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Genetic analysis of brown planthopper resistance in Thai cultivars, local varieties, and wild relatives of rice using molecular markers

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Abstract. *Saengprajak J, Phetsom J, Sangdee A, Rattanapan A, Saengprajak A, Thanyasiriwat T, Mahakham W, Theerakulpisut P. 2024. Genetic analysis of brown planthopper resistance in Thai cultivars, local varieties, and wild relatives of rice using molecular markers. Biodiversitas 25: 4870-4877.* The brown planthopper [*Nilaparvata lugens* Stål 1854] is prevalent in Asia as a major insect pest that causes significant damage to rice crops. This study aimed to identify cost-effective, eco-friendly methods for developing BPH (Brown Planthopper)-resistant rice varieties by examining the genetic basis of resistance in 40 rice germplasms, including Thai cultivars, local varieties, and wild relatives. Five DNA markers, including SSR (*bph2, Bph3, Bph15, Bph17*) and InDel (*Bph14*) markers, closely linked to major BPH resistance genes, were utilized to evaluate genetic diversity. Ten loci, with an average of 2 alleles per locus and 100% polymorphism, were identified. Of the 35 genotypes analyzed, each genotype contained one to five BPH resistance genes. Allele frequencies ranged from 34.92% to 42.86%, while expected heterozygosity varied between 0.242 (RM463) and 0.367 (RM16626), averaging 0.321. The polymorphic information content (PIC) values ranged from 0.388 to 0.437, with an average of 0.419, indicating moderate polymorphism and effective discriminating power for the selected BPH-specific markers. UPGMA analysis grouped the 40 rice genotypes into three main clusters, distinguishing them into resistant and susceptible groups. Our findings provide valuable information for selecting parental lines with BPH resistance, supporting the integration of marker-assisted selection (MAS) into rice breeding programs to develop BPH-resistant varieties.

Keywords: Brown planthopper (BPH), DNA markers, genetic diversity, BPH resistance gene, rice breeding

INTRODUCTION

Rice (*Oryza sativa* L.) is the main food source for half of the world's population, providing about 20% of their caloric intake (Fukagawa and Ziska 2019). Global rice production was 600 million tons in 2000 and is projected to increase by 1.5 times, reaching 904 million tons by 2030 (Nayak et al. 2021). Ninety percent of this rice, approximately 612 million tons grown on 143 million ha, is produced in Asia (Papademetriou 2000). The rising threat of pathogens and pests, driven by human mobility, global trade, and climate change, highlights the need for effective crop management strategies (Bebber et al. 2013).

Rice faces major pest threats in Southeast Asia, including the stem borer, Brown Planthopper (BPH), gall midge, and leafhopper. BPH [*Nilaparvata lugens* Stål 1854] is particularly destructive, causing hopper burn and transmitting diseases like ragged stunt virus and grassy stunt virus. Damage from BPH can vary from 20% to 80% in irrigated, wet-seeded, and off-season rice fields (Muduli et al. 2021; Listihani et al. 2022). BPH populations have different biotypes, classified as 1, 2, 3, and 4, with new biotypes recently identified in Thailand (Jairin 2021; Muduli et al. 2021). Although pesticides are commonly used for BPH control, their overuse has led to environmental pollution and the emergence of pesticide-resistant BPH strains, prompting a shift towards breeding for natural resistance in rice (Han et al. 2018). Developing resistant rice varieties is a sustainable and effective approach that brings both economic and environmental benefits (Sahu et al. 2022).

Breeding methods, including conventional and mutation breeding, marker-assisted selection (MAS), transgenics, and genome editing, have been used to develop BPH-resistant rice (Yadav et al. 2019). Among these, MAS has proven effective in creating rice cultivars with enduring BPH resistance. Genetic markers like Insertion-deletion polymorphisms (InDel), Simple Sequence Repeats (SSR), Single Nucleotide Polymorphisms (SNP), and Sequence-Related Amplified Polymorphisms (SRAP) are commonly used to study BPH resistance genetics in rice (Kusumawati et al. 2018; Yang et al. 2020; Zhang et al. 2020; Chaerani et al. 2021; Pannak et al. 2023).

More than 43 host plant resistance genes have been discovered and utilized in breeding efforts, primarily located on chromosomes 3, 4, 6, and 12 (Jiang et al. 2018).

These genes include dominant and recessive types like *bph4*, *bph5*, *bph7*, *bph8*, *bph19*, *bph25*, and *bph29*. Genes such as *bph2*, *Bph3*, *Bph14*, *Bph15*, and *Bph17* show great potential for improving BPH resistance and are widely investigated in breeding programs (Jiang et al. 2018; Nguyen et al. 2019; Sansanoh et al. 2019; Yang et al. 2020). However, resistance in some rice varieties can decrease due to biotype variations (Muduli et al. 2021). The incomplete resistance of current rice varieties to all BPH biotypes underscores the need for new resistance sources, leading to efforts to identify and transfer resistance genes from potential donors to elite susceptible varieties (Kumar et al. 2020; Sahu et al. 2022). The genetic diversity found in Thai cultivars, local varieties, and wild rice in Thailand is important due to their adaptability, economic value, cultural significance, ecological roles, nutritional benefits, and contribution to agricultural biodiversity (Pathaichindachote et al. 2019; Tanaporn et al. 2021; Saengprajak et al. 2024).

Genetic diversity, especially resistance genes, is essential in selecting mother plants for rice breeding programs. This study aimed to investigate the genetic basis of BPH resistance within diverse rice germplasm, including Thai cultivars, local varieties, and wild rice relatives using SSR and InDel markers The results provide basic information to support the development of BPH-resistant rice varieties in the future.

MATERIALS AND METHODS

Plant materials

Forty rice genotypes were examined comprising Thai farmers' cultivars (C1-C8), local varieties with BPH gene differentials (L1-L28), wild species (W1-W1), and standard BPH-resistant and susceptible lines (RC and TN1) (Table 1). Farmers in Khon Kaen and Mahasarakham Provinces, Thailand, provided the rice cultivars. The local varieties with BPH gene differentials, wild species, and standard BPH-resistant and susceptible lines were sourced from the Pathum Thani Rice Research Center, Thailand. Approximately 20 seeds from each rice sample were soaked for 48 hours and then germinated in soil-filled culture trays in the greenhouse of the Department of Biology, Faculty of Science, Mahasarakham University, Thailand.

Procedures

Genomic DNA isolation

The genetic analysis was performed in the Laboratory of Molecular Genetics, Department of Biology, Faculty of Science, Mahasarakham University, Maha Sarakham, Thailand. Fresh young leaf samples (0.2 g) were collected from 15-day-old seedlings comprising five plants of each genotype. The samples were finely powdered with liquid nitrogen, and genomic DNA was subsequently extracted from the homogenized material using the PureDireX Genomic DNA Isolation Kit (Plants) from Thermo Fisher Scientific Co., Ltd. by the manufacturer's instructions.

The quality of the extracted genomic DNA was initially assessed by running the dissolved DNA on a 1.0% agarose gel using 1X TBE (Tris-Borate-EDTA) buffer. Subsequently, DNA quantification was performed using a Nanodrop Spectrophotometer (NND-1 NDL-PLUS-GL, Thermo Fisher Scientific Co., Ltd., Waltham, MA, USA). The genomic DNA was diluted to 50 ng/µL with nuclease-free water (Invitrogen™, Thermo Fisher Scientific Co., Ltd.) and stored at -20°C for further analysis.

DNA amplifications and electrophoresis

A genotypic evaluation of the 40 rice genotypes was conducted using four SSR markers and one InDel marker tightly linked to major BPH resistance genes, including *bph2*, *Bph3*, *Bph14*, *Bph15*, *and Bph17*. The SSR and InDel primer pairs were selected based on the genetic mapping of rice from previous research (Table 1). Polymerase chain reactions (PCRs) were conducted using thermal cyclers (Biometra TAdvanced, Bio-Active Co., Ltd.) with a total reaction volume of 20 µL, following the protocol outlined by Kanngan et al. (2023). Each reaction comprised 4 µL of 50 ng/µL genomic DNA, 0.5 µL of 50 mM $MgCl_2$, 2.0 µL of 10X PCR buffer, 0.4 µL of 10 mM dNTPs, 1.0 µL of 100 µM each forward and reverse primers, 1 µL of DMSO, and 1.0 U of nano*Taq* hot-start DNA polymerase (Bio-Helix Co., Ltd., Taiwan). The final volume was adjusted to 20 µL with nuclease-free H_2O.

The amplification program started with an initial denaturation at 95°C for 5 min, followed by 35 cycles of 30 s at 95°C for denaturation, 30 s for primer annealing at 55°C/58°C/60.5°C depending on the specific marker (Table 2), and 30 s at 72°C for extension, with a final extension at 72°C of 10 min. The PCR products were kept at 4°C until gel electrophoresis. The amplified DNA fragments were then fractionated by electrophoresis on a 4.0% (w/v) agarose gel with 1X TBE buffer. Electrophoresis was conducted at a constant voltage for 90-120 min at 100 V. The size of each DNA band produced was estimated using a 100 bp DNA ladder RTU (Bio-Helix Co., Ltd.). The gel electrophoresis results were visualized using Visafe Green Gel Stain (Vivantis®, Malaysia) and photographed under an ultraviolet transilluminator. All PCR assays were conducted twice to validate the results.

Data analysis

The banding patterns generated by each SSR and InDel primer for the 40 rice genotypes were individually assessed. The sizes of the amplified fragments were determined by comparing their migration distances relative to molecular weight markers and a 100 bp DNA ladder, with calculations performed using PhotocaptMW software. The presence of a band at a specific base pair position was recorded as "1" while the absence of a band at that position was recorded as "0" (zero). Genetic similarities between genotypes for each germplasm were then assessed using Sneath and Sokal's simple matching (SM) coefficient (Sneath and Sokal 1973). The data were converted into a binary matrix, which was then used to construct a dendrogram employing the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) through the SAHN (Sequential, Hierarchical, Agglomerative, and Nested Clustering) module in NTSYSpc 2.1 software (Rohlf 1998).

Table 1. The 40 rice genotypes used in this study

ID	Genotype	Location
C1	Riceberry	Ban Non-u-dom, Chumphae, Khon Kaen Province
C2	Thubthim chumpae	Ban Non-u-dom, Chumphae, Khon Kaen Province
C3	Nheuw daeng	Ban Paeng, Kosum Phisai, Maha Sarakham Province
C4	Hom nin	Ban Non Rasi, Khwao Rai, Kosum Phisai, Maha Sarakham Province
C5	Mali 105	Ban Ku Thong, Chiang Yuen, Maha Sarakham Province
C6	Jao daeng	Ban Non-u-dom, Chumphae, Khon Kaen Province
C7	Kam Sirithon	Ban Non-u-dom, Chumphae, Khon Kaen Province
C8	Khokho 6	Ban Tha Song Khon, Mueang Maha Sarakham, Maha Sarakham Province
L1	Khawklang	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L2	Matan	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L3	Namkhang	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L4	Lepchang	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L5	Luang yai	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L6	Yayfak	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L7	Makhai	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L8	Luang thong	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L9	Dhodam	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L10	Kaew	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L11	Khaw mali	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L12	Khoncud	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L13	Phuang ngein	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L14	Fimai	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L15	Khaw hom	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L16	Daeng noy	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L17	Luk non	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L18	Phra in	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L19	Lueng bun ma	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L29	Lon khrok	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L21	Mali hom	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L22	Homchan	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L23	Sao hi	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L24	Hom phama	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L25	Taban	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L26	Hang mani	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L27	Mak bid	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
L28	Nheuw dho daeng	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
W1	*Oryza rufipogon* Griff.	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
W2	*Oryza officinalis* Wall. ex Watt	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Than Province
RC	PTB33 (BPH resistance)	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province
SC	TN1 (BPH susceptible)	Pathum Thani Rice Research Center, Rangsit, Thanyaburi, Pathum Thani Province

Notes: C1-C8 represent cultivated rice from farmers, L1-L28 denote local varieties, W1-W2 are wild species, and RC and SC are standard BPH-resistant and susceptible lines, respectively

Table 2. SSR and InDel markers used in the study

Gene	Marker	Chr.	Sequences	Types of marker	Ta (°C)	References
bph2	RM463	12	F: 5′-TTCCCCTCCTTTTATGGTGC-3′	SSR	55.0	Sun et al. (2006)
			R: 5′-TGTTCTCCTCAGTCACTGCG-3′			
Bph3	RM588	3	F: 5′-TCTTGCTGTGCTGTTAGTGTACG-3′	SSR	58.0	Jairin et al. (2007)
			R: 5′-GCAGGACATAAATACTAGGCATGG-3′			
Bph14	IN76-2	3	F: 5′-CTGCTGCTGCTCTCGTATTG-3′	InDel	60.5	Du et al. (2009)
			R: 5′-CAGGGAAGCTCCAAGAACAG-3′			
Bph15	RM261	4	F: 5′-CTACTTCTCCCCTTGTGTCG-3′	SSR	58.0	Hu et al. (2015)
			R: 5′-TGTACCATCGCCAAATCTCC-3′			
Bph17	RM16626	4	F: 5′-ACATGATTGCTGGCTTGCTTACC-3′	SSR	58.0	Sun et al. (2005)
			R: 5′-GCCACGCAGTGTTGTTTCAGC-3′			

The polymorphic information content (PIC) was computed to evaluate the discriminatory power of each marker using the formula from Roldan-Ruiz et al. (2000) as PIC = 2fi(1-fi), where fi represents the frequency of present marker bands and (1-fi) represents the frequency of absent marker bands. The expected heterozygosity (H_e) was calculated using the formula $H_e = 1-\sum Pi^2$, where *Pi* denotes the frequency of the i^{th} allele for the germplasm, and $\sum Pi^2$ represents the sum of the squares of all allele frequencies (Kalinowski et al. 2007).

RESULTS AND DISCUSSION

DNA profiling

Forty rice genotypes were assessed for BPH resistance using five molecular markers linked to major resistance genes: *bph2*, *Bph3*, *Bph14*, *Bph15*, and *Bph17*. These markers, including four microsatellite markers and one InDel marker, showed 100% polymorphism across the genotypes, detecting 10 alleles. Each marker identified 2 alleles per locus, with amplified fragment sizes ranging from 90 to 210 bp (Figure 1). Representative banding patterns and polymorphisms observed with primers RM588, RM261, and RM16626 are shown in Figures 1a, b, and c, respectively. The results revealed that seven genotypes-Khawklang (L1), Namkhang (L3), Luang Yai (L5), Khoncud (L12), Fimai (L14), *O. officinalis* (W2), and PTB33 (RC)-contained all five BPH resistance genes. Ten genotypes (25%) carried four resistance genes: Riceberry (C1), Lepchang (L4), Yayfak (L6), Makhai (L7), Dhodam (L9), Kaew (L10), Phuang Ngein (L13), Khaw Hom (L15), Luk Non (L17), and *O. rufipogon* (W1). Five genotypes (12.5%) carried three resistance genes: Nheaw Daeng (C3), Khaw Mali (L11), Daeng Noy (L16), Mali Hom (L21), and Lon Khrok (L29). Seven genotypes (17.5%) contained two resistance genes: Kam Sirithon (C7), Matan (L2), Luang Thong (L8), Phra In (L18), Luang Bun Ma (L19), Hom Phama (L24), and Nheaw Dho Daeng (L28). Six genotypes (15%) carried only one resistance gene: Thubthim Chumpae (C2), Hom Nin (C4), Jao Daeng (C6), Khokho 6 (C8), Sao Hi (L23), and Mak Bid (L27). Finally, five genotypes (12.5%)-Hang Mani (L26), Hom Chan (L22), Mali 105 (C5), Taban (L25), and TN1 (SC)-lacked BPH resistance genes entirely (Figure 2).

This genetic evaluation provides essential insights for breeding rice varieties with improved BPH resistance, addressing both current and future agricultural needs (Liu et al. 2022). These markers, strongly associated with BPH resistance, serve as valuable tools in identifying and selecting resistant genotypes, particularly against the brown planthopper, a major pest in rice production (Kanngan et al. 2023).

Genetic diversity

The percentages of gene frequency, PIC values, and expected heterozygosity (He) for each primer are summarized in Table 3. Gene frequencies ranged from 34.92% to 42.86%, with an average of 39.99%. Primer RM16626, tightly linked to the *Bph17* gene, exhibited the highest gene frequency at 42.86%, while primer RM463, associated with the *bph2* gene, showed the lowest gene frequency at 34.92%. The PIC values for polymorphic loci varied between 0.388 and 0.437, with an average of 0.419.

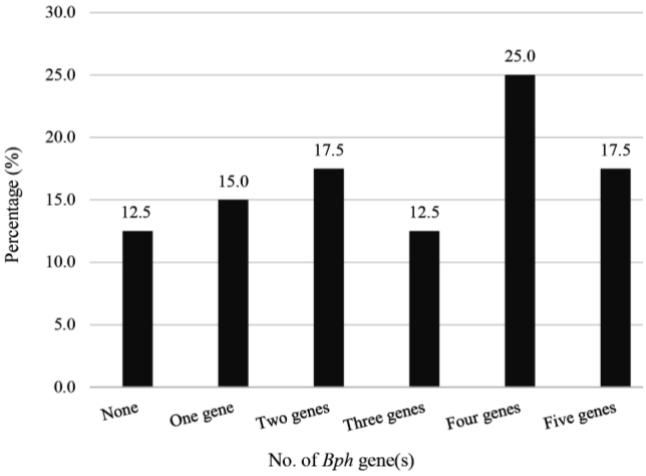

Figure 2. The percentage of *Bph* genes present in 40 rice genotypes

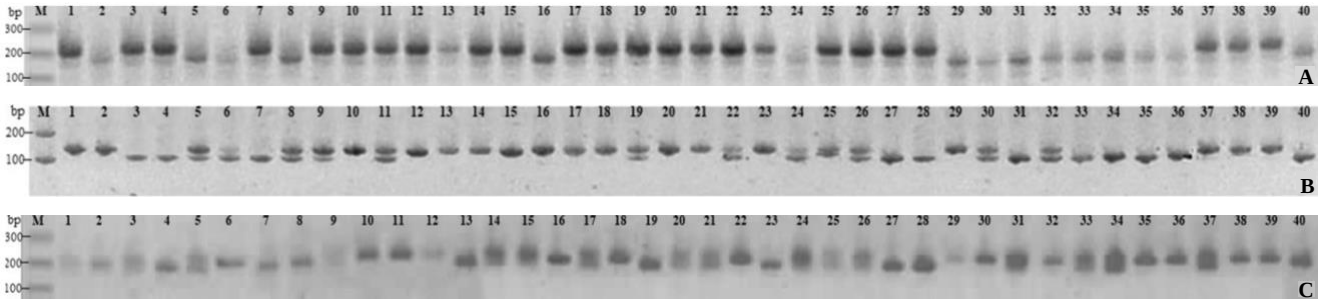

Figure 1. PCR amplified products of three genes. A. *Bph3* at locus RM588 on chromosome 3; B. *Bph15* at locus RM261 on chromosome 4; C. *Bph17* at locus RM16626 on chromosome 4. Lane M represents the 100 bp DNA ladder, while lanes 1 to 40 correspond to the genotypes listed in Table 1

Among these, primer RM16626 demonstrated the highest PIC value of 0.437, whereas primer RM463 had the lowest at 0.388. The expected heterozygosity (He) ranged from 0.242 to 0.367, with an average of 0.321. Primer RM16626 also recorded the highest He value, while RM463 had the lowest.

The variability in gene frequencies highlights the diversity in the presence of specific genes or markers among the rice genotypes studied. The high gene frequency (42.86%) observed for primer RM16626 suggests the prevalence of the *Bph17* gene, which is recognized for its role in conferring resistance to the brown planthopper. By contrast, the lower gene frequency (34.92%) of primer RM463 indicated less commonality of the *bph2* marker among the studied rice genotypes; all rice genotypes contained between zero and five *Bph* genes, consistent with previous reports. Ramkumar et al. (2016) found that 137 out of 260 rice cultivars had at least one BPH resistance gene using SNP markers, with *Bph10* being the most prevalent and *Bph20* the least. Some cultivars had combinations of two or three resistance genes. Vang et al. (2020) reported that 10 varieties showed sustained resistance to some BPH populations using SSR markers, while other varieties lacked five specific genes. Kanngan et al. (2023) found that 139 of 143 upland rice varieties harbored between one and five BPH resistance genes, as determined through SSR and InDel marker analysis.

The PIC measures the informativeness of a marker by evaluating the number of alleles at a locus and their respective frequencies (Chesnokov and Artemyeva 2015). According to Botstein et al. (1980), a PIC value of $\geq$0.5 indicates high informativeness, values between 0.5 and 0.25 suggest moderate informativeness, and a PIC value <0.25 provides little information. In this study, all markers were deemed moderately informative, as their PIC values were below 0.5, with an average PIC value of 0.4744. Kanngan et al. (2023) reported similar findings, with PIC values ranging from 0.4460 to 0.4984 and an average of 0.4744. In contrast, Moonsap et al. (2019) observed lower PIC values among Indo-China rice varieties, ranging from 0.07 (UBC818) to 0.38 (UBC808), with an average of 0.26. Conversely, Pathaichindachote et al. (2019) recorded slightly higher PIC values, averaging 0.56. Jegadeeswaran et al. (2024) observed a wider range of PIC values, from 0.359 (ISSR 890) to 0.846 (ISSR 826), in rice genotypes from Tamil Nadu Agricultural University, India, with an average of 0.666. These values align with the findings of Kumbhar et al. (2015) and Zayed et al. (2023) in their studies of rice landraces, improved varieties, and hybrids. Several factors, such as sample size, species breeding behavior, genotyping techniques, and marker locations significantly influence average PIC values and genetic diversity (Chen et al. 2017).

Gene diversity or expected heterozygosity (H_e), is a standard measure for assessing genetic variation within a population (Kanaka et al. 2023). In this study, the H_e value among the markers varied from 0.242 (RM463) to 0.367 (RM1162), with a mean value of 0.321 (Table 3 and Figure 3). This relatively low average heterozygosity suggested limited genetic variation within the tested rice genotypes, highlighting the need for diverse genetic resources and effective markers in rice breeding programs (Suvi et al. 2020). Singh et al. (2018) reported similar results, with an average H_e of 0.310 across various rice markers, suggesting a narrow genetic base. Choudhury et al. (2023) reported an average gene diversity of 0.420 in rice landraces under farmer management, further supporting the need for incorporating diverse genetic materials to enhance genetic variability. Factors such as sample size, selection of markers, and breeding practices influence the observed heterozygosity, underscoring the importance of utilizing a broad spectrum of genetic resources in breeding programs (Singh et al. 2024).

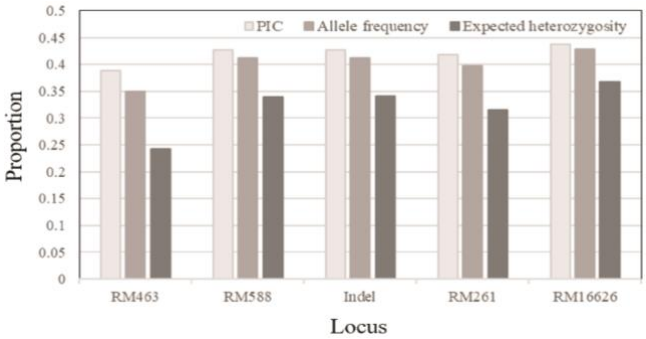

Figure 3. Histograms representing PIC, allele frequency, and expected heterozygosity of the associated markers

Table 3. SSR and InDel markers are tightly linked to the *Bph* resistance genes

Gene	Marker	Sequences	Expected size (bp)		Gene frequency (%)	PIC	He
			Resistant allele	Susceptible allele			
bph2	RM463	F: 5′-TTCCCCTCCTTTTATGGTGC-3′ R: 5′-TGTTCTCCTCAGTCACTGCG-3′	200	210	34.92	0.388	0.242
Bph3	RM588	F: 5′-TCTTGCTGTGCTGTTAGTGTACG-3′ R:5′-GCAGGACATAAATACTAGGCATGG-3′	90	110	41.27	0.427	0.339
Bph14	IN76-2	F: 5′-CTGCTGCTGCTCTCGTATTG-3′ R:5′-CAGGGAAGCTCCAAGAACAG-3′	190	180	41.21	0.428	0.341
Bph15	RM261	F: 5′-CTACTTCTCCCCTTGTGTCG-3′ R: 5′-TGTACCATCGCCAAATCTCC-3′	130	120	39.68	0.418	0.315
Bph17	RM16626	F: 5′-ACATGATTGCTGGCTTGCTTACC-3′ R: 5′-GCCACGCAGTGTTGTTTCAGC-3′	200	190	42.86	0.437	0.367
		Mean			39.99	0.419	0.321

Similarity coefficient analysis and clustering

The genetic similarity coefficients among the rice genotypes varied from 0.10 to 0.90 (data not shown). A UPGMA dendrogram was constructed using NTSYSpc 2.1 software and genetic similarity coefficients derived from four SSRs and one InDel markers. The cluster analysis classified the 40 rice genotypes into three primary clusters (I, II, and III) based on a genetic similarity coefficient of 0.51 (Figure 4). Cluster I included 19 genotypes: Khawklang (L1), Fimai (L14), Namkhang (L3), Luang Yai (L5), Khoncud (L12), PTB33 (RC), *O. officinalis* (W2), Kaew (L10), Lepchang (L4), Dhodam (L9), Phuang Ngein (L13), Riceberry (C1), Yayfak (L6), Daeng Noy (L16), Matan (L2), Khaw Hom (L15), Makhai (L7), Luk Non (L17), and *O. rufipogon* (W1). Cluster II consisted of 18 genotypes: Khaw Mali (L11), Hom Chan (L22), Mali 105 (C5), Thubthim Chumpae (C2), Hang Mania (L26), TN1 (SC), Sao Hi (L23), Taban (L25), Luang Bun Ma (L19), Lon Khrok (L29), Khokho 6 (C8), Nheaw Dho Daeng (L28), Hom Phama (L24), Jao Daeng (C6), Nheaw Daeng (C3), Hom Nin (C4), Kam Sirithon (C7), and Mali Hom (L21). Cluster III, the smallest, comprised 3 local varieties: Luang Thong (L8), Phra In (L18), and Mak Bid (L27).

Cluster analysis is used to present the complex relationships among populations of diverse origins in a more simplified manner (Lakshmi et al. 2021). The dendrogram grouped the 40 rice genotypes into three main clusters (Figure 4). Cluster I is the largest cluster was subdivided into four subclusters (i) I-A (8 genotypes, mostly with all five *Bph* genes, except Kaew with four); (ii) I-B (6 genotypes, primarily with four *Bph* genes, except Daeng Noy with three); (iii) I-C (2 genotypes, one with two and one with four genes); and (iv) I-D (3 genotypes, all with four *Bph* genes). Cluster II, comprising 18 genotypes, was divided into three subclusters: II-A and II-C (genotypes with varying resistance genes) and II-B (15 genotypes with one to three *Bph* genes, except five genotypes lacking any *Bph* genes (i) Homchan; (ii) Mali 105; (iii) Thubthim chumpae; (iv) Hang mania; (v) TN1, and Taban). Cluster III, the smallest, contained three local genotypes (Luang thong, Phra in, and Mak bid) with two resistance genes, except for Mak bid, which only had the *Bph15* gene on chromosome 4. Overall, Cluster I mostly included resistant genotypes, Cluster II ranged from moderate resistance to susceptibility, and Cluster III contained mostly susceptible genotypes.

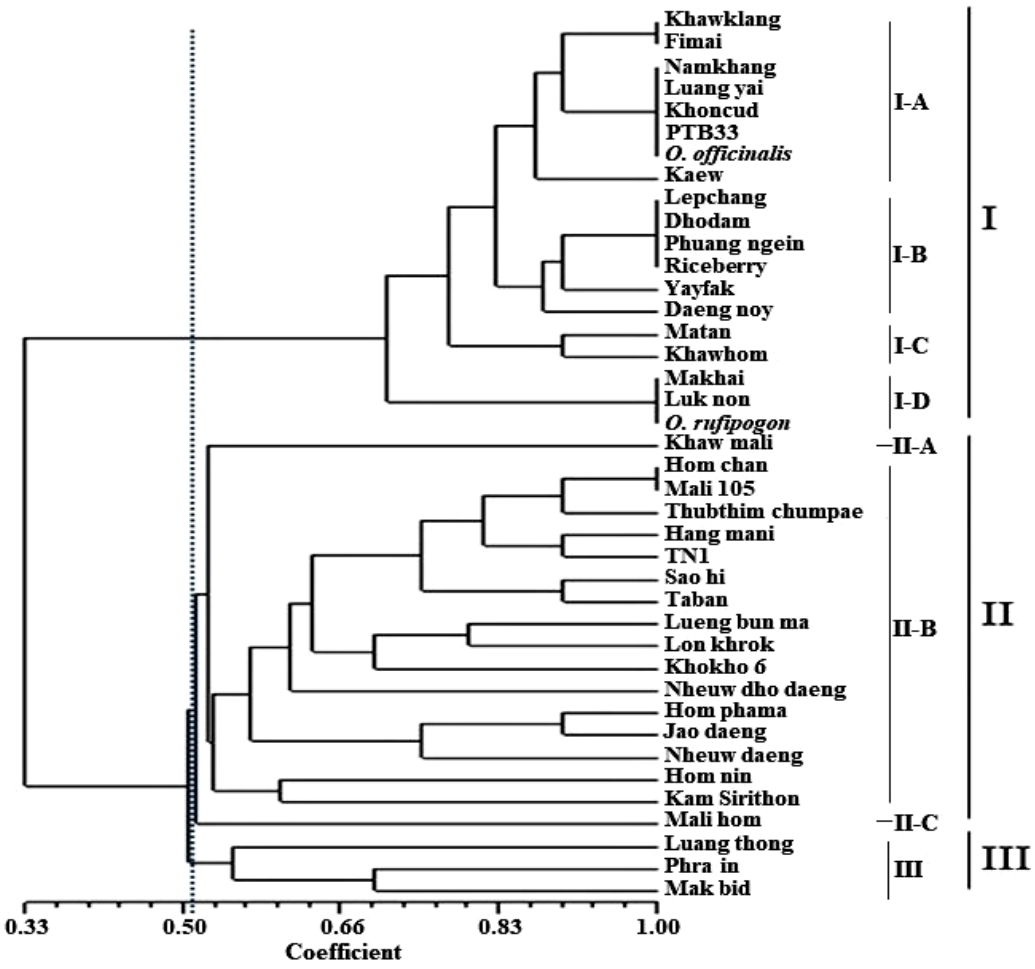

Figure 4. A UPGMA dendrogram illustrating the genetic diversity of the 40 rice genotypes

Several studies have extensively researched and applied these selected genes in breeding projects, particularly in Southeast Asia. *Bph3* is renowned for its wide-ranging resistance to all biotypes of BPH. It has been effectively utilized to create superior rice lines, serving as a valuable source of germplasm for breeding programs (Sansanoh et al. 2019). Nguyen et al. (2019) showed that rice genotypes carrying a combination of the *bph2*, *Bph3*, and *Bph17* genes had strong resistance to the Koshi-2013 BPH population. Rice varieties with the combined *Bph14* and *Bph15* genes have demonstrated considerable resistance to BPH (Li et al. 2011; Jiang et al. 2018; Haliru et al. 2020). This pyramiding strategy, combining multiple resistance genes, enhances resistance in rice breeding programs. Jiang et al. (2018) reported that rice lines with both *Bph14* and *Bph15* genes exhibited high resistance, reducing pest damage and improving yield stability. Li et al. (2019) found that this combination broadened resistance against different BPH biotypes. Haliru et al. (2020) confirmed the durability of BPH resistance across various environments.

Current research has genotyped Thai upland rice for five key *Bph* resistance genes: *bph2*, *Bph3*, *Bph14*, *Bph15*, and *Bph17*. Evaluations indicated that these selected genotypes were resistant to BPH (Kanngan et al. 2023), aligning with other reports highlighting the importance of incorporating multiple resistance genes to achieve robust and durable resistance in rice breeding programs. Gene pyramiding, combining multiple resistance genes into a single variety, has proven effective in enhancing resistance levels and broadening the spectrum of resistance against various BPH biotypes (Deng et al. 2024). This approach is critical for developing resilient rice genotypes capable of withstanding pest pressures in diverse agroecological environments.

In conclusion, this study conducted a genetic analysis of BPH resistance genes across 40 rice genotypes, including Thai cultivars, local varieties, and wild rice species, using SSR and InDel markers. The results identified DNA markers closely linked to BPH resistance genes and highlighted significant genetic diversity through genetic indices, such as polymorphism rate, allele frequency, PIC, and H_e. Cluster analysis grouped the rice genotypes into three main clusters, distinguishing between resistant and susceptible groups. Our findings suggested that local varieties, such as Khawklang, Fimai, Namkhang, Luang yai, Khoncud, and the wild rice *O. officinalis* are not just promising sources of BPH resistance but also hold great potential for selection and integration into future BPH resistance breeding programs, inspiring and motivating further research and development in this field.

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