest whereas CH1 was the highest in yield among the genotypes. Also, NPT18 and NI were the least and the most fertile genotypes identified, respectively.

Table 11 Mean squares (MS), minimum and maximum mean values, and overall means, standard errors (ES) and coefficients of variation (CV) of 17 morpho-agronomic traits of the 24 rice genotypes.

Traits	MS (df=23)	Minimum		Maximum		Mean±SE	CV (%)
		Value	Cultivar	Value	Cultivar		
Days to flowering	1081.24**	75.33	CO39	149.67	KDML105	106.97±2.97	4.80
Total number of tillers	21.05**	3.33	NERICA3	14.89	CNT1	8.11±1.14	24.30
No. of productive tillers	16.64**	3.13	NERICA3	10.78	NPT8	6.73±1.15	29.60
Plant height (cm)	1940.85**	68.69	CO39	167.00	AZUCENA	111.43±5.72	8.90
Culm length (cm)	1596.25**	46.96	CO39	137.78	AZUCENA	85.01±4.74	9.70
Flag-leaf length (cm)	144.79**	32.58	IR64	56.29	CH1	43.77±4.09	16.20
Panicle length (cm)	50.43**	17.56	KOH1	32.09	NPT8	26.42±1.73	11.40
Total number of spikelets	13726**	61.33	NI	342.56	CH1	205.12±17.67	14.90
No. of fertile spikelets	7820.19**	55.89	KOH1	277.22	CH1	151.00±16.22	18.60
Spikelet fertility (%)	289.47**	57.24	NPT18	95.18	NI	74.67±4.23	9.80
100-seed weight (gm)	0.39**	1.68	CH3	3.15	NERICA3	2.48±0.95	6.70
Seed width (mm)	0.24**	2.04	NPT8	3.01	TW1	2.37±0.06	4.00
Seed length (mm)	5.71**	6.55	TW1	10.85	TDK5	9.22±0.13	2.40
Seed shape	2.31**	2.18	TW1	5.00	NPT8	3.99±0.09	3.80

Table 11 (Continued)

Traits	MS (df=23)	Minimum		Maximum		Mean±SE	CV (%)
		Value	Cultivar	Value	Cultivar		
Biomass (gm)	1699.46**	8.87	KOH1	90.45	TDK5	48.88±10.53	37.30
Yield per plant	118.53**	5.04	KOH1	29.06	CH1	16.25±3.74	39.90
Harvest index (%)	524.16**	15.26	TDK1	66.10	CO39	37.89±5.24	23.90

**=significant at 0.01 probability level

Phenotypic correlation

In order to assess traits association, phenotypic correlation analysis was done and the result is depicted in Table 12. Generally the result showed high order of correlation between most of the traits under study. Days to flowering was highly and significantly correlated with plant height (0.883), culm length (0.893), biomass (0.772) and harvest index (-0.732). Similarly, yield per plant was significantly correlated with total number of tillers (0.541), plant height (0.525), panicle length (0.649), total number of spikelets (0.585), number of fertile spikelets (0.539), biomass (0.716), seed length (0.606) and seed shape (0.622) towards the positive direction. Also, strong and significant association of harvest index was detected with days to flowering (-0.732), plant height (-0.603), culm length (-0.601), total number of spikelets (-0.455), biomass (-0.705) and spikelet fertility (0.710). Spikelet fertility was negatively correlated to all traits under study except harvest index.

Table 12 Phenotypic correlation coefficients between 17 morpho-agronomic traits in the 24 rice genotypes.

	DF	**TNT**	**PT**	**PHT**	**CL**	**FLL**	**PNL**	**TSP**
TNT	0.023							
PT	-0.164	0.910**						
PHT (cm)	0.883**	-0.001	-0.156					
CL (cm)	0.893**	-0.059	-0.216	0.990**				
FLL (cm)	0.362	-0.303	-0.261	0.579**	0.540**			
PNL (cm)	0.451*	0.325	0.248	0.631**	0.518**	0.554**		
TSP	0.511*	0.017	-0.146	0.561**	0.525**	0.533**	0.530**	
FSP	0.382	-0.082	-0.251	0.514*	0.492*	0.587**	0.422*	0.924**
HSW (gm)	0.077	-0.028	0.003	0.202	0.126	0.328	0.550**	0.039
BM (gm)	0.772**	0.345	0.218	0.798**	0.770**	0.351	0.614**	0.614**
YLD (gm)	0.376	0.541**	0.497*	0.525**	0.464*	0.431*	0.649**	0.585**
HI (%)	-0.732**	-0.127	0.033	-0.603**	-0.601**	-0.139	-0.357	-0.455*
SW (mm)	-0.132	-0.302	-0.254	-0.375	-0.330	-0.178	-0.472*	-0.285
SL (mm)	0.413	0.198	0.165	0.667**	0.597**	0.476*	0.780**	0.300
SSH	0.337	0.301	0.262	0.601**	0.537**	0.387	0.706**	0.334
SPF	-0.428*	-0.194	-0.172	-0.257	-0.215	-0.020	-0.386	-0.387

Table 12 (Continued)

	FSP	HSW	BM	YLD	HI	SW	SL	SSH
TNT								
PT								
PHT (cm)								
CL (cm)								
FLL (cm)								
PNL (cm)								
TSP								
FSP								
HSW (gm)	-0.081							
BM (gm)	0.428*	0.231						
YLD (gm)	0.539**	0.166	0.716**					
HI (%)	-0.211	-0.185	-0.705**	-0.152				
SW (mm)	-0.379	0.219	-0.301	-0.431*	0.056			
SL (mm)	0.305	0.400	0.605**	0.606**	-0.252	-0.711**		
SSH	0.373	0.147	0.550**	0.622**	-0.209	-0.888**	0.947**	
SPF	-0.029	-0.252	-0.543**	-0.204	0.710**	-0.027	-0.138	-0.095

DF=days to flowering, TNT=total number of tillers, PT=number of productive tillers, PHT=plant height, CL=culm length, FLL=flag-leaf length, PNL=panicle length, TSP=total number of spikelets, FSP=number of fertile spikelets, HSW=hundred-seed weight, BM=biomass yield per plant, YLD=yield per plant, HI=harvest index, SW=seed width, SL=seed length, SSH=seed shape, SPF=spikelet fertility

**, *=significant at 0.01 and 0.05 probability levels, respectively

Euclidean distance

Euclidean distance matrix was produced by assuming 276 total possible pairwise combinations of the 24 rice genotypes (Table 13). The distance coefficients ranged from 1.142 for NERICA3-NERICA4 to 10.267 for KOH1-Azucena pairwise cultivar combinations with a mean of 5.172. In addition, TW1-TW2 (1.743), CH2-CH3 (1.848) and NPT4-NPT18 (1.976) were cultivars with smaller pairwise Euclidean distances. Similarly, the other higher distance values were that of KOH1 with CH1 (10.183), KDML105 (9.788) and SPR1 (9.368). KOH1 is generally the most diverged genotype from others with higher mean Euclidean distance of 7.426 while RD31 was the least with mean Euclidean distance of 4.41.

Table 13 Pairwise Euclidean distance coefficients for all possible combinations of the 24 genotypes using 17 morpho-agronomic traits.

	AZUCENA	CH1	CH2	CH3	CNT1	CO39	IR64	KDML105	KOH1	NERICA3	NERICA4
CH1	5.666										
CH2	7.283	6.102									
CH3	7.230	7.028	1.848								
CNT1	7.135	4.812	5.938	7.174							
CO39	9.399	8.192	3.249	3.775	7.147						
IR64	7.264	6.397	3.860	4.636	3.669	4.644					
KDML105	3.045	5.338	7.093	7.239	6.174	8.744	6.365				
KOH1	10.267	10.183	5.260	5.129	8.912	3.558	6.163	9.788			
NERICA3	6.499	5.965	5.763	5.857	6.596	6.193	5.558	6.225	6.945		
NERICA4	6.751	6.055	5.434	5.652	6.467	5.829	5.369	6.394	6.337	1.142	
NI	10.222	9.194	5.089	6.041	6.993	3.958	5.205	9.613	3.699	7.571	6.888
NPT13	6.563	6.980	4.821	4.852	5.415	5.111	3.100	6.072	5.962	4.331	4.435
NPT18	5.472	4.834	5.819	6.262	4.353	7.002	4.628	4.777	8.672	5.600	5.954
NPT4	6.278	5.636	5.880	6.265	4.546	6.781	4.522	5.596	8.130	5.550	5.802
NPT8	6.461	4.457	5.575	6.624	2.627	6.888	3.937	5.699	9.061	6.428	6.503
PSL60-2	5.122	4.686	5.389	5.873	3.599	7.315	3.820	4.186	8.901	6.340	6.468
PTT1	6.119	4.026	4.980	6.061	2.220	6.240	3.167	4.961	8.009	5.118	4.995
RD31	6.254	5.403	5.062	5.872	2.899	5.857	2.531	5.385	7.377	5.010	5.053
SPR1	5.280	4.087	6.547	7.219	3.516	7.886	4.740	4.035	9.368	5.324	5.554
TDK1	4.355	6.860	6.727	6.525	6.230	7.983	5.312	3.911	8.383	5.632	5.858
TDK5	5.677	5.513	6.445	6.941	3.926	7.737	4.252	4.450	9.020	6.173	6.342
TW1	7.513	8.012	5.572	5.462	7.397	5.528	5.851	7.214	5.516	6.152	6.116
TW2	7.159	7.123	5.429	5.599	6.654	5.708	5.619	6.543	6.150	6.180	6.134

Table 13 (Continued)

	NI	NPT13	NPT18	NPT4	NPT8	PSL60-2	PTT1	RD31	SPR1	TDK1	TDK5	TW1
CH1												
CH2												
CH3												
CNT1												
CO39												
IR64												
KDML105												
KOH1												
NERICA3												
NERICA4												
NI												
NPT13	6.346											
NPT18	8.145	4.021										
NPT4	7.624	4.050	1.976									
NPT8	7.685	4.780	3.314	4.445								
PSL60-2	8.021	4.866	3.215	3.725	3.459							
PTT1	6.730	4.269	3.676	4.347	2.142	3.285						
RD31	6.365	2.861	3.115	3.687	2.491	3.469	1.988					
SPR1	8.516	4.840	3.164	4.296	3.065	3.208	2.415	2.833				
TDK1	8.713	3.953	4.297	4.998	5.566	4.298	5.059	4.251	4.063			
TDK5	8.363	4.129	2.684	3.613	3.280	2.830	3.228	2.756	2.420	3.359		
TW1	6.272	4.430	5.007	4.524	7.034	6.640	6.594	5.600	6.927	5.845	6.229	
TW2	6.218	4.898	4.448	3.878	6.526	5.873	5.984	5.322	6.299	5.937	5.832	1.743

Two-step cluster analysis

The two-step cluster analysis automatically separated the entire genotypes into two groups. Subsequently, one-way ANOVA was run to declare whether any of the mean differences of the traits between the two groups were significant. Accordingly, the result showed that the between-group means were significant ($P<0.01$) for most of the traits except for number of total and productive tillers, number of fertile spikeletes, spikelet fertility and harvest index (Table 14). On average, the genotypes in cluster II were significantly earlier (91.8 vs. 113.2 days) and shorter (82.99 vs. 123.15 cm) than that of cluster I. Compared with cluster I, the genotypes in cluster II had significantly lower 100-seed weight (2.17 vs. 2.60), biomass (26.38 vs. 58.14 gm), grain yield (11 vs. 18.41 gm), and smaller seed length (7.30 vs. 10.01).

Table 14 Between-groups mean-squares and cluster means for 17 morpho-agronomic traits of the 24 rice genotypes.

Traits	MS (df=1)	Mean	
		Cluster 1 (n=17)	Cluster 2 (n=7)
Days to flowering	2272.02**	113.22	91.81
Total number of tillers	4.42	8.39	7.44
No. of productive tillers	0.83	6.85	6.44
Plant height (cm)	7998.04**	123.15	82.99
Culm length (cm)	5131.35**	94.40	62.23
Flag-leaf length (cm)	295.70**	46.02	38.30
Panicle length (cm)	317.06**	28.76	20.76
Total number of spikelets	18337.86*	222.86	162.05
No. of fertile spikelets	7793.82	162.57	122.92
Spikelet fertility (%)	151.47	73.06	78.59
100-seed weight (gm)	0.94**	2.60	2.17
Seed width (mm)	0.53**	2.27	2.60
Seed length (mm)	36.40**	10.01	7.30
Seed shape	11.66**	4.44	2.90
Biomass (gm)	5003.70**	58.14	26.38
Yield per plant (gm)	272.46**	18.41	11.00
Harvest index (%)	605.14	34.67	45.71

**, * = significant at 0.01 and 0.05 probability levels

Hierarchical cluster analysis

Hierarchical clustering was attempted by using paired group algorithm with different distance measures like Gower, Euclidean, Mahalanobis and Manhattan. The result showed that Gower, Euclidean and Manhattan distance measures yielded similar dendrogram topology and similar cluster membership of the rice genotypes; however, Mahalanobis distance measure yielded different dendrogram topology which was characterized by chaining of the genotypes. The dendrogram of the morpho-agronomic traits grouped the genotypes into two clusters with additional subgroups in each groups. Group I was composed of 17 genotypes and group II included 7 genotypes (Figure 8). Group I was made up of miscellaneous type of genotypes as it was composed of 10 *indica*, four NPT, one tropical *japonica* and two NERICA types. This group was further divided into four subgroups: subgroup 1 contained the two NERICAs; subgroup 2 was made up of the four NPTs and seven *indica* types; subgroup 3 contained a distinct cultivar CH1, this variety was characterized by longer flag-leaf, higher number of spikeletes and higher yield; and subgroup 4 included KDML105, Azucena and TDK1. The members of this subgroup were late maturing and tall plant types. However, group II contained four *japonica* and three *indica* types. This group also further divided in to three subgroups: subgroup 1 contained TW1 and TW2 (*japonica* types); subgroup 2 was made up of CH2, CH3 and CO39 (*indica* types), and subgroup 3 was composed of NI and KOH1, *japonica* types.

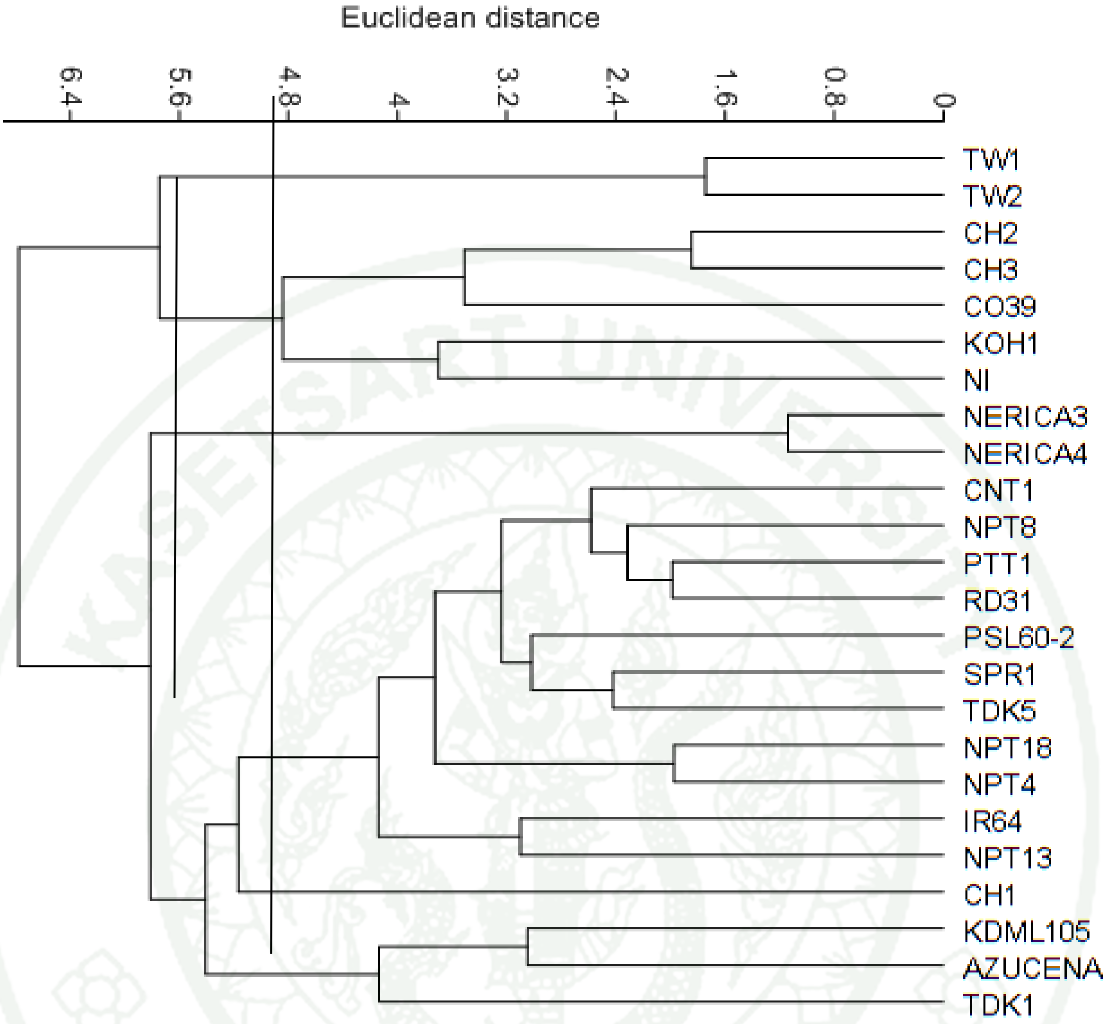


Figure 8 Dendrogram of the 24 rice genotypes based on 17 morpho-agronomic traits constructed by means of paired group algorithm and Euclidean distance.

Principal component analysis

The result of the principal component analysis (PCA) is depicted in Table 15. The objective of principal component analysis is reduction of dimensionality of a data set with a large number of correlated variables or traits (Jolliffe, 2002). PCA was carried out by using 24 genotypes and 17 traits. In the analysis a total of 17 PCs, equals to the number of traits, were extracted. However, the first five PCs with eigenvalues greater than 1 were retained. The result showed that 89.68 % of the variability was explained by the first five principal component (PC) axes (Table 15). Out of the five, the first and the second explained 44.52 and 16.64 % of the variation, respectively. Days to flowering, plant height, culm length, panicle length, biomass, seed length and shape were the important traits contributing to the first PC. In the second PC, however, number of total and productive tillers were important. Similarly, flag-leaf length, harvest index, seed width and spikelet fertility were important in the third axis. While only 100-seed weight was important in the fourth axis. Number of total and fertile spikelets, and yield per plant were the important traits contributing to the fifth PC. The first axis differentiated genotypes which were late flowering, tall, higher in biomass and grain yield, with slender (narrow and tall) seeds from genotypes which were early flowering, short, low biomass and grain yield with round (wide and short) seeds. The second axis however differentiated genotypes with higher number of total and productive tillers from those with low number of tillers.

Table 15 Eigenvalues, total variance, cumulative variance and eigenvectors for 17 morpho-agronomic traits of the 24 rice genotypes.

Traits	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**	**Axis 5**
Days to flowering	0.276	-0.212	-0.225	-0.111	-0.235
Total tillers	0.083	0.501	-0.213	-0.173	0.082
Productive tillers	0.035	0.543	-0.176	-0.061	0.110
Plant height (cm)	0.327	-0.161	-0.007	0.009	-0.223
Culm length (cm)	0.307	-0.201	-0.013	-0.041	-0.277
Flag-leaf length (cm)	0.219	-0.211	0.271	0.215	0.262
Panicle length (cm)	0.299	0.132	0.029	0.283	0.171
Total spikelets	0.262	-0.160	0.038	-0.290	0.431
Fertile spikelets	0.228	-0.172	0.266	-0.325	0.375
100-seed weight (gm)	0.101	-0.009	-0.124	0.709	0.244
Biomass (gm)	0.321	0.023	-0.230	-0.076	-0.014
Yield per plant (gm)	0.272	0.239	0.065	-0.101	0.299
Harvest index (%)	-0.218	0.147	0.439	0.070	0.143
Seed width (mm)	-0.196	-0.258	-0.334	0.175	0.284
Seed length (mm)	0.289	0.162	0.197	0.278	-0.216
Seed shape	0.278	0.231	0.254	0.077	-0.244
Spikelet fertility (%)	-0.151	0.007	0.496	-0.023	-0.138
Eigenvalue	7.568	2.829	2.099	1.498	1.251
Variance (%)	44.517	16.643	12.349	8.815	7.362
Cumulative variance (%)	44.517	61.16	73.509	82.324	89.685

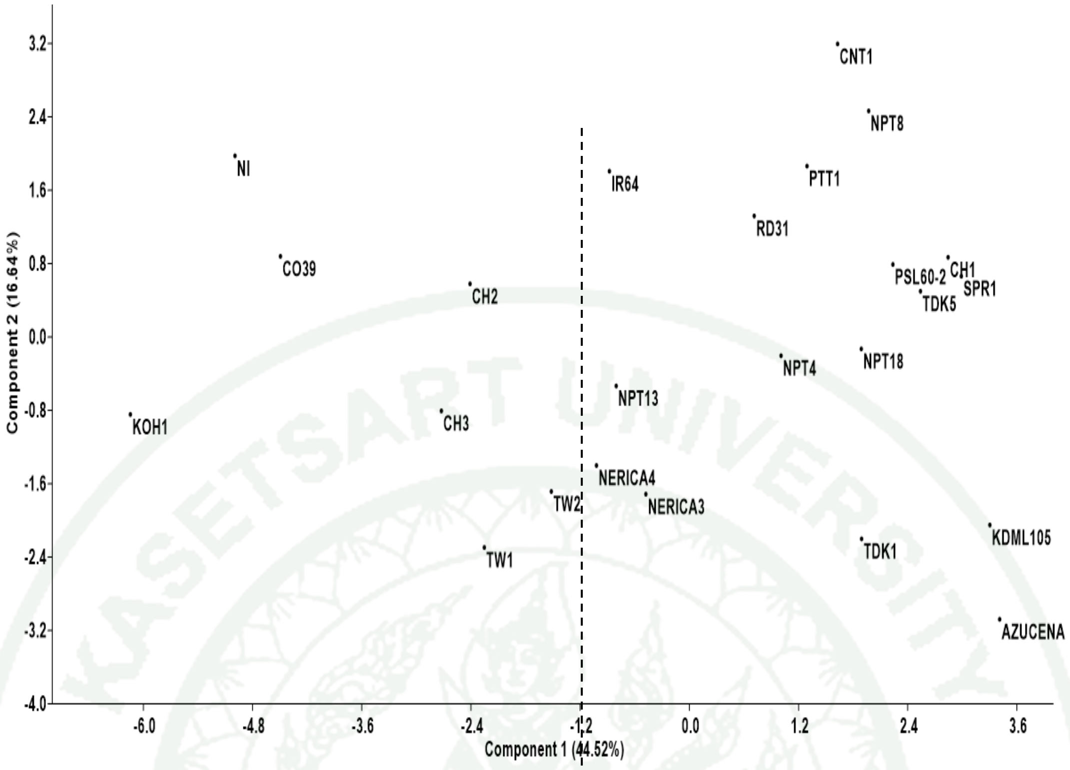


Figure 9 Scattered distribution of the 24 rice genotypes by using 17 morpho-agronomic traits on the first two principal component axes.

Generally, the PC analysis broadly grouped the genotypes based on zones of origin into temperate and tropical categories. The ones located to the left of the two dimensional plane being temperate with the exception of CO39, while those to the right being tropical in zone of origin (Figure 9).

III. Evaluation of BPH resistance in rice

Relative virulence of populations

The mean BPH damage-score and the relative virulence of the six BPH populations are depicted in Table 16 and Table 17. The damage-score ranged from 0 to 9 (Table 16). The virulence of BPH ranged from 45.45% for populations from Klong SamWa (BKK_F) and Chachoengsao farmer’s field to 81.82% for Chachoengsao Rice Research Center (Table 17).

Susceptibility of genotypes

The susceptibility also ranged from 0 to 100% (Table 17). Ptb33 and Rathu Heenati were resistant to all the BPH populations. RD31 was resistant to four and moderately resistant to two populations. IR64 was resistant to three and moderately resistant to three populations. While CNT1, NPT13 and NPT8 were not consistent in their reaction to the BPH populations studied. Both CNT1 and NPT13 were susceptible to BPH population from Chachoengsao Rice Research Center. On the other hand, CNT1 was resistant to two and moderately resistant to three BPH populations; while NPT13 was resistant to one and moderately resistant to four BPH populations. NPT8 was resistant, moderately resistant and susceptible to one, three and two population, respectively. Mudgo was moderately resistant only to the population from Pathum Thani farmer's field (Thanyaburi district), but susceptible to the rest of the BPH populations. Azucena, CH1, NERICA3, NERICA4 and TN1 were susceptible to all the six populations.

Table 16 Mean of BPH damage-score of the six populations on the 22 tested genotypes of rice.

Genotype	BPH population					
	PTT_R	CCS_R	BKK_R	PTT_F	CCS_F	BKK_F
Azucena	9	9	9	9	9	7
CH1	7	9	9	9	9	9
CH2	7	9	9	7	3	7
CNT1	3	7	5	3	5	5
CO39	7	7	7	9	5	3
IR64	5	5	3	5	3	1
KDML105	7	9	7	9	5	9
Mudgo	9	9	9	5	9	7
NERICA3	7	9	9	9	7	9
NERICA4	9	9	7	7	7	7

Table 16 (Continued)

Genotype	BPH population					
	PTT_R	CCS_R	BKK_R	PTT_F	CCS_F	BKK_F
NPT13	5	7	5	5	3	5
NPT18	7	9	9	5	7	5
NPT4	7	7	9	7	9	5
NPT8	7	7	5	5	3	5
PtB33	0	1	3	3	1	0
PTT1	7	7	5	9	5	7
Rathu Heenati	1	1	3	3	1	3
RD31	5	3	3	5	1	3
SPR1	7	9	5	9	7	7
TDK1	7	9	5	7	5	5
TDK5	7	9	9	9	9	5
TN1	9	9	9	9	9	9

PTT_R = Pathum Thani Rice Research center,
CCS_R = Chachoengsao Rice Research center,
BKK_R = Kasetsart University Bangkhen,
PTT_F = Pathum Thani farmer’s field,
CCS_F = Chachoengsao farmer’s field,
BKK_F = Khlong Sam Wa farmer’s field

When the reaction of the genotypes to BPH collected from research stations and farmers' fields was compared, IR64 was moderately resistant to population of Chachoengsao Rice Research Center but resistant to Chachoengsao farmer's field population. The Thai rice varieties CNT1, PTT1 and KDML105 (traditional variety) were susceptible to Chachoengsao Rice Research Center population but moderately resistant to Chachoengsao farmer's field (Bang Nam Priao district) population. However, PTT1 and SPR1 which were susceptible to Khlong Sam Wa farmer's field population were moderately resistant to Kasetsart University-Bangkhen population.

Table 17 Relative virulence of the six BPH populations and susceptibility of the 22 tested rice genotypes.

Genotype	BPH population						
	PTT_R	CCS_R	BKK_R	PTT_F	CCS_F	BKK_F	Susceptibility (%)
PtB33	R	R	R	R	R	R	0
Rathu Heenati	R	R	R	R	R	R	0
RD31	MR	R	R	MR	R	R	0
IR64	MR	MR	R	MR	R	R	0
CNT1	R	S	MR	R	MR	MR	16.7
NPT13	MR	S	MR	MR	R	MR	16.7
NPT8	S	S	MR	MR	R	MR	33.3
TDK1	S	S	MR	S	MR	MR	50
CO39	S	S	S	S	MR	R	66.7
NPT18	S	S	S	MR	S	MR	66.7
PTT1	S	S	MR	S	MR	S	66.7
CH2	S	S	S	S	R	S	83.3
KDML105	S	S	S	S	MR	S	83.3
Mudgo	S	S	S	MR	S	S	83.3
NPT4	S	S	S	S	S	MR	83.3
SPR1	S	S	MR	S	S	S	83.3

Table 17 (Continued)

Genotype	BPH population						
	PTT_R	CCS_R	BKK_R	PTT_F	CCS_F	BKK_F	Susceptibility (%)
TDK5	S	S	S	S	S	MR	83.3
Azucena	S	S	S	S	S	S	100
CH1	S	S	S	S	S	S	100
NERICA 3	S	S	S	S	S	S	100
NERICA 4	S	S	S	S	S	S	100
TN1	S	S	S	S	S	S	100
Virulence (%)	72.73	81.82	54.54	59.09	45.45	45.45	

PTT_R = Pathum Thani Rice Research center**,**
CCS_R = Chachoengsao Rice Research center,
BKK_R = Kasetsart University Bangkhen,
PTT_F = Pathum Thani farmer’s field,
CCS_F = Chachoengsao farmer’s field, BKK_F = Khlong Sam Wa farmer’s field,
S = susceptible, MR = moderately resistant, R = resistant.

Cluster and principal component (PC) analyses

Cluster analysis of the genotypes by using the mean BPH resistance score showed appreciable diversity among the genotypes tested. It divided the tested genotypes into three groups at about 30% similarity level (Figure 10). Group I contained 18 genotypes including the susceptible check TN1. Group II contained two genotypes, IR64 and RD31; while the third group was made up of the two resistant checks Rathu Heenati and Ptb33.

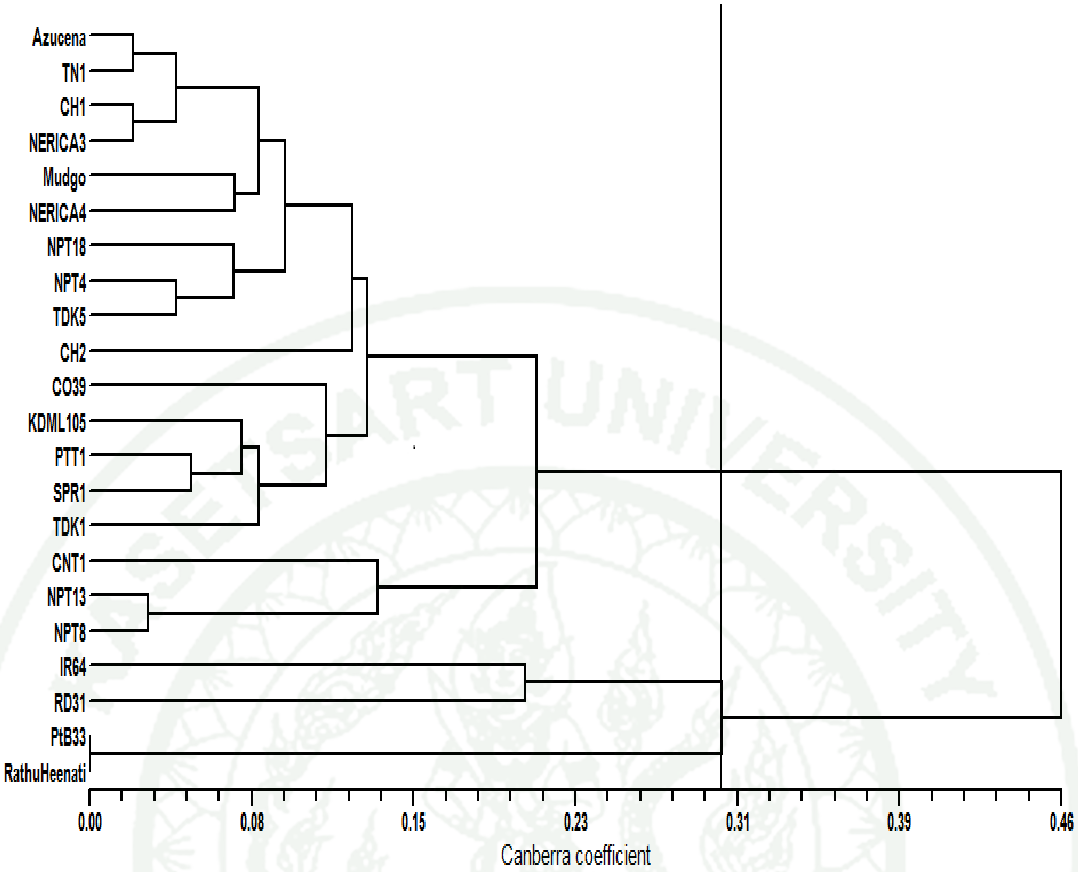


Figure 10 Dendrogram of the 22 rice genotypes based on the reaction to six BPH populations and constructed by means of paired group algorithm.

Cluster analysis to group the BPH populations was also executed by using the mean BPH resistance score of each location. The analysis showed a single group at 18% similarity level. However, it divided the populations into three groups at about 13% similarity level (Figure 11).

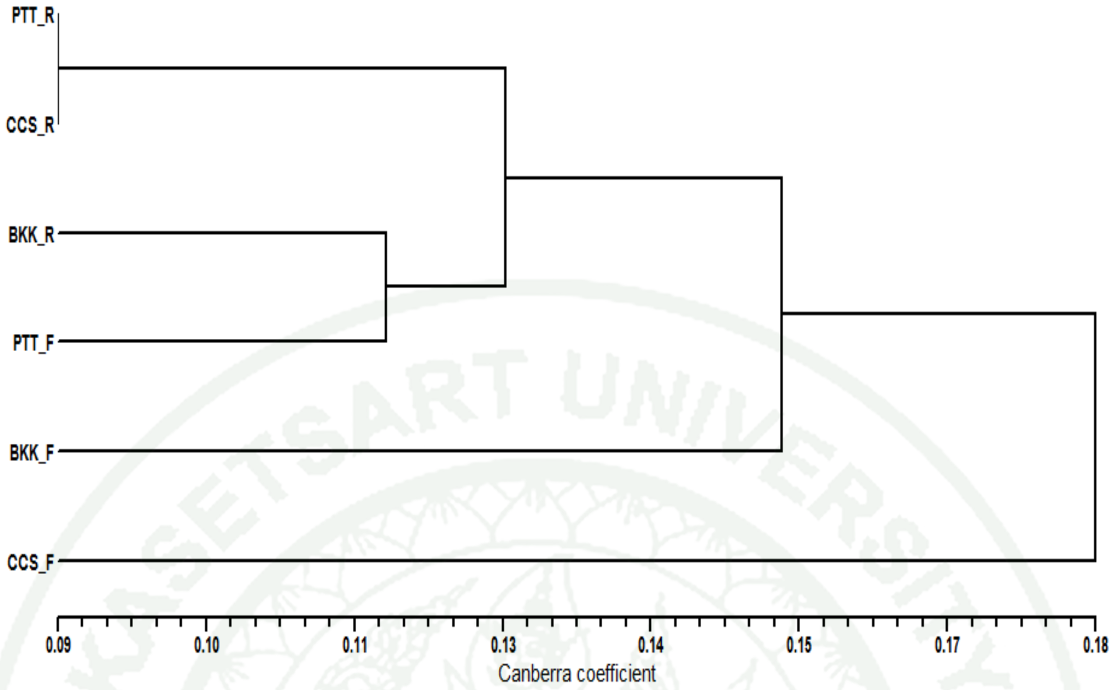


Figure 11 Dendrogram of the six BPH populations based on their virulence on the 22 rice genotypes and constructed by means of paired group algorithm.

The result of the principal component analysis showed that the first four PC axes were important in explaining the variability contained in the genotypes as they have eigenvalues greater than 1.0 (Table 18). Out of the four, the first and the second axes explained 76.88 and 8.18% of the variation, respectively. Together, the first two axes explained 85. 06% of the total variation. The first PC was very important in separating the genotypes studied (Figure 12).

Table 18 Eigenvalues, total variance, cumulative variance and eigenvectors for six BPH populations used for testing the resistance of the 22 rice genotypes.

BPH population	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**
PTT_R	0.398	0.151	0.225	-0.610
CCS_R	0.444	0.122	-0.188	-0.488
BKK_R	0.390	-0.416	-0.195	0.151
PTT_F	0.331	0.464	0.672	0.398
CCS_F	0.473	-0.607	0.202	0.241
BKK_F	0.399	0.454	-0.620	0.388
Eigenvalue	28.763	3.062	2.046	1.741
Variance (%)	76.875	8.184	5.468	4.652
Cumulative variance (%)	76.875	85.059	90.527	95.179

PTT_R = Pathum Thani Rice Research center, CCS_R = Chachoengsao Rice Research center, BKK_R = Kasetsart University Bangkhen, PTT_F = Pathum Thani farmer’s field, CCS_F = Chachoengsao farmer’s field, BKK_F = Khlong Sam Wa farmer’s field

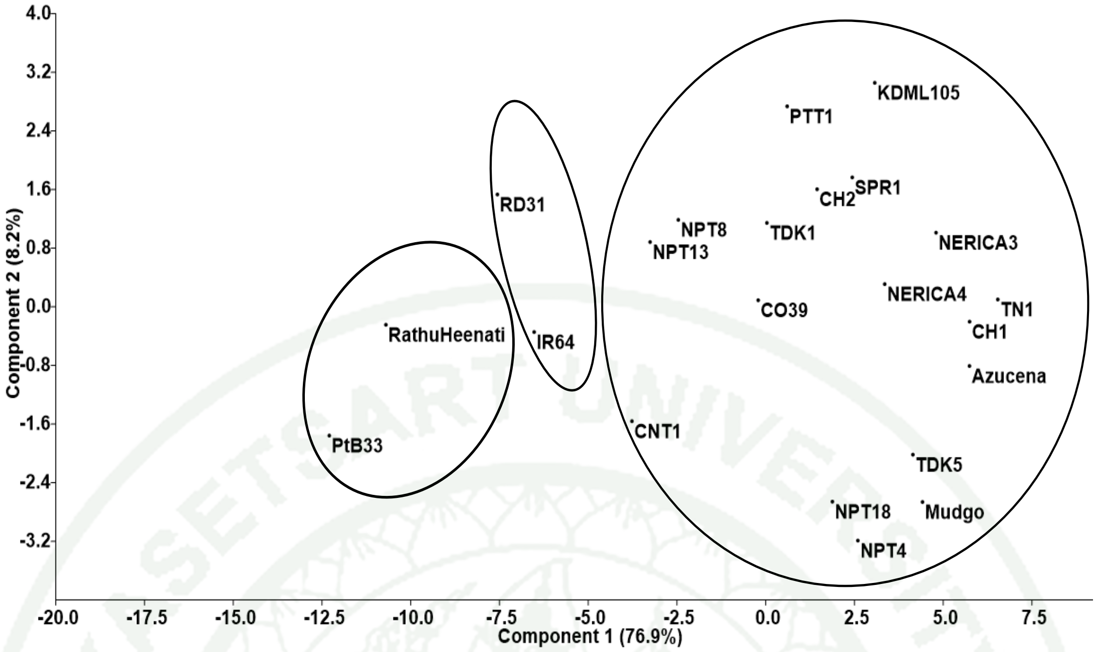


Figure 12 Scattered distribution of the 22 rice genotypes on the first two principal component axes.

Discussion

I. Genetic diversity analysis in rice using SSR markers

Plant breeding depends on the correct combination of specific alleles at the genetic loci present in a plants' genome (Hoisington *et al*., 1999). The knowledge of frequency and distribution of alleles is also important to identify genetically divergent parents; this information in turn assists plant breeders towards creating hybrid (in the F_1) and variability in the subsequent generations.

A mean of 4.966 SSR alleles per locus was reported in this experiment. In comparison with other works, the mean number of alleles estimated was smaller than the one reported by Alvarez *et al*. (2007), Giarrocco *et al*. (2007), Thomson *et al*. (2007) and Faivre-Rampant *et al*. (2010); and closer to that of Lapitan *et al*. (2010) and Saini *et al*. (2004).

Also, when compared with other findings, the mean H_e value is lower than the one reported by Alvarez *et al*. (2007). However, Thomson *et al*. (2007) reported a slightly lower H_e value (0.46). Most of the markers used for the study could be regarded as highly informative as 82.76% of them had high (greater than 0.5) H_e values. The genetic diversity of each SSR locus was found to be correlated (r=0.648) with the number of alleles (Na) detected per locus; the higher the H_e value is, the higher the Na per locus.

From the total combination of 276 pairwise unbiased minimum distance values, 40.94% had greater than 0.8 genetic distance coefficients indicating higher level of dissimilarity within the cultivars analyzed. The smallest (0.04) distance between the two NERICA cultivars showed that they shared higher degree of similarity as expected. They are sister lines and developed from the same parents (Samado *et al*., 2008). However, the highest genetic distance of the two NERICA cultivars, NERICA3 and NERICA4, from the cultivar TDK5 followed by TW2-

NPT13, NI-TDK5, NI-RD31 and Azucena-CH3 pairwise genetic distances suggesting the possibility of using them in further breeding programs.

The cluster analysis resulted into two conspicuous groups. Group I contained NERICA and *japonica* (both temperate and tropical) types. It is interesting to note that the *O. sativa* parent of upland NERICA types is *japonica* and these cultivars (NERICAs) are important in West Africa, where they were developed, and other upland rice growing sub-Saharan Africa countries (Samado *et al*., 2008), including Ethiopia. Most of the *indica* cultivars included in the second group are known for different traits of agronomic importance and cultivated in Asia. The 4 NPTs are breeding lines developed by the International Rice Research Institute (IRRI) during implementation of ideotype breeding (Kush, 2005).

The PCoA showed that the cultivars are generally placed to the left and to the right of the two-dimensional plane. Those which are located to the left are placed close to each other (aggregated), showing the similarity among them. The other cultivars which are placed to the right are somewhat scattered than the former. This shows the necessity of further dividing the member of this cluster into subgroups. The cluster analysis was in general agreement with PCoA in discriminating the cultivars.

II. Genetic diversity analysis in rice using morpho-agronomic characters

The significant ($P<0.01$) variation among the rice genotypes for all the morpho-agronomic traits and the range of values obtained for most of the traits indicates a sizable variability in the genotypes studied for the 17 traits considered. The correlation study showed that late flowering is important in attaining tall plant types, higher biomass and lower harvest index. It also showed that more number of tillers, tall plant types (increased height and panicle length), higher number of spikelets, heavier above ground mass, seed length and shape were the important traits in attaining higher yield per plant in rice. However, early flowering, short plant types (lower height and culm length), lower number of total spikelets, light above-ground mass but higher level of spikelet fertility are important to improve harvest index.

Significant correlations of yield and yield-related traits were also reported by other workers in inbred lines, hybrids and landrace rice (Chakravorty *et al*. 2013; Janwan *et al*., 2013 and Seesang *et al*., 2013).

The pairwise Euclidean distance coefficients estimated in this study ranged from 1.142 to 10.267 with a mean of 5.172. However, Caldo *et al*. (1996a) estimated a range of Euclidean distance varying between 2.23 and 16.71 with a mean of 7.55 for 78 improved rice genotypes by using 33 qualitative and quantitative traits. In a separate study of 81 ancestral lines of Philippines modern rice genotypes, Caldo *et al*. (1996b) computed Euclidean distance estimates ranging from 3.97 to 17.389 with a mean of 8.80 using 41 traits. The output of the hierarchical cluster analysis exhibited similar dendrogram topology and cluster membership of the rice genotypes for Gower, Euclidean and Manhattan distance measures. This confirms the stability of the dendrogram constructed. Generally, the first group was characterized by late flowering, tall plant types, higher biomass and grain yield, slender (narrow and long) and heavier seeds. Unlike the first group, the second group was characterized by early flowering, short plant types, lower biomass and grain yield, round- (wide and short) and light weighted- seeds. The two-step cluster analysis was similar to that of the hierarchical cluster analysis in grouping the genotypes into two groups and the cluster membership was also similar.

Principal components (PCs) are orthogonal and independent of each other (Mohammadi and Prasanna, 2003); they explain the variability which was not explained by the others. In this study, the total variability was explained by five PCs. This may indicate the contribution of many traits with higher level of correlation to explain the gross diversity. In a study of Caldo *et al*. (1996a), 75% of the variability was accounted for by seven PCs. In their work, Chakravorty *et al*. (2013) explained the total variability by six PC axes; Caldo *et al*. (1996b) explained 67% of the variation by 10 PCs. Jolliffe (2002) stated that PCs are ordered so that the first few retain most of the variation present in all of the original variables. In this experiment, the first and the second PCs explained 61.16% of the variability, the first being the most important. Accordingly, the traits included in the first PC especially those with comparatively high loadings (PCA coefficients) are important in separating the genotypes. Generally, the PC analysis showed high level of diversity of the rice genotypes as the entire variation cannot be explained by few principal components.

III. Evaluation of BPH resistance in rice

Varietal resistance to insects is the most practical and economical control measure (Brar *et al*., 2009). The development of new biotypes after the release of improved genotypes of rice that carry a single major insect resistance gene poses a great problem as the new biotypes have acquired virulence to the specific resistance gene (Fujita *et al*., 2009). This calls for the identification of several resistance genes (Brar *et al.*, 2009) and sequential release of varieties with major genes (Khush, 1979) to cope up with the development of new biotype population. A great emphasis has been given for organic farming as chemicals has adverse effects to the environment and non-target insects, and even to human health. Therefore, using BPH resistant varieties can go along with this system since no chemicals are involved in the control of the insect. Besides, it is economical to be practiced by farmers in developing countries because they are not expected to incur extra costs once they get the resistant varieties.

An attempt was made in order to determine the reaction of 22 rice genotypes to six BPH populations collected from three provinces of central Thailand. The result showed substantial difference among the populations used for the study. Population of Chachoengsao Rice Research Center was found to be the most virulent as compared to the others, as 18 (81.82 %) of the rice genotypes were found to be susceptible to this population. The next virulent population was that of Pathum Tani Rice Research center which was virulent over 16 (72.73%) of the rice genotypes. The result also showed differences in virulence of BPH populations collected from research station and farmer’s field of the same province. In this regard, the virulence of BPH from Chachoengsao Rice Research Center and farm were 81.82 and 45.45%, respectively; while that of Pathum Tani Rice Research Center and field were 72.73 and 59.09%, respectively. Similarly, the virulence of BPH population from Kasetsart University-

Bangkhen campus (representing Bangkok research station) was 54.54%, while that of the farmer’s field (Khlong SamWa district) was 45.45%. In all cases the populations collected from the rice research stations were relatively more virulent than the fields. Since the research stations are dealing with a number of rice genotypes as compared to farmers, BPH populations of the research stations may have the chance to adapt to more rice genotypes (hence virulent to more genotypes) than populations of the farms. Claridge *et al*. (1982) reported the occurrence of closely adapted BPH populations to different locally grown varieties within a small geographical area (about 100 km apart) in Sri Lanka.

Ptb33 and Rathu Heenati were resistant to all the six BPH populations. Both of them were included as resistant checks. The nature of resistance in Ptb33 is reported to be digenic and conditioned by *Bph3* and *bhp2* genes (Angeles *et al*., 1986) whereas *Bph3* gene is the source of resistance for Rathu Heenati (Lakshminarayana and Khush, 1977). Jairin *et al*. (2007a) reported the highly resistant reaction of Rathu Heenati (*Bph3*) to a number of BPH populations in Thailand. Although both IR64 and RD31 were resistant to some of the population used in the study, they were moderately resistant to two or three of the BPH populations. They could be regarded as moderately resistant genotypes. Moderate resistance of IR64 to BPH population of Pathum Thani was reported previously (Jairin *et al*., 2005). The source of resistance for IR64 is reported to be *Bph1* gene incorporated from the resistant parent Mudgo (Jena and Kim, 2010). In this study, however, Mudgo was found to be susceptible to all of the BPH populations except that of Pathum Thani farmer’s field (MR). Multiple QTLs conferring BPH resistance were detected by using mapping population derived from a cross of IR64 and Azucena (Alam and Cohen, 1998; Ramalingam *et al*., 2003; Soundararajan *et al*., 2004) most of which derived from IR64 (Alam and Cohen, 1998). IR64 was also reported to have slight to moderate levels of antibiosis, antixenosis and tolerance to BPH (Cohen *et al*., 1997). This explains why IR64 was resistant or moderately resistant to the populations investigated while its parent Mudgo was susceptible.

The genotypes CNT1, NPT13 and NPT8 were not consistent in their reaction to the BPH populations studied. On the one hand, they become resistant and moderately resistant to some populations; on the other hand, they still found to be susceptible to one or two of the populations. Both CNT1 and NPT13 were susceptible to the population from Chachoengsao research research center which was found to be most virulent according to this study. CNT1, once resistant to BPH, is now losing its resistance. Jairin *et al*. (2007b) also reported the break-down of the resistance of this variety within a few years by the occurrence of virulent BPH biotype. Therefore, the production of this variety is not dependable. The genotypes NERICA3, NERICA4, Azucena, CH1 and TN1 were susceptible to all the six populations. NERICA (New Rice for Africa) genotypes were developed by interspecific hybridization of African rice *O. glaberrima* and Asian rice *O. sativa* (Samado *et al*., 2008) under African environmental condition. BPH is not common in Africa (Catindig *et al*., 2009); it is therefore unlikely to get resistance to BPH in African varieties without operational selective pressure of the insect. Nevertheless, Liu *et al*. (2001) reported the existence of BPH resistance in one of the wild rice species, *Oryza eichingeri* (acc. 105159), found in Uganda, West Africa. TN1 was included as susceptible check as it does not have resistance gene to any of the BPH biotypes identified so far (Jena and Kim, 2010).

Cluster analysis by using the BPH-resistance (damage) score and Canberra distance coefficient divided the test genotypes into three groups. Group I, could be regarded as susceptible group, contained breeding lines (NTPs), varieties developed by interspecific crosses (NERICAs), genotypes once resistant but susceptible nowadays like CNT1, SPR1 and Mudgo (Jairin *et al*., 2007b; Khush, 1979), and traditional varieties without known resistance genes like KDML105, Azucena and TN1 (Cohen *et al*., 1997; Jairin *et al*., 2005; Jena and Kim, 2010). Group II contained two genotypes, IR64 and RD31, with moderate resistance. In areas with low insecticide use and presence of the potential actions of natural enemies, high levels of *N. lugens* (BPH) resistance may not be necessary (Bottrell and Schoenly, 2012; Cohen *et al*., 1997). Therefore, in such areas of central Thailand IR64 and RD31 could be used with caution. The third group, the resistant group, was made up of the two resistant checks Rathu Heenati and Ptb33. These two genotypes could obviously be used as parents in breeding for BPH resistance.

The principal component analysis showed that the first PC was very important in separating the genotypes studied based on their reaction to the BPH populations; clearly showing the pattern such that the genotype at the left side of the two dimensional plane (Figure 10) are most resistant and the resistance decreases towards the right hand side of the plane.

The study was conducted by using standard seed box screening test. This system offers the BPH a choice among the rice plants under investigation before initiating feeding (Horgan, 2009). The BPH, therefore, are free to move from plants to plants depending on the reaction of the plants or depending on preference of the BPH. This movement of the BPH inside the seed box is determined by the peculiarities, combinations and relative positions of the test genotypes (Horgan, 2009). Since these factors determine dispersion and settling of the BPH, the result would not be expected to be stable between successive tests. Thus, repeating the experiment by including more rice genotypes and BPH populations, and by rearranging the positions of the cultivars would be advisable.

CONCLUSION AND RECOMMENDATIONS

The 29 SSR markers provided considerable genetic resolution and this genetic diversity analysis showed higher level of allelic diversity in the cultivars analyzed. Due to the higher amount of hetrozygosity in the sample at each marker and the observed allelic diversity, most of the markers used in this study could be used in association and linkage studies. The result could be used for designing effective breeding programs. Cultivar combinations from the two clusters with higher value of dissimilarity coefficient could be used as parents as long as they have desirable combination of traits of agronomic importance; and thus heterotic expression in the F_1 hybrid and considerable variability in the subsequent segregating population may be expected. In this study, NERICA cultivars showed higher degree of dissimilarity from all of the other cultivars (especially from the second group). These cultivars could be used as parents for designing further breeding programs so as to come up with transgresive segregants.

The phenotypic diversity study showed the presence of considerable level of dissimilarity in the rice genotypes analyzed and the importance morpho-agronomic traits to study genetic diversity. Days to flowering, plant height, culm length, panicle length, biomass, seed length and shape were the important traits in differentiating the genotypes under study. The hierarchical and two-step cluster analyses were in general agreement with the PC analysis in grouping the genotypes into two clusters. Parental combinations from the two clusters with higher value of Euclidean distance (dissimilarity) coefficient could be used for genetic improvement by crossing. Generally KOH1 is the most diverged genotype from others. Thus crossing of this genotype with the likes of Azucena, CH1, KDML105 and SPR1 may result in heterotic expression in the F_1 hybrid and substantial variability in the subsequent segregating population.

Monitoring the virulence of BPH and the ability of the resistance genes may be necessary as there is a possibility of development of new BPH biotypes and the break-down of resistance genes as well. The cluster and PC analyses, in the BPH resistance study, were in agreement in explaining the variability contained in the genotypes. Rathu Heenati and Ptb33 could be used as sources of resistance for BPH in central areas of Thailand in order to curb the eminent development of new BPH biotypes and outbreak of the insect. Genotypes IR64 and RD31 could still be used with caution. However, it is important to study the reaction of more genotypes by rearranging their positions in the seed box and using additional BPH populations. Identification of biotypes could also be suggested. The differential response of the rice genotypes to BPH populations from research stations and farmers' fields of the same province also needs further investigation.

LITERATURE CITED

Acquaah, G. 2007. **Principles of Plant Genetics and Breeding**. Blackwell publishing, Oxford.

Ahmadian, A., B. Gharizadeh, A.C. Gustafsson, F. Sterky, P. Nyrén, M. Uhlén and J. Lundeberg. 2000. Single Nucleotide Polymorphism analysis by pyrosequencing. **Anal. Biochem.** 280: 103-110.

Alam, S. N. and M.B. Cohen. 1998. Detection and analysis of QTLs for resistance to brown planthopper, *Nilaparvata lugens,* in a doubled-haploid population. **Theor. Appl. Genet.** 97: 1370-1379.

Alvarez, A., J.L. Fuentes, V. Puldon, P.J. Gonez, L. Mora, M.C. Duque, G. Gallego and G.M. Tohme. 2007. Genetic diversity analysis of Cuban traditional rice (*Oryza sativa* L.) varieties based on microsatellite markers. **Genet. Mol. Biol.** 30: 1109-1117.

Angeles, E.R., G.S. Khush and E.A. Heinrichs. 1986. Inheritance of resistance to planthoppers and leafhopper in rice, pp 537-549. *In* IRRI, ed. **Rice genetics**. Proceedings of the international rice genetics symposium, 27-31 May 1985, International Rice Research Institute, Manila.

Asíns, M.J. 2002. Present and future of quantitative trait locus analysis in plant breeding. **Plant Breeding** 121: 281-291.

Athwal, D.S., M.D. Pathak, E. Bacalangco and C.D. Pura. 1971. Genetics of resistance to brown planthoppers and green leaf hoppers in *Oryza sativa* L. **Crop Sci.** 11: 747-750.

Benbouza, H., J.M. Jacquemin, J.P. Baudoin and G. Mergeai. 2006. Optimization of a reliable, fast, cheap and sensitive silver staining method to detect SSR markers in polyacrylamide gels. **Biotechnol. Agron. Soc. Environ.** 10: 77-81.

Bottrell, D.G. and K.G. Schoenly. 2012. Resurrecting the ghost of green revolutions past: The brown planthopper as a recurring threat to high-yielding rice production in tropical Asia. **J. Asia-Pacific Entomol.** 15: 122-140.

Brar, D.S., P.S. Virk, K.K. Jena and G.S. Khush. 2009. Breeding for resistance to planthoppers in rice, pp. 401-427. *In* K. L. Heong and B. Hardy, eds. **Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia**. International Rice Research Institute, Los Baños.

Caldo, R. A., L.S. Sebastian and J.E. Hernandez. 1996a. Multivariate analysis of phenotypic diversity of Philippine improved rice varieties. **Philipp. J. Crop Sci.** 21: 76-85.

_______, _______ and _______. 1996b. Morphology-based genetic diversity analysis of ancestral lines of Philippine rice cultivars. **Philipp. J. Crop Sci.** 21: 86-92.

Cao, Q., B.R. Lu, H. Xia, J. Rong, F. Sala, A. Spada and F. Grassi. 2006. Genetic diversity and origin of weedy rice (*Oryza sativa* f. spontanea) populations found in north-eastern China revealed by simple sequence repeat (SSR) markers. **Ann. Bot.** 98: 1241-1252.

Catindig, J. L.A., G.S. Arida, S.E. Baehaki, J.S. Bentur, L.Q. Cuong, M. Norowi, W. Rattanakarn, W. Sriratanasak, J. Xia and Z. Lu. 2009. Situations of planthoppers in Asia, pp. 191-220. *In* K.L. Heong and B. Hardy, eds. **Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia**. International Rice Research Institute, Los Baños.

Chakravorty, A., P.D. Ghosh and P.K. Sahu. 2013. Multivariate analysis of phenotypic diversity of landraces of rice of west Bengal. **American Journal of Experimental Agriculture** 3: 110-123.

Chelliah, S., L.T. Fabellar and E.A. Heinrichs. 1980. Effect of sub-lethal doses of three insecticides on the reproductive rate of the brown planthopper, Nilaparvata lugens, on rice. **Environ. Entomol.** 9: 778-780.

Chen, J. W., L. Wang, X.F. Pang and H. Pan. 2006. Genetic analysis and fine mapping of a rice brown planthopper (*Nilaparvata lugens* Stal) resistance gene *bph19(t)*. **Mol. Gen. Genomics** 275: 321-329.

Chen, X., S. Temnykh, Y. Xu, Y.G. Cho and S.R. McCouch. 1997. Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). **Theor. Appl. Genet.** 95: 553-567.

Claridge, M.F. and J. den Hollander. 1983. The biotype concept and its application to insect pest of agriculture. **Crop Prot.** 2: 85-95.

_______, _______ and I. Furet. 1982. Adaptations of brown planthopper (*Nilaparvata lugens*) populations to rice varieties in Sri Lanka. **Entomol. Exp. Appl.** 32: 222-226.

Cohen, M.B., S.N. Alam, E.B. Medina and C.C. Bernal. 1997. Brown planthopper, *Nilaparvata lugens*, resistance in rice cultivar IR64: mechanism and role in successful *N. lugens* management in Central Luzon, Philippines. **Entomol. Exp. Appl.** 85: 221-229.

Cuartero, J., M.C. Bolarin, V. Moreno and B. Pineda. 2010. Molecular tools for enhancing salinity tolerance in plants, pp. 373-405. *In* S.M. Jain and D.S. Brar, eds. **Molecular Techniques in Crop Improvement**, 2nd ed. Springer-Verlag, London.

Dawe, D. 2007. Agricultural research, poverty alleviation and key trends in Asia's rice economy, pp. 37-53. *In* J.E. Sheehy, P.L. Mitchell and B. Hardy, eds. **Charting New Pathways to C_4 Rice**. International Rice Research Institute, Los Baños.

Deen, R., K. Ramesh, S.K. Gautam, Y.K. Rao, V.J. Lakshmi, B.C. Viraktamath, D.S. Brar and T. Ram. 2010. Identification of new gene for BPH resistance introgressed from *O. rufipogon*. **Rice Genet. Newsl.** 25: 70-72.

Doyle, J.J. and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue. **Focus** 12: 13-15.

Duran, C., N. Appleby, D. Edwards and J. Batley. 2009. Molecular genetic markers: Discovery, applications, data storage and visualisation. **Curr. Bioinformatics** 4: 16-27.

Dyck, V.A. and B. Thomas. 1979. The brown planthopper problem, pp. 3-17. *In* IRRI, ed. **Brown Planthopper: Threat to Rice Production in Asia**. International Rice Research Institute, Los Baños.

Dyck, V.A., B.C. Misra, S. Alam, C.N. Chen, C.Y. Hsien and R.S. Rejesus. 1979. Ecology of the brown planthopper in the tropics, pp. 61-98. *In* IRRI, ed. **Brown Planthopper: Threat to Rice Production in Asia**. International Rice Research Institute, Los Baños.

Faivre-Rampant, O., G. Bruschi, P. Abbruscato, S. Cavigiolo, A.M. Picco, L. Borgo, E. Lupotto and P. Piffanelli. 2010. Assessment of genetic diversity in Italian rice germplasm related to agronomic traits and blast resistance (*Magnoporthe oryzae*). **Mol. Breeding** 27: 233-246.

FAO. 2013. **FAOSTAT**. Available source: http://www. faostat.fao.org/site/567/ default.aspx, April 14, 2013.

Fujita, D., K.K.M. Myint, M. Matsumura and H. Yasui. 2009. The genetics of host-plant resistance to rice planthopper and leafhopper, pp. 389-399. *In* K.L. Heong and B. Hardy, eds. **Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia**. International Rice Research Institute, Los Baños.

Gao, L.Z. 2003. The conservation of Chinese rice biodiversity: genetic erosion, ethnobotany and prospects. **Genet. Resour. Crop Evol.** 50: 17-32.

Giarrocco, L.E., M.A. Marassi and G.L. Salerno. 2007. Assessment of the genetic diversity in Argentine rice cultivars with SSR markers. **Crop Sci.** 47: 853-860.

Hammer, Ø., D.A.T. Harper, and P.D. Ryan. 2001. Past: Paleontological statistics software package for education and data analysis. **Palaeontologia Electronica** 4: 1-9.

Hashimoto, Z., N. Mori, M. Kawamura, T. Ishii, S. Yoshida, M. Ikegami, S. Takumi and C. Nakamura. 2004. Genetic diversity and phylogeny of Japanese sake-brewing rice as revealed by AFLP and nuclear and chloroplast SSR markers. **Theor. Appl. Genet.** 109: 1586-1596.

Heinrichs, E.A. 1986. Perspectives and directions for the continued development of insect-resistant rice varieties. **Agric. Ecosystems Environ.** l8: 9-36.

He, J., Y. Liu, Y. Liu, L. Jiang, H. Wu, H. Kang, S. Liu, L. Chen, X. Liu, X. Cheng and J. Wan. 2013. High-resolution mapping of brown planthopper (BPH) resistance gene *Bph27(t)* in rice (*Oryza sativa* L.). **Mol. Breeding** 31: 549-557.

Hirabayashi, H. and T. Ogawa. 1995. RFLP mapping of *Bph-1* (brown planthopper resistance gene) in rice. **Breed. Sci.** 45:369-71.

_______., E.R. Angeles, R. Kaji, T. Ogawa, D.S. Brar and G.S. Khush. 1998. Identification of brown planthopper resistance gene derived from *O. officinalis* using molecular markers in rice. **Breed. Sci.** 48 (Suppl): 82.

Hoisington, D., M. Khairallah, T. Reeves, J.M. Ribaut, B. Skovmand, S. Taba and M. Warburton. 1999. Plant genetic resources: what can they contribute toward increased crop productivity. **Proc. Natl. Acad. Sci. USA** 96: 5937-5943.

Holt, J., T.C.B. Chancellor, D.R. Reynolds and E.R. Tiongco. 1996. Risk assessment for rice planthopper and tungro disease outbreaks. **Crop Prot.** 15: 359-368.

Horgan, F. 2009. Mechanisms of resistance: a major gap in understanding planthopper-rice interactions, pp. 281-302. *In* K.L. Heong and B. Hardy, eds. **Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia**. International Rice Research Institute, Los Baños.

Hou, L. Y., P. Yu, Q. Xu, X. P. Yuan, H. Y. Yu, Y. P. Wang, C. H. Wang, G. Wan, S. X. Tang, S. T. Peng and X. H. Wei. 2011. Genetic analysis and preliminary mapping of two recessive resistance genes to brown planthopper, *Nilaparvata lugens* stål in rice. **Rice Science** 18: 238-242.

Huang, Z., G. He, L. Shu, X. Li and Q. Zhang. 2001. Identification and mapping of two brown planthopper resistance genes in rice. **Theor. Appl. Genet.** 102: 929-934.

Ikeda, R. and C. Kaneda. 1986. Genetic analysis of resistance to brown planthopper in rice, pp. 505-512. *In* IRRI, ed. **Rice Genetics**. Proceedings of the international rice genetics symposium, 27-31 May 1985, International Rice Research Institute, Manila.

IRRI. 2009. **Rice Knowledge Bank**. Available source: http://www.knowledgebank.irri.org/RiceDoctor/index.php?option=com_content&view=article&id=608&Itemid=2810, October 7, 2013.

______. 2002. **Standard evaluation systems for rice**. International Rice Research Institute, Los Baños.

Ishii, T., D.S. Brar, D.S. Multani and G.S. Kush. 1994. Molecular tagging of genes for brown planthopper resistance and earliness introgressed from *Oryza australiensis* into cultivated rice, *Oryza sativa*. **Genome** 37: 217-221.

Jairin J., K. Phengrat, S. Teangdeerith, A. Vanavichit and T. Toojinda. 2007a. Mapping of a broad-spectrum brown planthopper resistance gene, *Bph3*, on rice chromosome 6. **Mol. Breed.** 19: 35-44.

Jairin J., S. Teangdeerith, P. Leelagud, K. Phengrat, A. Vanavichit and T. Toojinda. 2007b. Detection of brown planthopper resistance genes from different rice mapping populations in the same genomic location. **Science Asia** 33: 347-352.

_______, T. Toojinda, S. Tragoonrung, S. Tayapat and A. Vanavichit. 2005. Multiple genes determining brown planthopper (*Nilaparvata lugens* Stal) resistance in backcross introgressed lines of Thai jasmine rice ‘KDML105’. **Science Asia** 31: 129-135.

Janwan, M., T. Sreewongchai and P. Sripichitt. 2013. Rice breeding for high yield by advanced single seed descent method of selection. **J. Plant Sci.** 8: 24-30.

Jena, K.K. and S.M. Kim. 2010. Current status of brown planthopper (BPH) resistance and genetics. **Rice** 3:161-171.

Jena, K.K., I.C. Pasalu, Y.K. Rao, Y. Varalaxmi, K. Krishnaiah, G.S. Khush and G. Kochert. 2002. Molecular tagging of a gene for resistance to brown planthopper in rice (*Oryza sativa* L.). **Euphytica** 129: 81-88.

_______, J.U. Jeung, J.H. Lee, H.C. Choi and D.S. Brar. 2006. High-resolution mapping of a new brown planthopper (BPH) resistance gene, *Bph18(t)*, and marker-assisted selection for BPH resistance in rice (*Oryza sativa* L.). **Theor. Appl. Genet.** 112: 288-297.

Jeon, Y.H., S.N. Ahn, H.C. Choi, T.R. Hahn and H.P. Moon. 1999. Identification of a RAPD marker linked to a brown planthopper resistance gene in rice. **Euphytica** 107: 23-8.

Jolliffe, I.T. 2002. **Principal Component Analysis**. 2nd ed. Springer-Verlag, New York.

Jones, N., H. Ougham and H. Thomas. 1997. Markers and mapping: we are all geneticists now. **New Phytol.** 137: 165-177.

Kabir, M.A. and G.S. Khush. 1988. Genetic analysis of resistance to brown planthopper in rice (*Oryza sativa* L.). **Plant Breed.** 100: 54-85.

Karp, A., O. Seberg and M. Buiatti. 1996. Molecular techniques in the assessment of botanical diversity. **Ann. Bot.** 78: 143-149.

Karp, A., S. Kresovich, K. Bhat, W. Ayad and T. Hodgkin. 1997. **Molecular tools in plant genetic resources conservation: a guide to the technologies**. IPGRI Technical Bulletin No. 2. International Plant Genetic Resources Institute. Rome.

Kawaguchi, M., K. Murata, T. Ishii, S. Takumi, N. Mori and C. Nakamura. 2001. Assignment of a brown planthopper (*Nilaparvata lugens* Stål) resistance gene *bph4* to the rice chromosome 6. **Breed. Sci.** 51: 13-8.

Khush, G.S. 1979. Genetics of and breeding for resistance to the brown planthopper, pp. 321-332. *In* IRRI, ed. **Brown Planthopper: Threat to Rice Production in Asia**. International Rice Research Institute, Los Baños.

Khush, G.S. 2005. What it will take to feed 5.0 billion rice consumers in 2030. **Plant Mol. Boil.** 59: 1-6.

Khush, G.S. and D.S. Brar. 1991. Genetics of resistance to insects in crop plants. **Adv. Agron.** 45: 223-74.

Kim, S.M. and J.K. Sohn. 2005. Identification of rice gene (*Bph1*) conferring resistance to brown planthopper (*Nilaparvata lugens* Stal) using STS markers. **Mol. Cells.** 20: 30-4.

Kisimoto, R. 1979. Brown planthopper migration, pp. 113-124. *In* IRRI, ed. **Brown Planthopper: Threat to Rice Production in Asia**. International Rice Research Institute, Los Baños.

Kojima, Y., K. Ebana, S. Fukuoka, T. Nagamine and M. Kawase. 2005. Development of an RFLP-based rice diversity research set of germplasm. **Breeding Sci.** 55: 431-440.

Kumar, L.S. 1999. DNA markers in plant improvement: An overview. **Biotechnol. Adv.** 17: 143-182.

Kuno, E. 1979. Ecology of the brown planthopper in temperate regions, pp. 45-60. *In* IRRI, ed. **Brown Planthopper: Threat to Rice Production in Asia**. International Rice Research Institute, Los Baños.

Lakshminarayana, A. and G.S. Kush. 1977. New genes for resistance to the brown planthopper in rice. **Crop Sci**. 17: 96-100.

Lapitan, V.C., D.S. Brar, T. Abe and E.D. Redoña. 2007. Assessment of genetic diversity of Philippine rice cultivars carrying good quality traits using SSR markers. **Breeding Sci.** 57: 263-270.

Li, R., L. Li, S. Wei, Y. Wei, Y. Chen, D. Bai, L. Yang, F. Huang, W. Lu, X. Zhang, X. Li, X. Yang and Y. Wei. 2006. The evaluation and utilization of new genes for brown planthopper resistance in common wild rice (*Oryza rufipogon* Griff.). **Molecular Plant Breed.** 4: 365-371.

Liu, G., H. Yan, Q. Fu, Q. Qian, Z. Zhang, W. Zhai and L. Zhu. 2001. Mapping of a new gene for brown planthopper resistance in cultivated rice introgressed from *Oryza eichingeri*. **Chinese Sci. Bull.** 46: 1459-1462.

Liu, Y., C. Su, L. Jiang, J. He, H. Wu, C. Peng and J. Wan. 2009. The distribution and identification of brown planthopper resistance genes in rice. **Hereditas** 146: 67-73.

Lu, Z.X. and K.L. Heong. 2009. Effects of nitrogen-enriched rice plants on ecological fitness of planthoppers, pp. 247-256. *In* K.L. Heong and B. Hardy, eds. **Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia**. International Rice Research Institute, Los Baños.

Maxted, N., L. Guarino, L. Myer and E.A. Chiwona. 2002. Towards a methodology for on-farm conservation of plant genetic resources. **Genet. Resources Crop Evol.** 49**:** 31-46.

McCouch, S.R., L. Teytelman, Y. Xu, K.B. Lobos, K. Clare, M. Walton, B. Fu, R. Maghirang, Z. Li, Y. Xing, Q. Zhang, I. Kono, M. Yano, R. Fjellstrom, G. DeClerck, D. Schneider, S. Cartinhour, D. Ware and L. Stein. 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). **DNA Research** 9: 199-207.

_______, X. Chen, O. Panaud, S. Temnykh, Y. Xu, Y.G. Cho, N. Huang, T. Ishii and M. Blair. 1997. Microsatellite marker development, mapping and applications in rice genetics and breeding. **Plant Molecular Biology** 35: 89-99.

Miller, M.P. 1997. **Tools for population genetic analysis (TFPGA), 1.3: A windows program for the analysis of allozyme and molecular population genetic data.** Distributed by the author. n.p.

Mochida, O. and T. Okada. 1979. Taxonomy and biology of *Nilaparvata lugens* (Hom., Delphacidae), pp. 21-43. *In* IRRI, ed. **Brown Planthopper: Threat to Rice Production in Asia**. International Rice Research Institute, Los Baños.

Mohammadi, S.A. and B.M. Prasanna. 2003. Analysis of genetic diversity in crop plants-salient statistical tools and considerations. **Crop Science** 43: 1235-1248.

Murai, H., Z. Hashimoto, P.N. Sharma, T. Shimizu, K. Murata, S. Takumi, N. Mori, S. Kawasaki and C. Nakamura. 2001. Construction of a high resolution linkage map of a rice brown planthopper (*Nilaparvata lugens* Stal) resistance gene *bph2*. **Theor. Appl. Genet.** 103: 526-32.

Murata, K., M. Fujiwara, H. Murai, S. Takumi, N. Mori and C. Nakamura. 2001. Mapping of a brown planthopper (*Nilaparvata lugens* Stal) resistance gene *Bph9* on the long arm of chromosome 12. **Cereal Res. Commun.** 29: 245-50.

Myint, K.K.M., D. Fujita, M. Matsumura, T. Sonoda, A. Yoshimura and H. Yasui. 2012. Mapping and pyramiding of two major genes for resistance to the brown planthopper (*Nilaparvata lugens* [Stal]) in the rice cultivar ADR52. **Theor. Appl. Genet.** 124: 495-504.

Nasu, S., J. Suzuki, R. Ohta, K. Hasegawa, R. Yui, N. Kitazawa, L. Monna and Y. Minobe. 2002. Search for and analysis of Single Nucleotide Polymorphisms (SNPs) in rice (*Oryza sativa*, *Oryza rufipogon*) and establishment of SNP markers. **DNA Research** 9: 163-171.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. **Proc. Natl. Acad. Sci. USA** 70: 3321-3323.

Nei, M. 1978. Estimation of average hetrozygosity and genetic distance from a small number of individuals. **Genetics** 89: 583-590.

Nemoto, H., R. Ikdea and C. Kaneda. 1989. New genes for resistance to brown planthopper, *Nilaparvata lugens* Stål, in rice. **Jpn. J. Breed.** 39: 23-28.

Pathak, M.D. and G.S. Khush. 1979. Studies of varietal resistance in rice to the brown planthopper at the International Rice Research Institute, pp. 285-301. *In* IRRI, ed. **Brown Planthopper: Threat to Rice Production in Asia**. International Rice Research Institute, Los Baños.

_______ and Z.R. Khan. 1994. **Insect Pests of Rice**. International Rice Research Institute, Manila.

Peakall, R. and P.E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. **Molecular Ecology Notes** 6: 288-295.

Pongprasert, S. and P. Weerapat. 1979. Varietal resistance to the brown planthopper in Thailand, pp. 273-283. *In* IRRI, ed. **Brown Planthopper: Threat to Rice Production in Asia**. International Rice Research Institute, Los Baños.

Powell, W., G.C. Machray and J. Provan. 1996. Polymorphism revealed by simple sequence repeats. **Trends Plant Sci.** 1: 215-222.

Rahman, L., W. Jiang, S.H. Chu, Y. Qiao, T.H. Ham, M.O. Woo, J. Lee, M.S. Khanam, J.H. Chin, J.U. Jeung, D.S. Brar, K.K. Jena and H.J. Koh. 2009. High-resolution mapping of two rice brown planthopper resistance genes, *Bph20(t)* and *Bph21(t)*, originating from *Oryza minuta*. **Theor. Appl. Genet**. 119: 1237-1246.

Ramalingam, J., C.M.V. Cruz, K. Kukreja, J.M. Chittoorr, J.L. Wu, S.W. Lee, M. Baraoidan, M.L. George, M.B. Cohen, S.H. Hulbert, J.E. Leach and H.Leung. 2003. Candidate defense genes from rice, barley, and maize and their association with qualitative and quantitative resistance in rice. **Mol. Plant-Microbe Interact.** 16: 14-24.

Rao, V.R. and T. Hodgkin. 2002. Genetic diversity and conservation and utilization of plant genetic resources. **Plant Cell Tiss. Org.** 68: 1-19.

Ravi, M., S. Geethanjali, F. Sameeyafarheen and M. Maheswaran. 2003. Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. **Euphytica** 133: 243-252.

Ren, X., X. Wang, H. Yuan, Q. Weng, L. Zhu and G. He. 2004. Mapping quantitative trait loci and expressed sequence tags related to brown planthopper resistance in rice. **Plant Breed.** 123: 342-8.

Renganayaki, K., A.K. Fritz, S. Sadasivam, S. Pammi, S.E. Harrington, S.R. McCouch, S.M. Kumar, and A.S. Reddy. 2002. Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from *Oryza officinalis* into cultivated rice. *O. sativa*. **Crop Sci.** 42: 2112-2117.

Rohlf, F.J. 1998. **NTSYS-pc numerical taxonomy and multivariate analysis system Version 2.02**. Applied Biostatistics Inc., Setauket, New York.

Saini, N., N. Jain, S. Jain and R.K. Jain. 2004. Assessment of genetic diversity within and among Basmati and non-Basmati rice varieties using AFLP, ISSR and SSR markers. **Euphytica** 140: 133-146.

Samado, E.A., R.G. Guei and N. Nguyen. 2008. NERICA: Origin, nomenclature and identification characteristics, pp: 10-19. *In* E.A. Somado, R.G. Guei and S.O. Keya, eds. **NERICA®: The New Rice for Africa – a Compendium**, Africa Rice Center, Cotonou, Benin.

Seesang, J., P. Sripicchitt, P. Somchit and T. Sreewongchai. 2013. Genotypic correlation and path coefficient for some agronomic traits of hybrid and inbred rice (*Oryza sativa* L.) cultivars. **Asian J. Crop Sci.** 5: 319-324.

Sharma, P.N., A. Torii, S. Takumi, N. Mori and C. Nakamura. 2004. Marker-assisted pyramiding of brown planthopper (*Nilaparvata lugens* Stal) resistance genes *Bph1* and *Bph2* on rice chromosome 12. **Hereditas** 136: 39-43.

Sharma, P.N., Y. Ketipearachchi, K. Murata, A. Torii, S. Takumi, N. Mori and C. Nakamura. 2002. RFLP/AFLP mapping of a brown planthopper (*Nilaparvata lugens* Stal.) resistance gene *Bph1* in rice. **Euphytica** 129: 109-17.

Shirasawa, K., L. Monna, S. Kishitani and T. Nishio. 2004. Single Nucleotide Polymorphisms in randomly selected genes among japonica rice (*Oryza sativa* L.) varieties identified by PCR-RF-SSCP. **DNA Research** 11: 275-283.

Shivrain, V.K., N.R. Burgos, H.A. Agrama, A. Lawton-Rauh, B. Lu, M.A. Sales, V. Boyett, D.R. Gealy and K.A.K. Moldenhauer. 2010. Genetic diversity of weedy red rice (*Oryza sativa*) in Arkansas, USA. **Weed Res.** 50: 289-302.

Sogawa, K. and C.H. Cheng. 1979. Economic thresholds, nature of damage and losses caused by the brown planthopper, pp. 125-142. *In* IRRI, ed. **Brown Planthopper: Threat to Rice Production in Asia**. International Rice Research Institute, Los Baños.

Soundararajan, R.P., P. Kadirvel, J.K. Gunathilagara and M. Maheswaran. 2004. Mapping of quantitative trait loci associated with resistance to brown planthopper in rice by means of a doubled-haploid population. **Crop Sci.** 44: 2214-20.

Stuber, C.W., M. Polacco and M.L. Senior. 1999. Synergy of empirical breeding, marker assisted selection and genomics to increase crop yield potential. **Crop Sci.** 39: 1571-1583.

Su, C.C., H.Q. Zhai, C.M. Wang, L.H. Sun and J.M. Wan. 2006. SSR mapping of brown planthopper resistance gene *Bph9* in Kaharamana, an indica rice (*Oryza sativa* L.). **Acta Genet. Sin.** 33: 262-8.

Sun, L.H., C.M. Wang, C.C. Su, Y.Q. Liu, H.Q. Zhai and J. Wan. 2006. Mapping and marker-assisted selection of a brown planthopper resistance gene *bph2* in rice (*Oryza sativa* L.). **Acta Genet. Sin.** 33: 717-23.

Sun, L., C. Su, C. Wang, H. Zhai and J. Wan. 2005. Mapping of a major resistance gene to the brown planthopper in the rice cultivar Rathu Heenati. **Breeding Sci.** 55: 391-396.

_______, Y. Liu, L. Jiang, C. Su, G. Wang, H. Zhai and J. Wan. 2007. Identification of quantitative trait loci associated with resistance to brown planthopper in the indica rice cultivar Col. 5 Thailand. **Hereditas** 144: 48-52.

Tanksley, S.D. 1983. Molecular markers in plant breeding. **Plant Mol. Boil. Rep.** 1: 3-8.

Temnykh, S., G. DeClerck, A. Lukashova, L. Lipovich, S. Cartinhour and S. McCouch. 2001. Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations and genetic marker potential. **Genome Research** 11: 1441-1452.

Temnykh, S., W.D. Park, N. Ayres, S. Cartinhour, N. Hauck, L. Lipovich, Y.G. Cho, T. Ishii and S.R. McCouch. 2000. Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). **Theor. Appl. Genet**. 100: 697-712.

Thomson, M.J., E.M. Septiningsih, F. Suwardjo, T.J. Santoso, T.S. Silitonga and S.R. McCouch. 2007. Genetic diversity analysis of traditional and improved Indonesian rice (*Oryza sativa* L.) germplasm using microsatellite markers. **Theor. Appl. Genet.** 114: 559-568.

Tu, M., B.R. Lu, Y. Zhu and Y. Wang. 2007. Abundant within-varietal genetic diversity in rice germplasm from Yunnan province of China revealed by SSR fingerprints. **Biochem. Genet.** 45: 789-801.

Visarto, P., M.P. Zalucki, H.J. Nesbitt and G.C. Jahn. 2001. Effect of fertilizer, pesticide treatment and plant variety on the realized fecundity and survival rates of brown planthopper, *Nilaparvata lugens* (Stal) (Homoptera: Delphacidae) - Generating outbreaks in Cambodia. **J. Asia-Pacific Entomol**. 4: 75-84.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. **Nucleic Acids Res.** 23: 4407-4414.

Wade, L.J., S. Fukai, B.K. Samson, A. Ali and M.A. Mazid. 1999. Rainfed lowland rice: physical environment and cultivar requirements. **Field Crop. Res.** 64: 3-12.

Weising, K., H. Nybom, K. Wolff and G. Kahl. 2005. **DNA Fingerprinting in Plants: Principles, Methods and Applications,** 2nd ed. Taylor and Francis group, Boca Raton.

Winter, P. and G. Kahl. 1995. Molecular marker technologies for crop improvement. **World J. Microb. Biot.** 11: 438-448.

Wu, C.J., G.H. Jiang, X. Li, T. Liu, C.G. Xu and Y.Q. He. 2005. Dynamic detection and analysis of QTL for resistance to the brown planthopper, using a doubled-haploid rice population. **Mol. Plant Breeding** 3: 456-462. (in Chinese with English abstract)

Wu, J.C., J.X. Xu, S.Z. Yuan, J.L. Liu, Y.H. Jiang and J.F. Xu. 2001. Pesticide-induced susceptibility of rice to brown planthopper, *Nilaparvata lugens*. **Entomol. Exp. Appl.** 100: 119-126.

Yang, H.Y., A.Q. You, Z.F. Yang, F.T. Zhang, R.F. He, L.L. Zhu and G.C. He. 2004. High-resolution genetic mapping at the *Bph15* locus for brown planthopper resistance in rice (*Oryza sativa* L.). **Theor. Appl. Genet.** 110: 182-191.

Yang, H., X. Ren, Q. Weng, L. Zhu and G. He. 2002. Molecular mapping and genetic analysis of a rice brown planthopper (*Nilaparvata lugens* Stal) resistance gene. **Hereditas** 136: 39-43.

Yeh, F.C., R.C. Yang, T.B. J. Boyle, Z.H. Ye and J.X. Mao. 1997. **POPGENE, the user-friendly shareware for population genetic analysis**. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Yin, J.L., H.W. Xu, J.C. Wu, J.H. Hu and G.Q. Yang. 2008. Cultivar and insecticide applications affect the physiological development of the brown planthopper, *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae). **Environ. Entomol.** 37: 206-212.

Yu, G.Q., Y. Bao, C.H. Shi, C.Q. Dong and S.Ge. 2005. Genetic diversity and population differentiation of Liaoning weedy rice detected by RAPD and SSR markers. **Biochem. Genet.** 43: 261-270.

Zhang, Y.M. 2006. Advances on methods for mapping QTL in plants. **Chinese Sci. Bull.** 51: 2809-2818.

Zhao, W., J.W. Chung, K.H. Ma, T.S. Kim, S.M. Kim, D.I. Shin, C.H. Kim, H.M. Koo and Y.J. Park. 2009. Analysis of genetic diversity and population structure of rice cultivars from Korea, China and Japan using SSR markers. **Genes and Genomics** 31: 283-292.

APPENDIX

Appendix Table 1 Number of alleles, information index and hetrozygosity values of SSR loci on rice chromosomes.

Chromosome	**Number of loci**	**Na**	**Ne**	**I**	**He**	**UHe**
1	4	4.75	2.87	1.193	0.621	0.635
2	3	5.00	4.05	1.481	0.751	0.768
3	1	3.00	2.60	1.010	0.615	0.631
4	2	6.50	4.51	1.620	0.763	0.780
5	4	4.00	2.69	1.112	0.611	0.627
6	3	3.67	2.38	0.986	0.539	0.551
8	1	3.00	1.72	0.709	0.417	0.427
9	2	5.00	3.68	1.399	0.714	0.729
10	1	6.00	4.88	1.679	0.795	0.812
11	5	6.20	3.97	1.525	0.717	0.733
12	3	5.67	3.44	1.349	0.648	0.662
Mean	2.64	4.80	3.34	1.28	0.65	0.67

Na = number of different allales, Ne = number of effective alleles,

I = Shannon's information index, He = expected hetrozygosity,

UHe = Unbiased expected hetrozygosity

Appendix Table 2 Mean values of the 24 rice cultivars for 17 traits used in the morpho-agronomic diversity analysis.

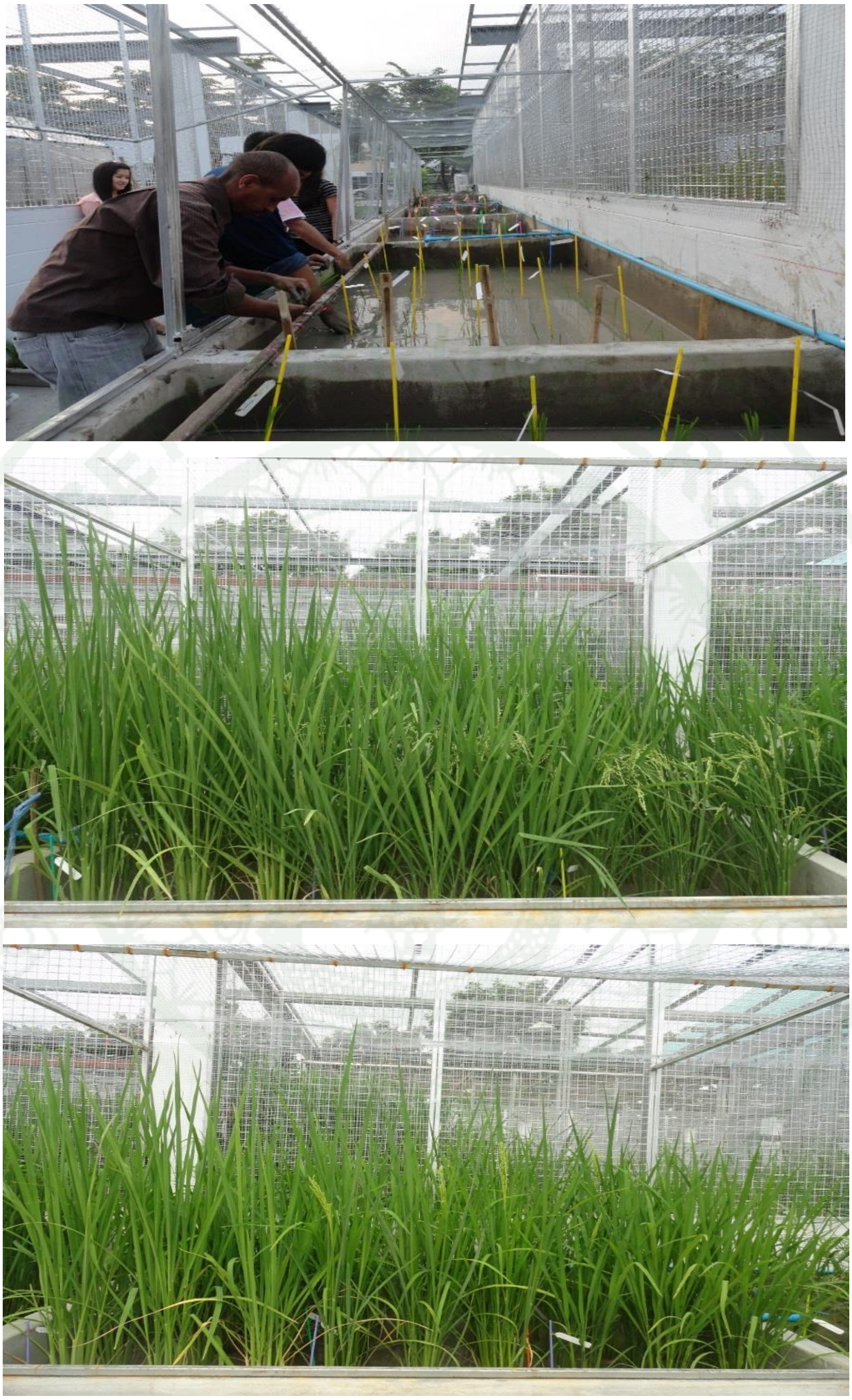
Cultivar	Days to flowering	Total number of tillers	No. of productive tillers	Plant height	Culm length
AZUCENA	149.00	6.33	3.39	167.00	137.78
CH1	97.33	9.67	8.33	115.37	86.49
CH2	91.00	8.33	6.89	82.06	61.12
CH3	88.67	5.89	4.33	86.89	67.17
CNT1	106.33	14.89	10.28	115.81	84.83
CO39	75.33	7.22	6.67	68.69	46.96
R64	105.33	10.22	8.11	102.43	76.89
KDML105	149.67	5.58	3.83	157.39	130.44
KOH1	79.67	5.44	4.72	69.90	52.34
NERICA3	88.00	3.33	3.13	108.52	79.34
NERICA4	88.33	3.78	3.44	101.28	72.84
NI	84.33	10.89	10.56	79.01	57.84
NPT13	111.00	6.33	5.67	105.80	78.10
NPT18	114.67	8.67	7.50	118.66	88.14
NPT4	113.67	9.97	6.47	108.71	77.44
NPT8	107.67	11.11	10.78	115.51	83.42
PSL60-2	115.33	10.22	7.56	134.10	106.73
PTT1	106.67	9.78	9.22	113.88	84.82
RD31	108.33	9.78	8.89	118.26	89.27
SPR1	109.33	8.56	8.00	137.44	108.36
TDK1	127.00	5.33	3.89	149.49	123.81
TDK5	127.00	9.00	7.89	123.89	96.00
TW1	111.67	6.76	5.33	93.72	71.72
TW2	112.00	7.56	6.56	100.63	78.42

Appendix Table 2 (Continued)

Cultivar	Panicle length	Total number of spikelets	No. of fertile spikelets	Spikelet fertility	100-seed weight
AZUCENA	29.22	289.30	239.97	83.07	2.04
CH1	28.88	342.56	277.22	80.77	2.31
CH2	20.93	206.56	171.89	83.42	1.77
CH3	19.72	210.78	179.00	81.17	1.68
CNT1	30.98	188.33	146.22	78.00	2.70
CO39	21.73	134.67	98.00	72.89	2.01
R64	25.54	140.78	108.00	76.51	2.54
KDML105	26.94	261.43	196.37	75.76	2.25
KOH1	17.56	62.67	55.89	89.08	2.28
NERICA3	29.17	220.56	182.17	82.80	3.15
NERICA4	28.43	191.56	172.56	90.13	3.03
NI	21.17	61.33	58.33	95.18	2.34
NPT13	27.70	150.89	99.44	66.32	2.68
NPT18	30.51	290.83	167.28	57.24	2.61
NPT4	31.33	288.53	183.14	62.28	2.88
NPT8	32.09	209.78	138.11	64.78	2.25
PSL60-2	27.37	256.78	189.22	73.79	2.39
PTT1	29.06	188.17	141.72	77.12	2.54
RD31	28.99	159.89	109.11	68.65	2.72
SPR1	29.08	224.61	157.56	69.58	2.85
TDK1	25.68	175.44	122.33	71.14	2.59
TDK5	27.89	209.22	133.22	64.12	2.74
TW1	22.00	212.22	133.44	62.92	2.52
TW2	22.21	246.11	163.89	65.46	2.56

Appendix Table 2 (Continued)

Cultivar	Seed width	Seed length	Seed shape	Biomass	Yield per plant	Harvest index
AZUCENA	2.20	9.19	4.18	58.31	15.44	27.26
CH1	2.10	10.22	4.88	63.57	29.06	45.09
CH2	2.10	8.09	3.86	26.18	12.48	47.20
CH3	2.11	7.93	3.77	20.04	8.64	38.96
CNT1	2.19	10.42	4.76	61.49	24.75	39.79
CO39	2.38	7.62	3.20	16.02	10.59	66.10
R64	2.16	9.82	4.55	34.83	14.26	39.31
KDML105	2.38	10.46	4.45	80.30	24.47	41.35
KOH1	2.85	7.17	2.52	8.87	5.04	53.40
NERICA3	2.38	9.79	4.11	23.00	12.43	54.22
NERICA4	2.48	9.92	4.01	24.55	13.53	55.30
NI	2.74	7.14	2.61	20.54	12.17	59.14
NPT13	2.39	9.74	4.08	26.41	7.60	29.46
NPT18	2.35	9.13	3.89	68.87	18.68	24.33
NPT4	2.58	8.88	3.45	64.46	16.30	24.69
NPT8	2.04	10.19	5.00	60.64	22.02	35.03
PSL60-2	2.09	9.95	4.77	77.40	19.67	26.31
PTT1	2.20	10.64	4.84	56.58	24.08	43.39
RD31	2.24	9.93	4.44	48.10	17.01	35.54
SPR1	2.20	10.84	4.93	74.67	25.05	32.92
TDK1	2.36	10.22	4.33	74.79	10.72	15.26
TDK5	2.26	10.85	4.79	90.45	17.96	20.06
TW1	3.01	6.55	2.18	41.26	10.61	24.68
TW2	3.00	6.61	2.20	51.71	17.47	30.51



Appendix Figure 1 Morpho-agronomic genetic diversity analysis in rice.

Appendix Figure 2 BPH collection by using susceptible rice seedlings

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