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Bph14 gene determining brown-planthopper (*Nilaparvata lugens* Stal) resistance in rice varieties (*Oryza sativa* L.)

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ABSTRACT

The brown planthopper (BPH) is one of the major insect pests of many rice areas in Vietnam [3]. Many researchers have reported that host plant resistance is the most effective way of controlling BPH, and thus breeding of insect resistance has taken priority in rice improvement programs [6]. In this study, we determined the presence of *bph14* gene in four rice varieties IRRI 352, BG 367-2, Sai Duong Kien An, Loc Nuoc. They were the BPH resistance rice varieties and their BPH resistant capacity were tested and supplied by Plant Resources Center, Science Institute of Agronomy, Hanoi, Vietnam. These rice varieties were cultivated and studied in Thua Thien Hue, Vietnam. From *cds* of *bph14* gene sequence (<http://www.ncbi.nlm.nih.gov>, accession number: FJ941067.1) we have designed primers to identify of *bph14* gene in these rice cultivars. Results showed that among these four rice varieties, *bph14* gene was detected in Sai Duong Kien An and Loc Nuoc but it was not detected in IRRI 352 and BG 367-2.

Key words: *bph14*, brown planthopper, brown planthopper resistance gene, BPH.

INTRODUCTION

Brown planthopper causes direct damage to the plant by sucking the phloem sap, feeds by phloem abstraction and causes hopper burn, and transmits viral diseases [2], [7]. Farmers used to chemical method for controlling this insect, which are expensive and harmful to the environment. The most economical and environment-friendly strategy to control this insect is to grow genetically resistant rice varieties [4], [6].

To date, 22 major BPH resistance genes have been identified from the gene pool of cultivated and wild species of *Oryza*. Of the 22 genes conferring resistance to brown planthopper, two resistance genes, *bph14* and *bph18* have been cloned [5].

According to Bo Du *et al* (2009), *bph14* was mapped on the long arm of chromosome 3. This BPH gene encodes a coiled-coil, nucleotide-binding, and leucine-rich repeat (CC-NB-LRR) protein. Sequence comparison indicates that *bph14* carries a unique LRR domain that might function in recognition of the BPH insect invasion and activating the defense response [8]. Expression of *Bph14* activates the salicylic acid signaling pathway and induces callose deposition in phloem cells and trypsin inhibitor production after planthopper infestation, leading to reduce the feeding, growth rate, and longevity of the BPH insects [1].

In this study, we determined *bph14* gene in four rice varieties IRRI 352, BG 367-2, Sai Duong Kien An, Loc Nuoc. The purpose of this study is to understand of the resistance mechanism of these lines and choose BPH resistance rice cultivars.

MATERIALS AND METHODS

Plant materials

Four rice (*Oryza sativa* L.) varieties IRRI 352, BG 367-2, Sai Duong Kien An, Loc Nuoc from Plant Resources Center (Science Institute of Agronomy, Hanoi, Viet Nam) were cultivated and studied in Thua Thien Hue province.

DNA isolation

Total genomic DNA was extracted from young leaves (20 days old). Young leaves were ground in liquid nitrogen. Powdered rice leaf was dispersed in eppendorf tube with 500 μ L extraction buffer (100 mM Tris.HCl, 500 mM NaCl, 50 mM EDTA, pH 7.5), and mixed well. Adding 50 mL SDS (Sodium dodecyl sulfate) 20% and incubated at 65°C for 30 minutes. The supernatant was extracted two times with an equal volume of phenol, phenol: chloroform (1:1, v/v) and chloroform. Nucleic acids was precipitated by adding an equal volume of cold ethanol 100%, and centrifuged at 12.000 rpm/4°C for 15 minutes. The pellet was washed by cold ethanol 70%, dried at room temperature, and then dissolved in TE buffer (10 mM Tris.HCl pH 7.5 and 1mM EDTA).

Isolate BPH resistance gene – *bph14*

Design primers

We used DNASIS to designed 4 primer pairs (Figure 1 and Table 1) for 4 overlapping sequences in *bph14* cds region.

Table 1. Sequences of specific primer pairs to *bph14* gene

Primer	Forward sequence	Reverse sequence	Fragment size (bp)
M1	ATGGCGGAGCTAATGGCCACCA	AGAGTTCTTTATATCATGGAECTCA	1491
M2	GATCATGAGATTGACGTGGAAA	AAGTCACTTAGCTTTGGTG	1541
M3	AGTCGATGGAECTCAAGGG	GATGAGTATGCTTGAGGCC	1025
M4	AATCTTGCTTAGGAGAGCTCGC	CTACTTCAAGCACATCAGC	919

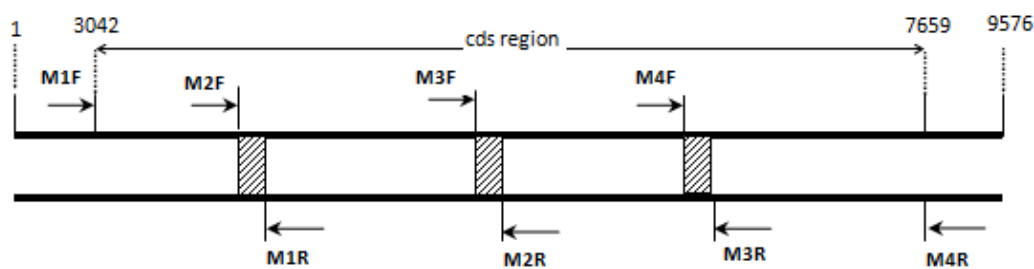



Figure 1. Diagram of primers on *bph14* gene (9576 bp)

 : Overlapping fragments

PCR amplification

Identification of the presence of *bph14* gene on the genome of some rice cultivars through determining the presence of four DNA sequences in genome. Specific primers to four DNA sequences were showed in Table 1 and Figure 1.

PCR amplifications were performed as follow: 50 μ L of reaction mixture containing 100 ng of total DNA, 10 pmol each of primers, 200 mM dNTPs, 1.5 mM MgCl₂, 10 μ L of 5 \times Taq polymerase buffer and 1.25 unit Taq polymerase (Promega). The polymerase chain reaction was conducted with thermocycler (Icycler, Bio-Rad), with the following temperature profiles: The initial denaturation was at 95 $^{\circ}$ C for 5 min, followed by 30 cycles of denaturing at 95 $^{\circ}$ C for 1 min, annealing at 55 $^{\circ}$ C for 1 min, extension at 72 $^{\circ}$ C for 1 min, and 10 min at 72 $^{\circ}$ C for final extension. The PCR products were electrophoresed with 1.2% agarose gel at 100 volts in 1 \times TAE buffer and stained with ethidium bromide (0.5 μ g/mL) for 15 min. The stained gel was photographed under UV light using gel documentation system (Biorad).

Cloning and sequencing of PCR products

PCR products with expected size from 1.2% agarose gel were purified using Gel Purification AccuPrep[®] Kit (Bioneer, Korea) and cloned into pTZ57R/T vector (Figure 2). Reaction mixture contained 0.54 pmol PCR products, 5 \times T4 ligation buffer, 4 units of T4 DNA ligase, 0.18pmol pTZ57R/T vector; the final volume was 10 μ L. The reaction was incubated at 4 $^{\circ}$ C for overnight, followed by transforming the reaction mixture into *E.coli* (DH5-alpha, Invitrogen) by heat shock method at 42 $^{\circ}$ C for 90 seconds and then at 4 $^{\circ}$ C for 3 min. Recombinants were selected by method of blue/white colonies. Recombinant *E. coli* cells were cultured in liquid LB broth with ampicillin at 37 $^{\circ}$ C for 15hours, and biomass was collected by centrifugation. Finally, plasmid DNA was extracted by AccuPrep[®] Plasmid Mini Extraction Kit (Bioneer, Korea). PCR products were sequenced by method of fluorescent dideoxy-terminator on CEQ machine (Ver. 7.0.55). These nucleotide sequences were compared with corresponding regions in *bph14* gene (<http://www.ncbi.nlm.nih.gov>, accession number: FJ941067.1).

RESULTS AND DISCUSSION

Amplification of cds region of *bph14* gene

We identified the *bph14* gene on the genome DNA of in four BPH resistance rice varieties through presence of four DNA sequences. The markers M1 were designed to amplify a 1485 bp, M2 (1541 bp), M3 (1025 bp), M4 (919 bp).

The results of amplification reactions were presented in Figures 2, 3, 4 and 5.

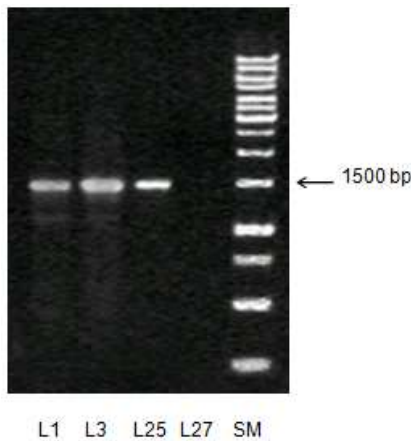


Figure 2. PCR products were amplified by M1 maker
SM: DNA marker (1kb DNA Ladder),
L1: IRRI 352, L3: BG 367-2, L25: Sai Duong Kien An, L27: Loc Nuoc

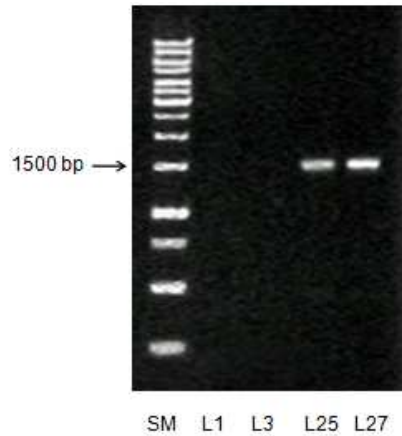


Figure 3. PCR products were amplified by M2 maker

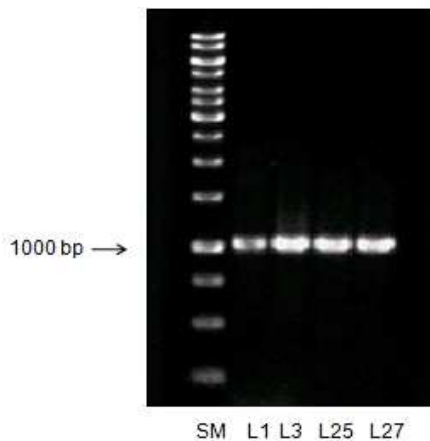


Figure 4. PCR products were amplified by M3 maker

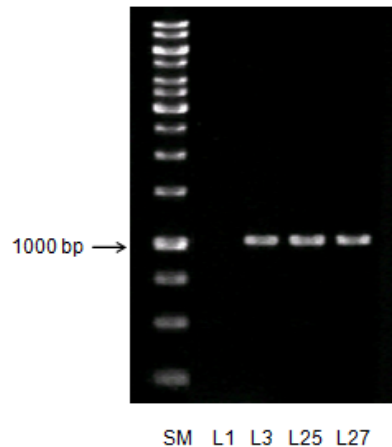


Figure 5. PCR products were amplified by M4 maker

The analysis of electrophoresis revealed that there were PCR products amplifying by primers M1, M2, M3 and M4 as expected.

Amplification of DNA from examined cultivars with the primer M1F/R gave rise approximate to 1500-bp product for IRRI 352, BG 367-2, and Sai Duong Kien An. Primer M2F/R gave rise approximate to 1500-bp product for Sai Duong Kien An and Loc nuoc. Primer M3F/R gave rise

approximate to 1000-bp product for four rice cultivars. Primer M4F/R gave rise approximate to 1000-bp product for BG 367-2, Sai Duong Kien An and Loc nuoc.

In rice cultivars, Sai Duong Kien An and Loc nuoc has four fragments. As a result, we came to the following conclusion Sai Duong Kien An and Loc nuoc cultivars contain the *bph14* gene on the genome.

Analysis of sequences of *bph14* gene

We cloned the PCR products amplified by specific primers of M1, M2, M3, M4 from Sai Duong Kien An cultivar into plasmids and analyzed their sequences. The overlapping sequences were ligated to a DNA fragment (4714 bp) called *bph14-25*. The nucleotide sequences homologized with corresponding regions in *bph14* gene (<http://www.ncbi.nlm.nih.gov>, accession number: FJ941067.1) (Figure 6, 7).

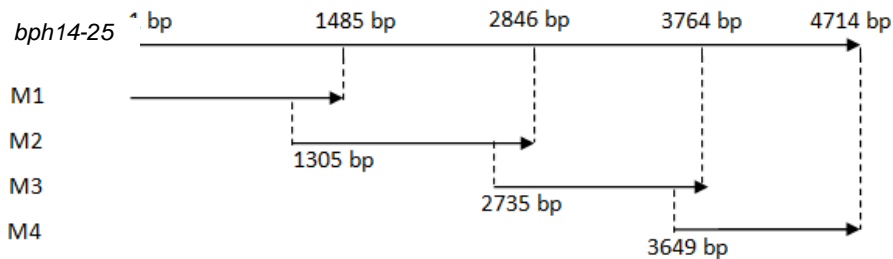


Figure 6. The length of *bph14-25* gen

Identities = 3981/4425 (90%), Gaps = 58/4425 (1%)

Query	1	ATGGCGGAGCTAATGGCCACCATGGTGGTCGGGCCACTGCTGTCCATGGTGAAGGACAAG	60
Sbjct	1	ATGGCGGAGCTAATGGCCACCATGGTGGTCGGGCCACTGCTGTCCATGGTGAAGGAGAAG	60
Query	61	GCCTCCAGCTACCTCCTGGAGCAGTACAAGGTGATGGAGGGCATGGAGGAGCAGCACGAG	120
Sbjct	61	GCCTCCAGCTATCTCATGGAGCAGTACAAGGTCATGGAGGGTATGGAGGAGCAGCACAAAG	120
Query	121	ATCCTCAAACGCAAGCTGCCAGCCATCCTCGACGTCATCGCCGACGCCGAGGAGCAGGCG	180
Sbjct	121	ATCCTCAAACGCAAGCTGCCAGCCATCCTCGACGTCATCGCCGACGCCGAGGAGCAGGCG	180
Query	181	GCTAAACACAGGGAAGGGGTGAAAGCATGGCTCGAGGCGCTCCGGAAGGTGGCCTACCAG	240
Sbjct	181	GCAAAACACAGAGAAGGGGCGAAAGCATGGCTGGAGGAGCTCCGGAAGGTGGCCTACCAG	240
Query	241	GCCAATGACGTCTTCGACGAGTTCAAGTACGAGGCACTCCGCCGCAAGGCCAAGG-----	295
Sbjct	241	GCCAATGATGTCTTCGACGAATTCAAGTACGAGGCCCTCCGCCGCAAGGCCAAGGCCAAT	300
Query	296	-GGCACTACAAGATGCTCAGCAGCATGGTTGTAATCAAGCTATTTCCTACTCACAACCGT	354
Sbjct	301	GGGCAGTATAAGATGCTCGGCA---TGGATGTAATAAAGCTCTTTCCTACTCACAACCGT	357
Query	355	ATTCTGTTTCAGTTATAGGATGGGCAACAAGCTCAGGATGATTCTGAATGCCATTGAAGTT	414
Sbjct	358	ATTGTGTTCCGTTACAGGATGGGCAACAAGCTCAGGATGATCCTGAATGCACATGAGGTC	417
Query	415	CTAATTGAAGAGATGAATGCCTTTAGGTTTAAATTCGACCAGAGCCACCAATGTCGTCC	474
Sbjct	418	CTAATTACAGAGATGAATGCCTTTAGGTTTAAATTCGACCAGAGCCACCAATGTCGTCC	477

Query	475	ATGAAATGGAGGAAGACAGATTCTAAAATCTCCGACCTTTCTTTGGACATTGCCAACAAC	534
Sbjct	478	ATGAAATGGAGGAAGACGGATTCTAAAATTTCTGAACATTCTATGGACATTGCCAACAACA	537
Query	535	TCAAGAAAGGAAGATAAACAGGAGATTGTCAGCAGATTGCTTGTTCAGCCAGCGAAGGG	594
Sbjct	538	TCAAGAGAGGAAGACAGACAGAAGATTGTCAAGTCATTGCTTTCTCAAGCCAGCAATGGG	597
Query	595	GATCTCACTGTTCTTCCCATTGTAGGAATgggggggATGGGCAAGACCACCTTAGCGCAG	654
Sbjct	598	GATCTCACTGTTTATTTCCCATTGTAGGAATGGGGGGGATGGGCAAGACCACCTTAGCGCAG	657
Query	655	CTCATTTACAATGACCCTGACATTGAGAAGCATTTCAGTTGCTGCTCTGGGTGTGTGT	714
Sbjct	658	CTCATTTACAATGACCCTCAAATTCAGAAGCATTTCAGTTGCTCTCTGTGGGTGTGTGTC	717
Query	715	TCCGACAACCTTCGATGTGGATTGCTGGCTAAAAGCATAGTTGAAGCAGCTCGCAAACAG	774
Sbjct	718	TCTGACAACCTTCGATGTGGATTGCTGGCCTAAAAGCATAGTTGAAGCAGCTCGCAAACAG	777
Query	775	AAGAATGATAACAGTGGAAAGTACTAAACAAGTCACCATTGGATGAACCTTAAAGAAGTTGTG	834
Sbjct	778	AAGAACTGTAA---TGAAAG-----GGCTGAATTTAAAGAAGTTGTG	816
Query	835	AGTGGGCAGAGGTACCTCCTCGTTTTGGATGATGTCTGGAACCGTGATGCTCGTAAGTGG	894
Sbjct	817	AATGGGCAGAGGTTCTCCTCGTATTGGATGACGTCTGGAACCGTGAGGCTAGTAAGTGG	876
Query	895	GAAGCGCTCAAGTCTACCTTCAGCACGGTGGCAGCGGTAGCTCAGTTTTGACAACAACCT	954
Sbjct	877	GAAGCGCTCAAGTCTACGTTTCAGCATGGTGGCAGCGGTAGCTCAGTTTTGACAACAACC	936
Query	955	CGTGATCAAGAAGTGGCTCAAGTGTGGCTCCAGCTCAAAAACCTTATGATCTCAAGAGA	1014
Sbjct	937	CGTGATAAAAACAGTTGCTGAAATAATGGCTCCACCTAAAGAAGTTCATCATCTCAAG-GA	995
Query	1015	CT-GAAGGAAAGCTTCATAGAGGAAATTATCAGGACAAGTGTCTTTCAGTTCACAACAAGA	1073
Sbjct	996	CTTGAATGAAACTTTATAAAGGAAATTATCGAGAGAAGTGTCTTCAATTCAGAAGAAGA	1055
Query	1074	---AAGGCCTCTGAGCTTCTCAAAATGGTTGGTGATATTGCCAAGAAATGTTCTGGTTC	1130
Sbjct	1056	GAAAAGGCAATCTGAGCTACTCGAAATGGTTGGTGATATTGCCAAGAAATGTTCTGGTTC	1115
Query	1131	CCCTTTAGCTGCAACAGCATTGGGCTCTACACTGCGTACGAAGACCACCAAGAAAGAATG	1190
Sbjct	1116	CCCTTTAGCTGCAACAGCATTGGGCTCTACACTGCGTACAAAGACCACCAAGAAAGAATG	1175
Query	1191	GGAGGCTATATTAAGCAGAAGCACAATTTGCGATGAGGAAAATGGAATTTTACCAATACT	1250
Sbjct	1176	GGAGGCTATATTAAGGAGAAGCACAATTTGTGATGAGGAAAATGGAATTTTACCAATACT	1235
Query	1251	CAAGCTCAGTTACAATTGCTTGCCATCATATATGCGGCAATGCTTTTCTTTTGTGCAAT	1310
Sbjct	1236	AAAGCTTAGTTACAATTGCTTGCCATCATATATGCGGCAGTGCTTTGCTTTCTGTGCTAT	1295
Query	1311	TTTCCCAAGGATCATGAGATTGACGTGGAAATGCTGATCCAGTTATGGATGGCCAATGG	1370
Sbjct	1296	TTTTCCAAAGGATCATGTGATTGATGTGGAAATGTTGATCCAATTATGGATGGCCAATTG	1355
Query	1371	TTTTATCCAGAGCAACAAGGAGAGTGCCCTGAAATCATTGGTAAAAGAATTTTCAGTGA	1430
Sbjct	1356	TTTTATCCAGAGCAACAAGGAGAGTGCCCTGAAATCAGTGGTAAAAGAATTTTCAGTGA	1415

Query	1431	GTTGGTGTCAAGGTCATTTTTTTCAGGATGCGAAAGGGATCCCGTTTGTAGTTCCATGATAT	1490
Sbjct	1416	GTTGGTGTCAAGGTCATTTTTTTCAGGATGTGAAGGGATCCCATTTGTAGTTCCATGATAT	1475
Query	1491	AAAGAACTCTAAGATTACTTGTAGATCCATGACCTTATGCATGATGTTGCACAATCCTC	1550
Sbjct	1476	AAAGAACTCTAAGATTACTGCTAAGATCCATGATCTTATGCATGATGTTGCACAATCTTC	1535
Query	1551	CATGGGAAAAGAATGCGCTGCTATAGATACAGAAGTTAGTAAAAGTGAGGATTTTCCTTA	1610
Sbjct	1536	CATGGGAAAAGAATGTGCTGCCATAGATTAGAAAAGTATTGGAAGTGAGGACTTCCCTTA	1595
Query	1611	TTCTGCTCGCCATCTATTTTTGTGTCAGGTGATAGACCAGAAGCTATTCGGACTCCTTCCCC	1670
Sbjct	1596	TTCCGCTCGCCATTTATTTTTGTGTCAGGTGATAGACCAGAAGTTATTCTTAATTCTTCCCT	1655
Query	1671	AGAGAAAGGATATCCAGGTATCCAAAACATTAATATGTT-CACGTTTCA--AATATTTGCA	1727
Sbjct	1656	AGAGAAAGGATATCCCGGTATCCAAAACATTGATATATTACTCGAAAAATGAAGATTTACA	1715
Query	1728	GAATGTATCAAAATACAGGTCATTGCGAGTATTAACAACGATGTGGGAAGGTTTCATTCCT	1787
Sbjct	1716	GAATTTATCAAAATACAGGTCATTGCGAGCATTAGA---GATCTGGGGAGGTATAATCCT	1772
Query	1788	GATACCAAAATATCATCATCACCTGAGGTATCTTGATCTCTCAGAAAGTGAAATTAAGC	1847
Sbjct	1773	GAAACCAAAATATCATCATCACCTGAGGTATCTTGATCTCTCATGGAGTGAAATTAAGC	1832
Query	1848	ACTTCCTGAAGACATAAGCATCCTATATCATTTGCAAACATTGAACCTTTCCTCGTTGTTT	1907
Sbjct	1833	ACTTCCTGAAGACATAAGCATCCTATATCATCTGCAAACGCTGAACCTTTCCTCACTGTAG	1892
Query	1908	ATCTCTCCGTCGACTTCCAAAGGGAATGAAGTACATGACCGCCCTCCGTCACCTTGACAC	1967
Sbjct	1893	CAATCTTCATCGACTTCCAAAGGGAACGAAGTACATGACTGCCCTCCGTCACCTGTACAC	1952
Query	1968	TCACGGATGTTGGAGTTTAGGAAGCATGCCTCCTGACCTCGGACACCTCACTTGCCTACA	2027
Sbjct	1953	TCACGGATGTGGGAGGTTAAAAGCATGCCTCCGAACCTCGGACACCTCACTTGCCTACA	2012
Query	2028	GACGCTTACATGCTTTGTAGCCGGTACTTGCTCTGGCTGCAGTGATTTGGGAGAGCTGCG	2087
Sbjct	2013	GACGCTTACATGCTTTGTAGCTGGTCTTGCTCTGGCTGCAGTGATTTGGGAGAGCTGCG	2072
Query	2088	GCAGTTGGACCTTGGTGGTCTGACTAGAGCTAAGAAAACCTGAAAATGTGACAAAAGCTGA	2147
Sbjct	2073	GCAGTCGGACCTTGGTGGTCTGACTAGAGCTAACACAACCTGAAAATGTGACAAAAGCTGA	2132
Query	2148	TGCAAAAGCAGCAAATCTCGGAAAGAAGGAAAAACTGACCAAATTGACCTTAATATGGAC	2207
Sbjct	2133	TGCAAAAGCAGCAAATCTCGGAAAGAAGGAAAAACTGACCGAATTGAGCTTAGGATGGGC	2192
Query	2208	TGATCAGGAGTACAAGGAGGCACAGAGTAATAATCATAAAGAGGTGCTGGAAGGTCTCAC	2267
Sbjct	2193	TGATCAGGAGTACAAGGAGGCACAGAGTAATAATCATAAAGAGGTGCTGGAAGGTCTCAT	2252
Query	2268	GCCTCACGAGGGGCTCAAGGTTCTGAGTATATATCACTGTGGGAGCAGTACATGTCCAAC	2327
Sbjct	2253	GCCTCACGAGGGGCTCAAGGTTCTGAGTATATATAGCTGTGGGAGCAGTACATGTCCAAC	2312
Query	2328	TTGGATGAATAAACTGCGGGACATGGTGGGGCTTGAGTTAAATGGTTGCAAAAATCTCGA	2387

Sbjct	2313	GTGGATGAATAAACTGCGGGACATGGTGAAGCTTAAGTTATATGGTTGCAAAAATCTCAA	2372
Query	2388	GAAGCTTCCTCCGTTGTGGCAGCTACCGGCTCTACAAGTTCTTTGCCGGAAGGACTGGG	2447
Sbjct	2373	GAAGCTTCCTCCATTGTGGCAGCTGACAGCTCTAGAAGTTCTTTGGCTTGAAGGACTGGA	2432
Query	2448	TAGTTTAAATTGCTTGTTCACCTGTGacacacacacacacCCTTCACATTTTGCAGACTGAA	2507
Sbjct	2433	TAGTGTAATTGCTTGTTCACAGTGGCAGCACACACCCCTTTAAATTCTGCAGACTGAA	2492
Query	2508	GGAGCTAACCTTGTCTGATATGACAAAATTTTGAGACATGGTGGGACACAAATGAGGTACA	2567
Sbjct	2493	GAAGCTTAACGTGTGTGATATGAAAAATTTTGAGACATGGTGGGACACAAATGAGGTAAA	2552
Query	2568	AGGAGAAGAGCTGATGTTTCTGAGGTTGAAAAGCTGTCAATCGAAAGTTGCCATAGGCT	2627
Sbjct	2553	AGGAGAAGAGCTGATATTTCTGAGGTTGAAAAGCTGTTAATCAAACGTTGCCGTAGGCT	2612
Query	2628	AACTGCCTTGCCAAAAGCATCAAATGCGATTTTCTGAAATCGTCCGGCGAAGTTAGCACCGT	2687
Sbjct	2613	AACTGCCTTACCAAAGCGTCAAATGCGATT-----TCTGGCGAAGTTAGCACCAT	2663
Query	2688	GTGTCGTTCTGCATTCCAGCATTGAAGGAAATGAAATTATATGATTTGCGTATCTTTCA	2747
Sbjct	2664	GTGTCGTTCTGCATTCCAGCATTGAAGGTAATGAAATTATATGATTTGGATATCTTTCT	2723
Query	2748	GAAATGGGAGGCAGTCGATGGAATCCAAGGGAGGAGGCAACATTTCTCAGCTTGACAA	2807
Sbjct	2724	GAAATGGGAGGCAGTCGATGGAATCAAAGGGAGGAGGTAACATTTCTCAGCTTGACAA	2783
Query	2808	ATTAGAAATCAGACAGTGCCAGAGCTGACTACTCTACCTGAAGCACCAAAGCTAAGTGA	2867
Sbjct	2784	ATTAGTAATCGGACGGTGCCAGAATTGACTACTCTACCTAAAGCACCAAAGCTAAGTGA	2843
Query	2868	CTTAGAGATATCTAAAGGCAATCAACAAATATCCCTACAGGCAGCCAGCAGACATATTAC	2927
Sbjct	2844	CTTAAACATATGTGAAGTCAATCAGCAAATATCCCTACAGGCAGCCAGCAGATATATTAC	2903
Query	2928	TTCATTGTCCAGTCTCGTTCTGCAATTTGTCCACTGATGACACAGAAACAGCATCGGTGGC	2987
Sbjct	2904	TTCATTGTCCAGTCTCCATCTGTTTGTGCAACTGATGACACAGAAACACATCGGTGGC	2963
Query	2988	CAAGCAACAAGATTGAGTGAATTTGGTGATTGAGGATGAGAAATGGAGTCATAAATCTCC	3047
Sbjct	2964	CAAGCAACAAGATTTGAGTGAATTTGGTGATTGAGGATGAGAAATGGAATCATAAATCTCC	3023
Query	3048	CCTGGAACCTTATGGTCTTGAGTCGGTGCAACCTTTTATTCTCTCACCAAGTGCACTGGC	3107
Sbjct	3024	CCTGGAACCTTATGGACTTAACTGGCTGCAACCTTTTATTCTCTTACCCTAGTGCGTTGGC	3083
Query	3108	TCTGTGGACATGTTTTGCTCAGCTCCTAGATCTGAAAATTCGGTATGTTGATGCGCTTGT	3167
Sbjct	3084	TCTGTGGACATGTTTTGTTTCTCAGCTCCTAGATCTGAAAATTTAGCCAAGTCGATGCGCTTGT	3143
Query	3168	CAGCTGGCCAGAAGAGGTGTTCCAGGGCTTAGTTTCTTGAGGAAGTTAGAGATTTCTGT	3227
Sbjct	3144	CGACTGGCCAGAAAGGGTGTCCGGGGCTTGGTTTCTTGAGGAAGTTACATATTGTTCA	3203
Query	3228	ATGCGAGAATCTGACAGGACACACACAAGCTCGTGGGCAATCTACACCCGCACCAAGTGA	3287
Sbjct	3204	ATGCAAGAATCTGACAGGACTCACACGAGCTCGTGGGCAATCTACACCCGCACCATGTTGA	3263
Query	3288	ACTCCTGCCACGTTTGGAGTCCCTAGAGATAACGTGTTGTGATTCTATTGTGGAGTCCC	3347

Sbjct	3264	 ACTCCTGCCACGTTTGGAGTCCCTAGAGATAAACCAATTGTGATTCTTTTGTAGAGGTCCC	3323
Query	3348	CAATCTACCGCGTCTCTCAAGCTATTAGAAATTAGGGGGTGCCCCGGCCTGGAGTCCAT	3407
Sbjct	3324	 CAACCTACCGACGTCTCTCAAGCTATTACAAATTTGGAATTGCCATGGCCTGAAGTCCA-	3382
Query	3408	CGTATTCAATCAGCAGCAGGATAGGACGATGTTGGTGAGTGCAGAAAGCTTTGCAGAGCA	3467
Sbjct	3383	 --TATTCAGCCAGCACAGGAGACGATGATGTTGGTGAGTGCAGAAAGCTTCGCACAGCC	3440
Query	3468	GGATAAGTCATCGTTAATATCAGGGTCCACAAGCGAGACCAACGATCACGTCTTCCACG	3527
Sbjct	3441	 GGACAAGTCA--TTAACATCAGGGTCCACCAGCGAGACCAGCGATCACGTCTTCCACG	3497
Query	3528	CCTAGAATCTCTTGTAAATAAATTTGGTGCATCGTTTGGAGGTTCTCCATCTTCTCCGTC	3587
Sbjct	3498	 CCTAGAATCTCTAGAAATAGGGTGTTCGATGTTTGGAGGTTCTCCATCTTCTCCGTC	3557
Query	3588	CATCAAGAAATTGGGTATTTATAGCTGTGAAAACTTCGGTCCCTCTCAGTAAAGCTGGA	3647
Sbjct	3558	 CATCAAGAAATTGGATATTTATCGCTGTGAAAACTTCAGTCACTCTCAGGAAAGCTGGA	3617
Query	3648	TGCCGTTTCGAGAATTAAGTATCAGACATTGCGGGAGCTTGAAATCACTGGAATCTTGCTT	3707
Sbjct	3618	 TGCCGTTTCGAGCATTAAATATCAGCTGTTGCGGGAGCTTGAAATCACTGGAATCTTGCTT	3677
Query	3708	AGGAGAGCTCGCGTCGCTGCAACAACCTCAAACCTTTTGGATTGCAAGAGCCTGGAATCCTT	3767
Sbjct	3678	 AGGAGAGCTCCCGTCGCTGCAACAACCTCAGCCTTTTGGATTGCAAGAGCCTGGTATCCTT	3737
Query	3768	GCCGAAGGGGCTCAAGCATACTCATCTCTTACATCTCTTGAAATTCGTGGTTGTTCTGG	3827
Sbjct	3738	 GCCGAAGGGGCTCAAGCATACTCATCTCTTACATCTCTTGAAATTCGTATTGTTCTGG	3797
Query	3828	TATAAAGGTGCTTCCACCGAGCCTACAGCAACGTCTGGATGACATCGAGGACAAAGAACT	3887
Sbjct	3798	 TATAAATTTGCTTCCACCGAGCCTGCAGCAACGTCTGGATGACATCGAGAATAAAGAACT	3857
Query	3888	AGATGCCTGCTATGAAGGTAATCTTCAGTTTCTTAACCGTGACATTTAGTGGTAAAAG	3947
Sbjct	3858	 AGATGCCTGCTATGAAGGTAATCTTCAGTTTCTTAACCGTGACATTTAGTGGTAAAAG	3917
Query	3948	TTTCGAGTTTCGTGTCTAGAACCCTAGTCAACCATTAATATGATTATATGTACATAGAG	4007
Sbjct	3918	 TTTCGAGCGTGGTGTCTAGAACCCTAGTCAACCATTAATATGATTATTTGTACATAGAG	3977
Query	4008	TACAATGCGCATTCACTAACTCACTTCTGCAGCTGTGTCATCTAAACCTTTAAACTTTGA	4067
Sbjct	3978	 TACAATGCGCATTCACTAACTCACTTCTGCAGCTGTGTCATCTAAACCTTTAAACTTTGA	4037
Query	4068	GTTGCATTTGGGTATCTAATCGCATGCAAAGGAATTTAGTTATATCTCCCGTAGCCATTC	4127
Sbjct	4038	 GTTGCATTTGGGTATCTAATCGCATGCAAAGGAATTTAGTGATATCTCCAGTAGCCATTC	4097
Query	4128	CTTATATGTGATGATCTCTTCCCTGTGATTATGCTTGTAGTTTGGACTATGTAATTAAT-T	4186
Sbjct	4098	 CTTATATATGATGATCTCTTCCCTGTGATTATGCTTGTAGTTTGGACTATGTAATTAAT	4157
Query	4187	TTTGCCGGGT-GACTATGTAATTACATGACTTCATTTAGTCGCCAGGTGTGGCATCATGC	4245
Sbjct	4158	 TTTGCCGGGTGACTATGTAATTAATTGACTTCATTTAGTCGCCAAGTGTGGCATCATGC	4217

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Query  4246  AATTATTATGGCAAAGCTGTATTAGTCATGATCGAAGCCACTTGGTGAACCTTATTCCTG  4305
      |||
Sbjct  4218  AATTATTATGGCAAAGCTGTATTAGTCATGATCGAAGCCACTTGGTGAACCTTATTCCTG  4277

Query  4306  CTACTTTGAACCAATACTCATTGATTATTTCCCTTTAAGCGTTTGATATGGACGACAGTT  4365
      |||
Sbjct  4278  CTACTTTGAATCAATACTCATTGATCATTTCCTTTAAGCGTTTGATATGGACAACACTT  4337

Query  4366  TAAATTTGCAGAGCTAACTAACGCAGCGCTTGTCTTTACATTTCT  4410
      |||
Sbjct  4338  TAAATTCGCAGAGCTAACTAACGCAGCGCTTGTCTTTACATTTCT  4382

```

Figure 7. Nucleotide sequence of *bph14-25* gene

CONCLUSION

Sai Duong Kien An and Loc Nuoc were cultivated and studied in Thua Thien Hue province. We tested their capacity BPH resistance to BPH populations in Thua Thien Hue, the results showed that two rice varieties resisted to BPH. This study revealed that *bph14-25* gene was detected in Sai Duong Kien An and Loc Nuoc cultivars, and nucleotide sequence of *bph14-25* was similar to nucleotide sequence of *bph14* (90%). These rice varieties are the important materials for growing and regenerating of the BPH resistant rice varieties with high yield in Thua Thien Hue.

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