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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 present 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 µL 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^{o}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^{o}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	AGAGTTCTTTATATCATGGAACTCA	1491
M2	GATCATGAGATTGACGTGGAAA	AAGTCACTTAGCTTTGGTG	1541
M3	AGTCGATGGAACTCCAAGGG	GATGAGTATGCTTGAGGCCC	1025
M4	AATCTTGCTTAGGAGAGCTCGC	CTACTTCAAGCACATCAGC	919

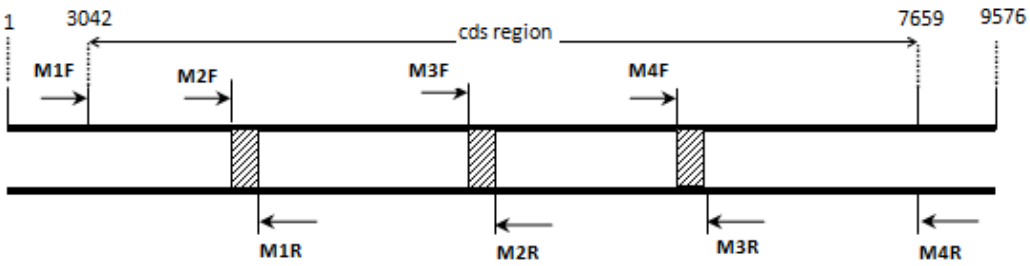

Figure 1. Diagram of primers on *bph14* gene (9576 bp)

: *Overlapping fragements*

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 µL of reaction mixture containing 100 ng of total DNA, 10 pmol each of primers, 200 mM dNTPs, 1.5 mM MgCl2, 10 µL of 5×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^{o}C for 5 min, followed by 30 cycles of denaturing at 95^{o}C for 1 min, annealing at 55^{o}C for 1 min, extension at 72^{o}C for 1 min, and 10 min at 72^{o}C for final extension. The PCR products were electrophoresed with 1.2% agarose gel at 100 volts in 1× TAE buffer and stained with ethidium bromide (0.5 µg/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× T4 ligation buffer, 4 units of T4 DNA ligase, 0.18pmol pTZ57R/T vector; the final volume was 10 µL. The reaction was incubated at 4^{o}C for overnight, followed by transforming the reaction mixture into *E.coli* (DH5-alpha, Invitrogen) by heat shock method at 42^{o}C for 90 seconds and then at 4^{o}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^{o}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.

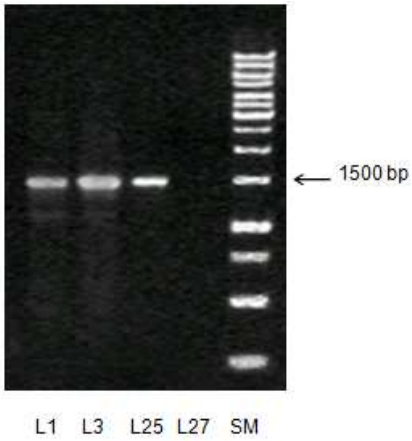

Figure 2. PCR products were amplified by M1 maker
SM: DNA marker (1kb DNA Ladder),
L1: IRRI 352, L3: BG 367-2, L25: Sai Duong Kien An, L27: Loc Nuoc

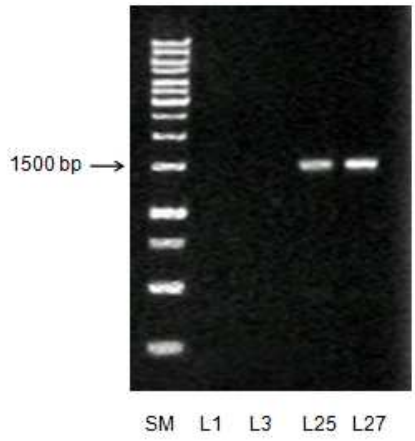

Figure 3. PCR products were amplified by M2 maker

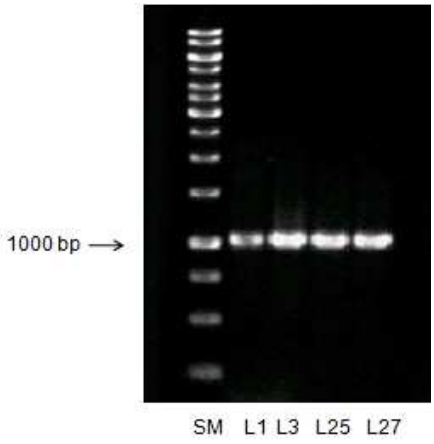

Figure 4. PCR products were amplified by M3 maker

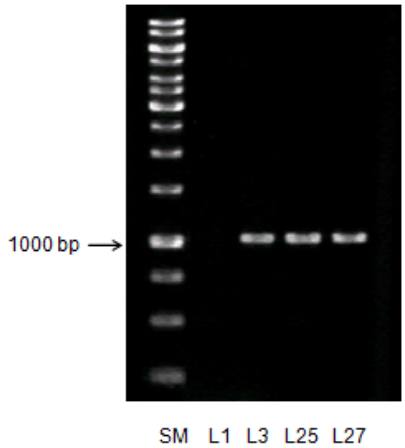

Figure 5. PCR products were amplified by M4 maker

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).

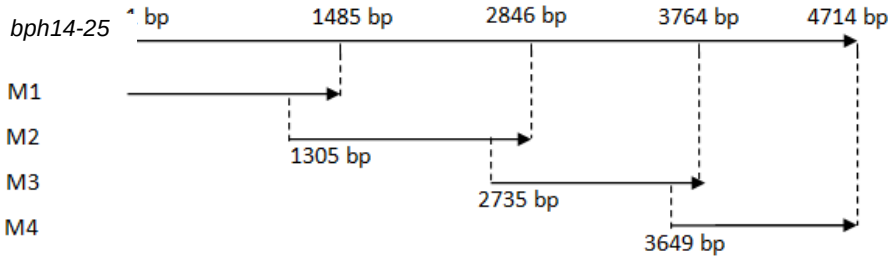

Figure 6. The length of *bph14-25* gen

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

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Query  2748  GAAATGGGAGGCAGTCGATGGAACTCCAAGGGAGGAGGCAACATTTCCTCAGCTTGACAA  2807
              |||||||||||||||||||||||| |||||||||| ||||||||||||||||||||||
Sbjct  2724  GAAATGGGAGGCAGTCGATGGAACTCAAAGGGAGGAGGTAACATTTCCTCAGCTTGACAA  2783

Query  2808  ATTAGAAATCAGACAGTGCCCAGAGCTGACTACTCTACCTGAAGCACCAAAGCTAAGTGA  2867
              ||||| |||| ||| |||||||||  |||||||||||||| |||||||||| ||||||||
Sbjct  2784  ATTAGTAATCGGACGGTGCCCAGAATTGACTACTCTACCTAAAGCACCAAAACTAAGTGA  2843

Query  2868  CTTAGAGATATCTAAAGGCAATCAACAAATATCCCTACAGGCAGCCAGCAGACATATTAC  2927
              |||| | |||| | ||| |||||| |||||||||||||||||||||||||| |||||||
Sbjct  2844  CTTAAACATATGTGAAGTCAATCAGCAAATATCCCTACAGGCAGCCAGCAGATATATTAC  2903

Query  2928  TTCATTGTCCAGTCTCGTTCTGCATTTGTCCACTGATGACACAGAAACAGCATCGGTGGC  2987
              ||||||||||||||  ||||  |||||| ||||||||||||||||| ||||||||||
Sbjct  2904  TTCATTGTCCAGTCTCCATCTGTTTTTGTCAACTGATGACACAGAAACAACATCGGTGGC  2963

Query  2988  CAAGCAACAAGATTCGAGTGATTTGGTGATTGAGGATGAGAAATGGAGTCATAAATCTCC  3047
              ||||||||||||||| ||||||| |||||||||||||||||||||| ||||||||||||
Sbjct  2964  CAAGCAACAAGATTTGAGTGAATTGGTGATTGAGGATGAGAAATGGAATCATAAATCTCC  3023

Query  3048  CCTGGAACTTATGGTCTTGAGTCGGTGCAACCTTTTATTCTCTCACCCAAGTGCACTGGC  3107
              ||||||||||||||| ||| | | | |||||||||||||||||| |||| |||||  ||||
Sbjct  3024  CCTGGAACTTATGGACTTAACTGGCTGCAACCTTTTATTCTCTTACCCTAGTGCGTTGGC  3083

Query  3108  TCTGTGGACATGTTTTGCTCAGCTCCTAGATCTGAAAATTCGGTATGTTGATGCGCTTGT  3167
              |||||||||||||||||| |||||||||||||||||||||| |   | || ||||||||||
Sbjct  3084  TCTGTGGACATGTTTTGTTCAGCTCCTAGATCTGAAAATTAGCCAAGTCGATGCGCTTGT  3143

Query  3168  CAGCTGGCCAGAAGAGGTGTTCCAGGGCTTAGTTTCCTTGAGGAAGTTAGAGATTTCTGT  3227
              |  |||||||||  |||||||| |||||| |||||||||||||||||| | |||  |
Sbjct  3144  CGACTGGCCAGAAAGGGTGTTCCGGGGCTTGGTTTCCTTGAGGAAGTTACATATTGTTCA  3203

Query  3228  ATGCGAGAATCTGACAGGACACACACAAGCTCGTGGGCAATCTACACCCGCACCAAGTGA  3287
              |||| |||||||||||||| ||||| |||||||||||||||||||||||||| ||||
Sbjct  3204  ATGCAAGAATCTGACAGGACTCACACGAGCTCGTGGGCAATCTACACCCGCACCATGTGA  3263

Query  3288  ACTCCTGCCACGTTTGGAGTCCCTAGAGATAACGTGTTGTGATTCTATTGTGGAGGTCCC  3347
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                ||||||||||||||||||||||||||||||||    |||||||||| |||| ||||||||
Sbjct  3264  ACTCCTGCCACGTTTGGAGTCCCTAGAGATAAACCATTGTGATTCTTTTGTAGAGGTCCC  3323

Query  3348  CAATCTACCGGCGTCTCTCAAGCTATTAGAAATTAGGGGGTGCCCCGGCCTGGAGTCCAT  3407
              ||| ||||||| |||||||||||||||||| ||||| ||    ||||  |||||| ||||||
Sbjct  3324  CAACCTACCGACGTCTCTCAAGCTATTACAAATTTGGAATTGCCATGGCCTGAAGTCCA-  3382

Query  3408  CGTATTCAATCAGCAGCAGGATAGGACGATGTTGGTGAGTGCAGAAAGCTTTGCAGAGCA  3467
                ||||||  ||||| ||||| | || |||||||||||||||||||||||| ||| |||
Sbjct  3383  --TATTCAGCCAGCACCAGGAGACGATGATGTTGGTGAGTGCAGAAAGCTTCGCACAGCC  3440

Query  3468  GGATAAGTCATCGTTAATATCAGGGTCCACAAGCGAGACCAACGATCACGTCCTTCCACG  3527
              ||| ||||||   |||| |||||||||||| |||||||||| ||||||||||||||||||
Sbjct  3441  GGACAAGTCA---TTAACATCAGGGTCCACCAGCGAGACCAGCGATCACGTCCTTCCACG  3497

Query  3528  CCTAGAATCTCTTGTAATAAATTGGTGCGATCGTTTGGAGGTTCTCCATCTTCCTCCGTC  3587
              |||||||||||| | ||||   || |||||| |||||||||||||||||||||||||||||
Sbjct  3498  CCTAGAATCTCTAGAAATAGGGTGTTGCGATGGTTTGGAGGTTCTCCATCTTCCTCCGTC  3557

Query  3588  CATCAAGAAATTGGGTATTTATAGCTGTGAAAAACTTCGGTCCCTCTCAGTAAAGCTGGA  3647
              ||||||||||||||| ||||||| |||||||||||||| ||| ||||||| |||||||||
Sbjct  3558  CATCAAGAAATTGGATATTTATCGCTGTGAAAAACTTCAGTCACTCTCAGGAAAGCTGGA  3617

Query  3648  TGCCGTTCGAGAATTAAGTATCAGACATTGCGGGAGCTTGAAATCACTGGAATCTTGCTT  3707
              |||||||||||| ||||| ||||||   |||||||||||||||||||||||||||||||||
Sbjct  3618  TGCCGTTCGAGCATTAAATATCAGCTGTTGCGGGAGCTTGAAATCACTGGAATCTTGCTT  3677

Query  3708  AGGAGAGCTCGCGTCGCTGCAACAACTCAAACTTTTTGATTGCAAGAGCCTGGAATCCTT  3767
              |||||||||| ||||||||||||||||  |||||||||||||||||||||| ||||||
Sbjct  3678  AGGAGAGCTCCCGTCGCTGCAACAACTCAGCCTTTTTGATTGCAAGAGCCTGGTATCCTT  3737

Query  3768  GCCGAAGGGGCCTCAAGCATACTCATCTCTTACATCTCTTGAAATTCGTGGTTGTTCTGG  3827
              ||||||||||||||||||||||||||||||||||||||||||||||||  |||||||||
Sbjct  3738  GCCGAAGGGGCCTCAAGCATACTCATCTCTTACATCTCTTGAAATTCGTTATTGTTCTGG  3797

Query  3828  TATAAAGGTGCTTCCACCGAGCCTACAGCAACGTCTGGATGACATCGAGGACAAAGAACT  3887
              ||||||  |||||||||||||||| |||||||||||||||||||||| | ||||||||
Sbjct  3798  TATAAATTTGCTTCCACCGAGCCTGCAGCAACGTCTGGATGACATCGAGAATAAAGAACT  3857

Query  3888  AGATGCCTGCTATGAAGGTAATCTTCAGTTTCTTAACCGTGTACCATTTAGTGGTAAAAG  3947
              |||||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  3858  AGATGCCTGCTATGAAGGTAATCTTCAGTTTCTTAACCGTGTACGATTTAGTGGTAAAAG  3917

Query  3948  TTTCGAGTTTCGTGTCTAGAACCCCTAGTCAACCATTAATATGATTATATGTACATAGAG  4007
              |||||||  | |||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  3918  TTTCGAGCGTGGTGTCTAGAACCCCTAGTCAACCATTAATATGATTATTTGTACATAGAG  3977

Query  4008  TACAATGCGCATTCATAACTCACTTCTGCAGCTGTGTCATCTAAACCCTTTAAACTTTGA  4067
              |||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  3978  TACAATGCGCATTCATAACTCACTTCTTCAGCTGTGTCATCTAAACCCTTTAAACTTTGA  4037

Query  4068  GTTGCATTTGGGTATCTAATCGCATGCAAAGGAATTTAGTTATATCTCCCGTAGCCATTC  4127
              |||||||||||||||||||||||||| ||||||||||||| |||||||| |||||||||
Sbjct  4038  GTTGCATTTGGGTATCTAATCGCATACAAAGGAATTTAGTGATATCTCCAGTAGCCATTC  4097

Query  4128  CTTATATGTGATGATCTCTTCCTGTGATTATGCTTGCTAGTTTGGACTATGTAATTAA-T  4186
              ||||||| |||||||||||||||||||||||||||||||||||||||||||||||||| |
Sbjct  4098  CTTATATATGATGATCTCTTCCTGTGATTATGCTTGCTAGTTTGGACTATGTAATTAATT  4157

Query  4187  TTTGCCGGGT-GACTATGTAATTACATGACTTCATTTAGTCGCCAGGTGTGGCATCATGC  4245
              |||||||||| |||||||||||  |||||||||||||||||| ||||||||||||||
Sbjct  4158  TTTGCCGGGTCGACTATGTAATTAATTGACTTCATTTAGTCGCCAAGTGTGGCATCATGC  4217
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Query  4246  AATTATTATGGCAAAGCTGTATTAGTCATGATCGAAGCCACTTGGTGAACTTATTCCCTG  4305
             ||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  4218  AATTATTATGGCAAAGCTGTATTAGTCATGATCGAAGCCACTTGGTGAGCTTATTCCCTT  4277

Query  4306  CTACTTTGAACCAATACTCATTGATTATTTCCCTTTAAGCGTTTGATATGGACGACAGTT  4365
             |||||||||| ||||||||||||| |||||||||||||||||||||||||||| ||| ||
Sbjct  4278  CTACTTTGAATCAATACTCATTGATCATTTCCCTTTAAGCGTTTGATATGGACAACACTT  4337

Query  4366  TAAATTTGCAGAGCTAACTAACGCAGCGCTTGTCTTTACATTTCT  4410
             |||||| |||||||||||||||||||||||||||||||||||||
Sbjct  4338  TAAATTCGCAGAGCTAACTAACGCAGCGCTTGTCTTTACATTTCT  4382
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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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