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THESIS

GENOMIC AND FUNCTIONAL ANALYSIS OF SESQUITERPENE SYNTHASE AND TERPINOID INVOLVED IN RICE BROWN PLANTHOPPER RESISTANCE

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Brown planthopper (BPH) is the most serious insect pest for rice production worldwide. The most effective way to prevent rice plant from destructive damage caused by BPH is identification of resistance genes and other related genes involved in BPH resistance mechanisms of rice. More than 20 BPH-resistance genes have been localized on specific region of rice chromosome using molecular genetic analysis and genomic tools. In this study, BPH-resistance genes from Sri Lanka rice cultivar Rathu heenati (RH) were investigated by whole genome strategy.

Single-feature polymorphism (SFP) mapping was used to localize 84 candidate resistance related genes on chromosomes 1, 2, 3, 4, 6, 8, 10, and 12. An expression analysis of 15 selected candidate genes in the KDML105 (KD) and the isogenic line (IL) identified Os04g27430 as the BPH-resistance related gene. Os04g27430 encodes a putative *sesquiterpene synthase* (*STPS*) gene that was induced by BPH feeding in ILs. Functional SNPs in 5’UTR and exon 5 in the susceptible rice line were identified, which might explain the differential response of Os04g27430 during BPH infestation. Os04g27430 may play role in antixenosis mechanism of rice against BPH attacking.

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Wintai Kamolsukyunyong
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LIST OF ABBRREVIATIONS

AFP = antixenosis of the BPH feeding preference

ATHB-1 = *Arabidopsis thaliana* homeobox protein 1

bp = base pair

BPH = brown planthopper

CC-NB-LRR = coiled-coil nucleotide binding and leucine-rich repeat

cDNA = complimentary deoxyribonucleic acid

cDNA-AFLP = cDNA-amplified fragment length polymorphism

D = days of BPH infestation

DC = days of control condition

DNA = deoxyribonucleic acid

DTPS = diterpene synthase

FDR = false discovery rate

FPP = farnesyl diphosphate

GPP = geranyl diphosphate

GGPP = geranylgeranyl diphosphate

HCS = Homchonlasit

Indel = insertion/ deletion

ILs = Isogenic lines

JA = jasmonic acid

JHN = Jaohomnin

Kb = kilobase

KDML105/KD = Khao Dok Mali 105

LOX = lipoxiginase

MeJA = methyl jasmonate

min = minute

ml = milliliter

mM = millimolar

LIST OF ABBRREVIATIONS (Continued)

MM = mismatch probe
MTPS = monoterpene synthase
NILs = near isogenic lines
Nip = Nipponbare
P = maize activator P
PCR = polymerase chain reaction
PK2 = Pinkaset2
PK3 = Pinkaset3
PL2 = Phitsanulok2
PM = perfect match probe
QTL = quantitative trait loci
RBR = Riceberry
RDA = representational difference analysis
RH = Rathu heenati
RM = rice microsatellite marker
RNA = ribonucleic acid
RT-PCR = Reverse transcriptase polymerase chain reaction
SA = salicylic acid
SBF-1 = silencer-binding factor 1
STPS = sesquiterpene synthase
SFP = single feature polymorphism
SNP = single nucleotide polymorphism
SSR = simple sequence repeat
TF = transcription factor
TN1 = Taichung native 1
TPS = terpene synthase
UBN = Ubon Ratchathani
µg = microgram
µl = microlite
µM = micromolar

GENOMIC AND FUNCTIONAL ANALYSIS OF SESQUITERPENE SYNTHASE AND TERPINOID INVOLVED IN RICE BROWN PLANTHOPPER RESISTANCE

INTRODUCTION

Rice is the most important cereal plant of Thailand and the world. Its grain production was consumed and exported as the most value agricultural product of Thailand. However some rice production was loss by several abiotic and biotic threats each year especially insect pest is one of the most important factor reduced rice production for almost every season.

Brown planthopper (BPH), *Nilaparvata lugens* Stål, is one of the most serious insect pests in rice, causing huge yield losses in Asian rice-growing areas each year. It feeds on the plant's phloem and causes decreasing leaf area, plant height, dry weight, leaf and stem nitrogen concentration, chlorophyll contents, and photosynthetic rate, but increase in free amino acid, sucrose, and leaf iron content (Lou et al. 2005a). BPH can be found at all stage of plant development (Dale 1994). It became a major threat to rice production in the early 1970s, when new high-yielding varieties were introduced. The most considerable way for BPH controlling is developing resistance rice cultivars. To date, a number of BPH resistance genes have been localized in rice genome. *Bph3*, a single dominant gene confers broad-spectrum BPH resistance against BPH populations found in Thailand from rice cultivar Rathu Heenati (RH) of Sri Lanka, has been mapped on the short arm of rice chromosome 6, however, the *Bph17* gene of the same rice cultivar was also mapped on rice chromosome 4 by another research group (Jairin et al 2007a, Sun et al 2005). Until now the gene confer resistance to BPH from this rice cultivar still not be identified.

OBJECTIVES

The overall objectives of this research are to identify and functional analysis of the BPH-resistance-related gene(s) in rice. To do these, genomic tools such as genomic DNA analysis by microarray hybridization will be conducted.

The specific objectives are:

1. To identify the BPH-resistance-related genes by single feature polymorphism (SFP) mapping between BPH susceptible rice cultivar, KDML105 (KD) and its BPH resistance isogenic lines (ILs).

2. To characterize genomic, function and role of rice sesquiterpene synthase gene, LOC_Os04g27430, identified by SFP mapping on BPH resistance mechanism.

LITERATURE REVIEWS

1. Brown planthopper (BPH) resistance genes in rice

The brown planthopper (BPH), Nilaparvata lugens Sta◦l, is one of the most destructive insect pests for rice (*Oryza sativa* L.). The target organ of BPH is highly specific to phloem of rice as the feeding behavior of phloem sucker. This feeding activity, sucking assimilates translocating in the phloem sieve elements, cause considerable physiological damage to the rice plants by nutrients loss and disrupting physiological processes and consequently reduces the growth and development of rice (Watanabe and Kitagawa 2000). The susceptible rice varieties always face severe yield losses each year from BPH attacking. BPH does not only make physiological damage to rice plants but also acts as the vector for several rice viruses (Heinrichs et al., 1985). In addition, a hopper burn condition, drying of the leaves and tiller wilting, is a damage of rice plants which were feed by large number of BPH.

Since the severe damage of rice by the BPH outbreak is the major cause for rice production loss in South-East Asia and world wide, along with the hazardous effects of using pesticide to protect rice crop, identification of BPH resistance genes is promoted by researchers for using in breeding program and studying their functions. To date, more than 20 resistance genes and several quantitative trait loci (QTL) associated with BPH resistance have been reported and reviewed (Alam and Cohen, 1998a; Chen et al., 2005; Huang et al., 1997; Huang et al., 2001; Ishii et al., 1994; Jairin et al., 2005; Jena et al., 2003; Jena et al., 2006; Jeon et al., 1999; Kawaguchi et al., 2001; Liu et al., 2001; Mei et al., 1996; Murai et al., 2001; Murata et al., 1998; Murata et al., 2001; Renganayaki et al., 2002; Sharma et al., 2003a, b; Soundararajan et al., 2004; Su et al., 2002; Sun et al., 2005; Wang et al., 2001; Xu et al., 2002; Yan et al., 2002; Yang et al., 2002). Among these, *Bph3*, a broad-spectrum dominant resistance gene of rice variety Rathu Heenati (RH), was finely mapped on the short arm of rice chromosome 6 (Jairin et al 2007a). According to the rice genomic sequence, Bph3 was localized to approximately 190 kb region flanked by two microsatellite markers, RM19291 and RM8072. Moreover, this same genomic region is encompassing *bph4* gene, the BPH resistance gene from another rice cultivar, Babawee, too (Kawaguchi et al. 2001, Jairin et al. 2010). On the other hand, Sun et al (2005) localized BPH resistance gene of RH on the short arm of rice chromosome 4 between RM8213 and RM5953 and tentatively designated as *Bph17* gene.

2. Brown planthopper responsive genes in rice

In recent decade, identification of gene(s) conferring resistance to brown planthopper have been studied intensively and progress were accomplished in mapping of more than twenty *Bph* genes located around rice genome. Moreover, several molecular biology techniques were used to identify candidate genes (Zhang et al 2004, Yuan et al 2004, Yuan et al 2005, Wang et al 2005, Cho et al 2005, Park et al 2007), and study the rice response reaction against BPH infestation (Hao et al 2008, Xia et al 2008)

Zhang et al (2004) used cDNA array hybridization technique to study the expression of several abiotic and biotic stress genes when rice were attracted by BPH and found that the rice reactions against BPH were complex and have crosstalking each other between stress responsive genes. Yuan et al (2004) and Yuan et al (2005) used suppression subtraction hybridization techniques to identify the rice genes that were induced or suppressed by BPH feeding. As the results, they found that several defense-related genes played important role in respond to BPH. Park et al (2008) used representational difference analysis (RDA) technique to identify genes which were induced in near isogenic lines (NILs) by BPH feeding and mapped those genes onto the rice chromosome map. They found that the identified genes were mapped close to the regions that were reported to have *Bph* genes mapped, so these genes may be candidate for BPH resistance genes. Xia et al (2008) studied the level of salicylic acid (SA), jasmonic acid (JA), ethalene and H_2O_2 that rice released when were attracted by BPH compared to rice that were induced with β-glucosidase, elicitor in herbivorous signal transduction pathway, and found the same release pattern. Hao et al (2008) reported the callose deposition in sieve plate of phloem sap in resistance rice cultivar when were feed by BPH resulted in harden of rice plant for herbivorous to feed on. Moreover, candidate genes for BPH resistance were reported, for example, homolog of wheat Wir1 family protein (Yuan et al, 2004), and lipoxigenase (LOX) (Park et al 2008).

3. Plant defense mechanisms against brown planthopper

To date, information about plant response mechanisms against attacking herbivorous insects is relied mainly on the study of interaction between plants and chewing insects (Moran and Thompson 2001). The chewing insects cause extensive plant tissue damage during their feeding and thus activate the wound-signaling pathway, which is a part of the induced defense reactions (Walling 2000). However, in contrast to chewing insects, because of the limited tissue damage and prolonged stylet interactions with plant cells, plant’s responses to phloem feeding insects are difference (Walling 2000). Even phloem feeding insects cause little damage to plants, these minor injuries are still perceived as pathogenic by the defense response pathway of the host plant (Moran et al. 2002). Rice is not only providing a staple food to more than one third of the world population, but also being a model organism for monocotlydonous plants. The interaction between rice and BPH has the potential to serve an excellent model system for understanding the genetic basis of plant defense against phloem-feeding insects.

4. Molecular response of BPH in exposure to resistance rice variety

Although BPH interacts with host rice plants at molecular and cellular levels, the overall response of BPH when infests on resistance rice is not well understood. Yang et al. (2006) used the cDNA-amplified fragment length polymorphism (cDNA-AFLP) to identify the differentially expressed genes in the insects feeding on three rice variety differ in BPH resistance, two resistance, B5 and MH63, and one susceptible, TN1. 61 cDNA bands of BPH were up- or down- regulated when change from susceptible to resistance host plants. Sequence analysis showed that the genes involved in signaling, stress response, gene expression regulation, detoxification and metabolism were regulated by host resistance. They identified four genes, a putative B subunit of phosphatase PP2A, a nemo kinase, a cytochrome P450 monooxygenase and a prolyl endopeptidase, as the defensive response of BPH to host rice resistance.

5. Biotypes of BPH

The biotypes of BPH show significant virulence differences among rice varieties, although some biotypes also differ in minor morphological and chemical features (Sogawa, 1978; Claridge and Hollander, 1980). Four BPH biotypes are well known in rice. Biotypes 1 and 2 are widely distributed in Southeast and East Asia, and usually occur on the rice varieties TN1 and Mudgo, respectively (Claridge and Hollander, 1980; Jena and Kim, 2010). Biotype 3 is a laboratory biotype produced on the resistance variety ASD7, which contains the BPH resistance gene bph2 (Panda and Heinrichs, 1983). Biotype 4 is the most destructive biotype and occurs predominantly on the RH variety. This biotype occurs on the Indian subcontinent and is thus referred to as the South Asian biotype (Claridge and Hollander, 1980; Jena and Kim, 2010). The discovery of many loci for resistance genes in rice has led to biotype 1 being considered a population that cannot infest any rice variety having major resistance genes, while biotype 2 is a population capable of infesting rice varieties having the *Bph1* resistance gene (Alam and Cohen, 1998b). Further studies have shown that *Bph1* provides resistance to biotypes 1 and 3; *bph2* provides resistance to biotypes 1 and 2; any of *Bph3*, *bph4*, *bph8*, and *Bph9* provide resistance to biotypes 1, 2, 3, and 4; and *bph5*, *Bph6*, and *bph7* only provide resistance to biotype 4 (Khush and Brar, 1991; Panda and Khush, 1995)

6. Signaling pathway of rice during BPH attack

Systematic resistances were proposed to be the mechanism of some dicotyledonous plants against pathogens and insects (Ross 1961, Green and Ryan 1972, Karban and Carey 1984). Chemical signals from plant play role in coordinating the responses of plant to attacker. By careful examination of the signalling events related to systemic-induced resistance, chemical cascades involving salicylic acid

(SA) were found to responsible for many induced reactions against pathogens (Malamy et al. 1990, Metraux et al. 1990) and cascades involving jasmonic acid (JA) were found to involve in many induced responses against wounding and insect attack (Farmer et al. 1992). Moreover, abscisic acid and ethylene have also been identified as other molecules important for plant response. Even the important role of these chemical signals in signalling responses to herbivory has been highly conserved among plants families (Thaler and Bostock 2004, Stout et al. 2006), but the role that these molecules might play in cereals such as rice has been poorly understood until recently (Schweizer et al. 1998, Kogel and Langen 2005).

7. Cloning of BPH resistance genes

Advances in molecular biology and bioinformatics have paved the way for the cloning and understanding of the molecular mechanism of BPH resistance. Of several genes conferring resistance to BPH, only one resistance gene, *Bph14*, located on the long arm of chromosome 3 has been cloned (Du et al. 2009). The *Bph14* gene which confers resistance at seedling and maturity stages was cloned by map-based cloning approach. The *Bph14* gene encodes a coiled-coil nucleotide binding and leucine-rich repeat (CC-NB-LRR) protein. Sequence comparison indicates that the *Bph14* gene carries a unique LRR domain that might function in recognizing BPH invasions and in activating the defense response. The *Bph14* gene activates salicylic acid signaling pathway and induces callose deposition in phloem tissue that inhibits BPH feeding on the host plant (Du et al. 2009).

8. Single feature polymorphism (SFP)

Microarray is the technique widely used for effectively measuring the gene expression at the whole genome level. The data from microarray experiment help researchers to draw the whole picture of gene functions. In microarray experiment, two necessary components are probe, the gene-specific DNA fragments that spotted on the surface of array, and target, normally the RNA extracted from the experimental samples. The target are normally labeled and hybridized to probes on the array, the light signal generated were used for measuring the expression level of the genes. Affymetrix GeneChip Array of Affymetrix Inc, UAS is one platform of microarray technologies. It is the on-slide synthesis array platform which 25-bp oligonucleotides were synthesized as probe on the surface of array. One probe set consisted of two subsets called perfect match probe (PM) and mismatch probe (MM) complementary to each gene in the genome. The MM, almost identical to PM except the base 13 which will be mismatch to the PM, was hybridized to target in mismatch manner for negative control hybridization. One slide of Affymetrix GeneChip encompasses more than 1.3 million probes represents almost every genes in the genome of study organisms. For rice, the Affymetrix Rice Genome Array consisted of about 630,000 PM probe represented about 50,000 rice genes.

Recently, Affymetrix GeneChip Array has been used as genomic tool for identification of genetic variation at the whole genome level. By using DNA instead of RNA as target to hybridize with probe of the array, difference in hybridization intensity of each probe was the results of sequence variation between two experimental DNA samples compare to sequence on the probe. By using this principle, the whole genome genotypic variation can be revealed (Figure1) this variation called single feature polymorphism (SFP) was first studied in yeast (Winzeler et al, 1998) and later were widely used to identify genetic variation in many plant species such as Arabidopsis (Borevitz et al, 2003), barley (Rostoks et al 2005), rice (Kumar et al, 2007), cowpea (Das et al, 2008), and wheat (Coram et al, 2008). In yeast, Winzeler et al (1998) identified more than 4,000 SFPs located all around yeast genome which potentially converted to DNA marker for genome mapping. In the same way, several thousand SFPs were identified between two accessions of Arabidopsis and could be used for genome mapping and positional cloning of interested gene (Borevitz et at, 2003; Hazen et al, 2005). Kumar et al (2007) studied the effectiveness of SFP in identification of genetic variation in rice. They compared 3 parental rice varieties by DNA hybridization of Affymetrix Rice Genome Array and found that the technique was powerful identified variation between rice varieties either within the same sub species (japonica) or across sub species (indica and japonica).

SFPs have been used for a variety of purposes including detection of marker-trait associations, which sometimes involved construction of a molecular map followed by QTL interval mapping. For instance, in Arabidopsis, bulk segregant analysis has been used for mapping of circadian and developmental genes (Hazen et al., 2005), ion accumulation genes (Gong et al., 2004) and light-responsive QTLs (Wolyn et al., 2004). Similarly, in tomato, 17 SFPs were identified, which were tightly linked to a disease resistance locus (Zhu and Salmeron, 2007). Other important examples of the utility of SFPs are the development of a map of 34,000 SFP loci representing 11,000 genes in maize and that of 8500 SFP loci from 6000 genes in tomato (Zhu and Salmeron, 2007).

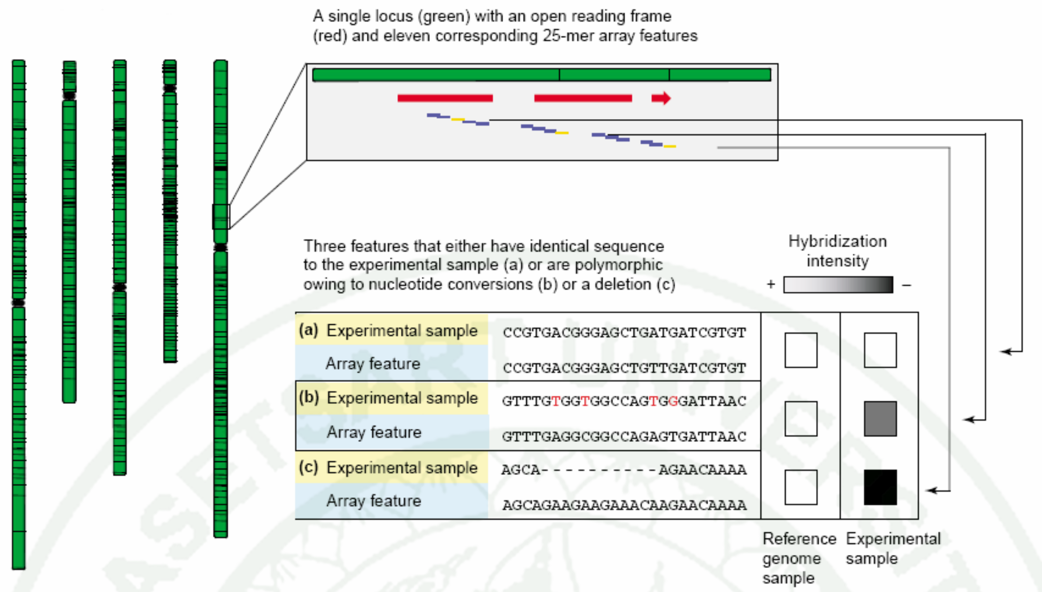


Figure 1 Diagram of sequence polymorphisms in a single gene detected by an oligonucleotide array. Each gene on the Arabidopsis ATH1 array is represented by 11 25-mer features. Differential hybridization intensity is interpreted as a sequence polymorphism. **Source: Hazen and Kay (2003)**

9. Terpene synthase (*TPS*) genes and indirect defense mechanism against insects in rice

Many plants release elevated levels of volatile organic compounds upon insect herbivory. These compounds function in indirect plant defense by attracting predators and/or parasitoids that are natural enemies of the feeding herbivores (Pachersky and Gershenzon, 2002). The molecular and genomic basis of indirect defense, i.e. how many genes are directly involved in the production of herbivory-induced volatiles and how the biosynthesis is regulated, is poorly characterized. Rice is an useful model for studying the molecular and genomic basis of indirect plant defense because of the availability of a full genome sequence and extensive genetic and genomic resources. For indirect defense, rice is well studied with respect to its interaction with BPH. Several volatile compounds including terpenoids were identified by BPH attacking (Lou et al. 2005b, 2006) however very little is known about how these compounds are synthesized and regulated in rice at the molecular level.

Terpenoids are the most common and diverse volatile compound groups among insect-induced plant volatiles (Pare and Tumlinson, 1999). All terpenoids are synthesized by the action of terpene synthase (*TPS*) genes which convert geranyl diphosphate (GPP), farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP) to monoterpene C10), sesquiterpene (C15) and diterpepe (C20) respectively. The rice genome contains a large number of *TPS* genes (The rice genome annotation database). The molecular and biochemical function for the majority of rice *TPS* genes still remains unknown.

MATERIALS AND METHODS

Part I: Single feature polymorphism (SFP) mapping of isogenic rice lines

1. Plant materials

For SFP mapping, KDML105 (KD), the Thai elite aroma rice variety and its 4 isogenic lines (ILs) resistant to BPH were used as experimental samples for DNA hybridization of Affymetrix Rice Genome Array. The ILs were developed by backcrossing of KDML105 with Rathu heenati (RH), the Sri Lanka BPH resistance variety. The four BC_3F_5, UBN3078-101-342-4-162 (IL162), UBN3078-101-342-4-283 (IL283), UBN3078-101-342-6-302 (IL302) and UBN3078-101-342-6-308 (IL308), showed highly resistance to BPH were selected as ILs and used in this study

2. Rice genome array

The rice genome array (www.affymetrix.com) contains ~630,000 perfect match (PM) probes or features represents about 50,000 distinct rice genes were used in this study. The arrays were hybridized with genomic DNA of KD and a pool of 4ILs. Two biological replicating experiments were conducted.

3. DNA extraction, probe generation and array hybridization

Single leaf of each IL was cut and pooled together, two pools were collected (IL1, IL2). Leave from two single plants of KD were collected separately (KD1, KD2). Four DNA samples were isolated by DNeasy Plant Mini Kit (Qiagen) and biotin (biotin-dCTP) labeled by BioPrime labeling kit (Invitrogen). For each sample, all of labeled reaction products were used for hybridization to Affymetrix rice genome array. The hybridization and array washing was performed according to standard RNA protocol as described in Affymetrix manual.

4. Data analysis

The data quality was assessed from expression report generated by GCOS1.3 software using the default parameter set for rice genpme array. The raw intensity values of all the probes present on the chip was transferred from .cel files into .txt file by using the tools available on GCOS1.3 software. The intensity values of PM (perfect match) probes was extracted and subjected to data analysis (Kumar et al, 2007) by using the SFP tools available in the RiceGeneThresher database (http://rice.kps.ku.ac.th/Site/index.html). The normalized hybridization intensity data of four arrays (IL1, IL1, KD1 and KD2) of the predicted SFP were separately identified from the whole array dataset. Average hybridization intensity of KDML105 and ILs were calculated as:

KDaverage = (KD1 + KD2)/2

ILaverage = (IL1 + IL2)/2

And finally, the hybridization fold change of each SFP was calculated as:

Fold change = the average with higher volume/the average with less volume

5. PCR of predicted SFP, DNA sequencing and sequence comparison

Primers flanking the predicted SFP were designed by primer3 program (http://frodo.wi.mit.edu/primer3/) and primer blast tools (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) based on Nipponbare (Nip) genomic sequence (http://rice.plantbiology.msu.edu/). The primers were designed to obtain 300 – 600 bp DNA fragments encompassing the predicted SFP regions. The primers used for SFP confirmation were listed in the Appendix Table A1. Genomic DNA was extracted from young leaves of KD and RH using the DNeasy Plant mini Kit (Qiagen, Valencia, CA, USA). PCR was performed in 25 µl reaction mixtures containing 50 ng of genomic DNA template, 0.1 mM of dNTPs, 0.25 mM $MgCl_2$ and 1x Thermophilic DNA polymerase buffer (Fermentas). The reaction mixtures were pre-heat at 94 ºC for 2 min and then the PCR reaction was carried out for 35 cycles with the following conditions: 94 ºC for 30 sec, 55 ºC for 30 sec and 72 ºC for 1 min, and final extension at 72 ºC for 5 min. Desired PCR bands were cut from 1% agarose gel and DNA fragments were purified by Gel/PCR DNA fragments Extraction Kit (Geneaid) and subjected to sequencing. The purified PCR products were sequenced in both directions with an automatic sequencer using the ABI PRISM^{TM} Big Dye^{TM} Terminator cycle (First BASE Laboratories, Salangor, Malaysia). Sequences were viewed using Chromas (http://technelysium.com.au). The sequence of predicted SFP from KD and RH were compared using ClustalW program available at The European Bioinformatics Institute, EMBL-EBI (http://www.ebi.ac.uk/Tools/msa/clustalw2) to identified DNA polymorphism at 25-bp probe region.

6. Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of SFP-containing genes

KD and IL162 were used as the plant materials for the expression analysis of SFP-containing genes by RT-PCR. Fifteen SFP-containing genes that have been confirmed for their sequence polymorphism were investigated. Total RNA was isolated from leaves of 2-week-old seedling of the two lines by using TRIzol® RNA Isolation reagents (Life Technologies, Carlsbad, CA, USA). The purified RNA was reverse-transcribed into cDNA by using ImProm-II^{TM} Reverse Transcription System (Promega, Madison, WI, USA) and the resulted cDNA products were PCR amplified by using KAPA 2G Robust HS PCR Kit with dNTPs (KAPA Biosystems, MA, USA) according to the manufacturer’s instructions. All the RT-PCR assays were carried out in triplicate.

Part II: Genomic and functional analysis of rice terpene synthase (*TPS*) gene, LOC_Os04g27430

1. Plant materials

Resistance to BPH was used as the basis for a study of the genomic level and expression of the Os04g27430 gene in 18 rice varieties/ lines. The BPH-resistant rice varieties used were: Mudgo (contained *Bph1*), ASD7 (contained *bph2*), RH (contained *Bph3*, *Bph17*), PTB33 (contained *bph2*, *Bph3*), ARC10550 (contained *bph5*), Pokkali (contained *Bph9*) and Phitsanulok2 (PL2) (contained *Bph3*) (Jairin et al. 2007a, Jairin et al 2007b, Jena and Kim 2010, Nemoto et al. 1989, Sun et al. 2005, unpublished data). The ILs of KD which inherited BPH resistance from RH were: UBN3078-101-342-4-143 (IL143), IL162, IL302 and IL308 (Jairin et al. 2009). The BPH moderately susceptible rice variety was Pinkaset3 (PK3) (unpublished data). The BPH-susceptible rice varieties were Pinkaset2 (PK2), Homchonlasit (HCS), Riceberry (RBR), Jaohomnin (JHN) and KD (unpublished data). Two standard BPH susceptible rice varieties, TN1 and Nip, were also used as control.

2. Insect and BPH treatment

The BPH population collected from Ubon Ratchathani province (UBN_BPH) was used for BPH feeding experiments in this study. The insects were reared on rice cultivar TN1 in plastic cages (30 × 30 × 40 cm^3). The rice plants were maintained in a control climate room at 25 ± 2ºC and 12-hr light-dark period. The pre-germinated seeds of the test varieties/ lines were individually sown in 7×24 m^2 plots. Twenty days after sowing, the seedlings were transfer into the BPH-testing cages and infested with the third to forth instar nymphs of BPH at a number of ten nymphs per seedling for two days and the plant tissues were collected for total RNA isolation. For the control plants, the seeding was transfer into another cage without BPH infestation.

3. RT-PCR analysis of *TPS* genes

KD and IL308 were used as the plant materials for the expression analysis of Os04g27430 and other rice *TPS* genes by RT-PCR. Three *TPS* genes (Os04g27430, Os07g11790 and Os08g07100) were investigated. Rice plants were infested by BPH for 1, 2, 3, 4 and 8 days respectively with 3 and 8 days control condition. Total RNA was obtained by using TRIzol® RNA Isolation reagents (Life Technologies, Carlsbad, CA, USA). The purified RNA was reverse-transcribed into cDNA by using ImProm-IITM Reverse Transcription System (Promega, Madison, WI, USA) and the resulted cDNA products were PCR amplified by using KAPA 2G Robust HS PCR Kit with dNTPs (KAPA Biosystems, MA, USA) according to the manufacturer’s instructions. All the RT-PCR assays were carried out in triplicate.

4. Expression of Os04g27430 in response to abiotic stresses

RH was used as the plant material for the expression analysis of Os04g27430 in response to abiotic stresses by RT-PCR. Two-week-old RH seedlings were subjected to several stresses. For SA and MeJA, the seedlings were sprayed with 1 mM and 250 µM salicylic acid and methyl jasmonate, respectively; for the salt treatment, the seedlings were transplanted into 150 mM NaCl solution; and for wounding, the seedlings were cut into small pieces in distilled water. Total RNA was isolated by using TRIzol® RNA Isolation reagents (Life Technologies, Carlsbad, CA, USA). The purified RNA was reverse-transcribed into cDNA by using ImProm-IITM Reverse Transcription System (Promega, Madison, WI, USA) and the resulted cDNA products were PCR amplified by using KAPA 2G Robust HS PCR Kit with dNTPs (KAPA Biosystems, MA, USA) according to the manufacturer’s instructions. All the RT-PCR assays were carried out in triplicate.

5. Genomic and full-length cDNA re-sequencing of Os04g27430

Sequence data of Os04g27430 gene from the Rice genome Annotation Project (http://rice.plantbiology.msu.edu) was used for primer design to cover the whole genomic region of the gene by Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) (primer name; Os04g27430_1 to Os04g27430_8 in Appendix Table A1). Genomic DNA from KD, RH and IL162 were used as the template for genomic amplification of the Os04g27430 gene and the PCR products were subject to DNA sequencing by the ABI PRISM^{TM} Big Dye^{TM} Terminator cycle (First BASE Laboratories, Salangor, Malaysia). Sequencing results of the gene from each rice variety were assembled by the CAP3 sequence assembly program (Huang and Madan 1999). Total RNA of rice plants feed by BPH for two days was isolated from KD, RH and IL162 by TRIzol® reagent (Life Technologies, Carlbad, CA, USA). Overlapping partial cDNA fragments of the Os04g27430 gene were amplified by RT-PCR using 2 overlapping primer pairs (primer name; Os04g27430_exon 1-4 and Os04g27430_exon 3-6 in Appendix Table A1). The resulting cDNA products were sequenced and assembled by the same methods as genomic fragments. The protein a sequence of re-sequenced cDNA was translated by using “translate tools” available at ExPASy Bioinformatics Resource Portal (http://www.expasy.org).

6. Sequence analysis at 5’upstream region and exon 5 of the gene Os04g27430

Genomic DNA was isolated from the rice varieties/ lines tested, the 5’ upstream region of the Os04g27430 gene was amplified using primer pairs Os04g27430_8 and cDNA of the expressed Os04g27430 was amplified by RT-PCR using primer pairs Os04g27430_exon 3-6(Appendix Table A1). PCR products of genomic and cDNA were sequenced (First BASE Laboratories, Salangor, Malaysia), and the sequences of all rice varieties/ lines were compared by the ClustalW program.

7. Phylogenetic analysis

A set of monoterpene synthase (*MTPS*), sesquiterpene synthase (*STPS*) and diterpene synthase (*DTPS*) genes from eight higher plant genomes (*Zea mays*, *Artemisia annua*, *Oryza sativa*, *Zingiber zerumbet*, peppermint tree (Mentha x piperita), *Gossypium* hirsutum, *Arabidopsis thaliana*, *Lavandula angustifolia*) was collected using the rice Os04g27430 protein sequence as query to perform BLASTP searches against the NCBI database. Multiple sequence alignments and phylogenetic trees were analyzed using a robust phylogenetic tree analysis web service (http://www.phylogeny.fr) with the “One click” mode. This mode contained multiple sequences aligned using the MUSCLE program, the aligned sequences were curate using GBLOCK, the phylogeny was identified using PhyML and tree rendered using TreeDyn (Dereeper et al., 2008).

8. Antixenosis of BPH feeding preference (AFP) test

Two-week-old seedling of test couple plants were introduced into the test 6-inch-tall plastic cups covered with fine, light transmitting plastic. Each rice plant was supported on spongy merged with distilled water. A total of eight cups were used for each pair of tested genotypes. To observe the host selection of the BPH, a total of 10 second- and third-instar BPH nymphs were placed in each cup and allowed to choose host plants on which to feed and reproduce over a 120-h period. The BPH insects that settled on each plant were counted at 1, 2, 3, 4, 5, 6, 7, 8, 24, 48, 72, 96 and 120 h after release.

RESULTS AND DISCUSSION

Results

Part I: Single feature polymorphism (SFP) mapping of isogenic rice lines

1. Hybridization, data quality and SFP prediction

The biotin labeled probes generated from labeling of genomic DNA was hybridized to Affymetrix rice expression array. Following hybridization the preliminary data quality was assessed from GCOS1.3 software (Affymetrix) generated expression report. The average background, noise (RawQ) and the call rate was comparable between the two rice samples, KD and ILs, and also between their biological replicates (data not shown). However to get better assessment of data quality the raw intensity data of only perfect match (PM) probes of rice samples, KD and ILs, were log2 transformed and study by density plot (Figure 2A) and pair-wise scatter plots (Figure 2B) respectively. The results obtained from density plot indicated no major deviations as most of features/probes have log2 intensity values between 6 and 9 with the average log2 intensity of about 8. Moreover only few features/probes have log2 intensity less than 6 and more than 10. For scatter plot study 12000 randomly chosen features were plotted against each other for all pair-wise combinations. No major variation was observed between biological replicates of KD as most of the features were falling along the diagonal. However there are some variation were observed between biological replicates of ILs. These variations may affect the quality of predicted SFPs.

SFPs were called by using the siggene package (www.bioconductor.org) and SAM procedure available in the RiceGeneThresher database (Table 1 and Figure 3). SAM procedure allows the users to choose the delta value, a threshold for the SAM d-statistics, so as to get a suitable number of significant SFPs with a tolerable false discovery rate (FDR) for future study. In this study, delta value of 2.0 was chosen to get the predicted 157 SFPs with 30% FDR (Appendix Table A2).

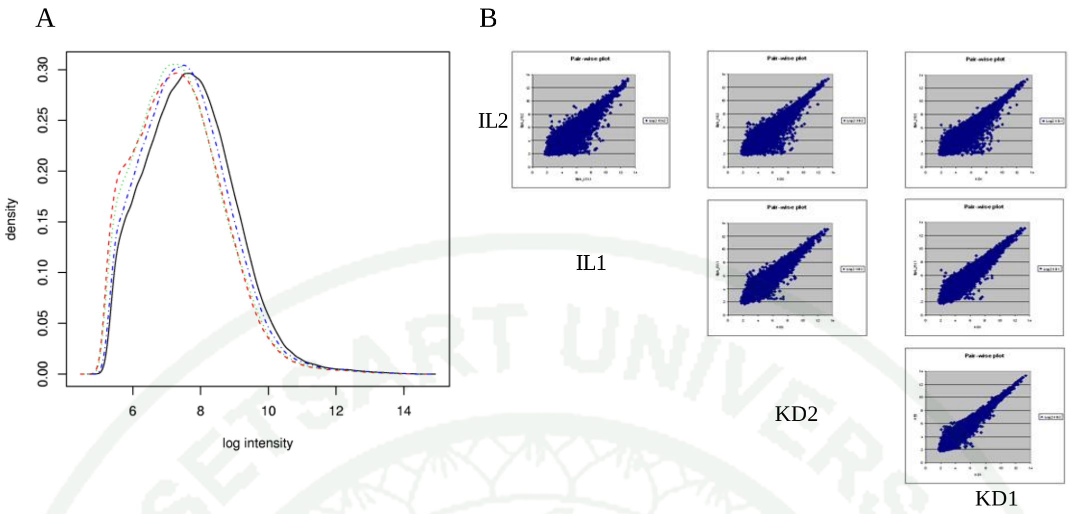


Figure 2 Assessment of the data quality for genomic DNA hybridization of Affymetrix rice genome array. A) Density plots for the raw PM probes intensity data. B) Pair-wise scatter plot for the raw PM probes intensity data across all arrays.

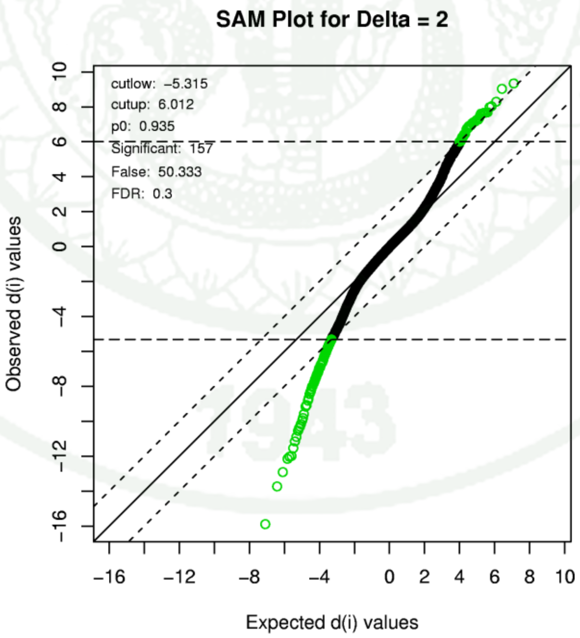


Figure 3 SAM plot of normalized data for KD&IL pair. Observed d-statistics (y-axis) is plotted against the expected d-statistics (x-axis) as determined by permutations and SFPs exceeding the threshold are shown in gray

Table 1 Number of gene-chip predicted SFPs called at different threshold (delta) in datasets. p0 = the prior probability of the proportion of SFP in the null datasets; Called = the number of SFP at each threshold; False = the number of SFP in the mean permuted datasets; FDR = false discovery rate

Delta	p0	FALSE	Called	FDR
0.1	0.935	17576	25331	0.648
1.1	0.935	163.167	500	0.305
2	0.935	50.333	157	0.3
3	0.935	10	42	0.223
4	0.935	3.833	21	0.171
4.9	0.935	2.333	14	0.156
5.9	0.935	1.167	7	0.156
6.9	0.935	0.333	2	0.156
7.8	0.935	0.167	1	0.156
8.8	0.935	0	0	0

2. Genomic location and functional classification of predicted SFPs

Only 99 SFPs had unique locations in the rice nuclear genome; the remaining 58 SFPs were located in multiple locations, no locations, or in organellar genomes (Appendix Table A3). The 99 SFPs were located on 84 annotated genes throughout the rice genome (Figure 4). There is no SFP was found on chromosome 5, 7 and 11. Five SFPs were located on chromosome 6 and were all outside the critically *Bph3* mapped location (Jairin et al. 2007a). By contrast, half of the SFPs were located on chromosome 4 (Appendix Table A4), where another BPH resistance gene from RH (*Bph17*) as well as other BPH resistance genes (*Bph12(t)*, *Bph15*, *Bph20(t)*, *Qbph2*, and *Qbph4*) have been previously mapped (Sun et al. 2005, Yang et al. 2002, Yang et al. 2004, Rahman et al. 2009, Huang et al. 2001, Liu et al. 2009).

The SFP-containing genes were classified into various functional groups (Figure 5). The largest group contained genes with unknown functions such as expressed proteins, hypothetical proteins, and uncharacterized proteins (Appendix Table A5). Transposons and retrotransposons formed the second largest group. The most significant finding was the identification of 10 genes that encode metabolic enzymes in the third most abundant group, which included three genes encoding terpene synthases (*TPS*). These enzymes are involved in the biosynthesis of secondary metabolites known as terpenoids, a large group of volatile compounds involved in defense mechanisms against plant herbivores (Schnee et al. 2006, Yuan et al. 2008). The fourth most abundant group included seven R gene-like sequences on chromosomes 3, 4, 8, and 10. These findings suggest that several minor quantitative trait loci (QTLs) may strengthen *Bph3* in terms of stable BPH resistance in RH and ILs. The last three groups contained genes involved in protein phosphorylation processes, transcription factors, and transporters.

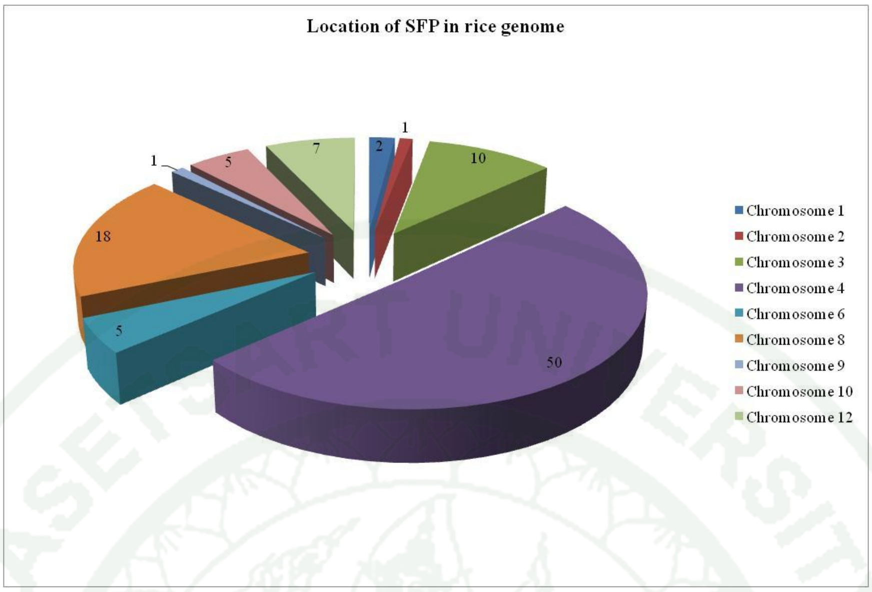


Figure 4 Pie chart depicting the chromosomal location of 99 predicted unique SFPs. Numeric characters in the pie charts indicate the number of SFPs or genes located or classified in that chromosome.

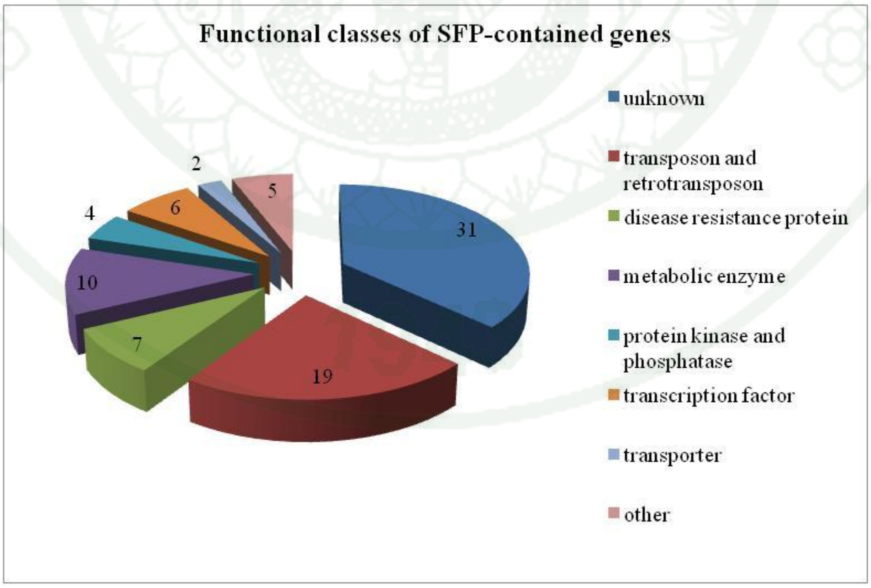


Figure 5 Pie chart showing the functional classification of 84 SFP-containing genes. Numeric characters in the pie charts indicate the number of SFPs or genes located or classified in that class.

3. SFP varidation by sequence comparison

To test the sensitivity of gene chip predicted SFPs data sequence comparison and normalized hybridization intensity fold change were studied. As no sequence information was available for rice varieties KD and RH; the donor of ILs, so fragments flanking the SFPs were amplified from their respective genome by PCR and their sequences was compared to verify polymorphism.

A total of 87 predicted SFPs were validated by comparing the hybridization intensity fold change of each probe with the results of sequence comparisons and PCR amplifications of the fragments flanking the SFPs. The hypothesis was stated that if sequence from respective genome identical to sequence on the gene chip, hybridization intensity must higher than sequence from respective genome which contains polymorphism with sequence on the gene chip.

3.1 Identification of 69-bp deletion in plastid genome of KD

Thirteen of 157 predicted SFPs were located in plastid genome (Appendix Table A3). SFP number 1, 4, 5, 6, 15, 34, 49, 72, 82 and 120 came from the probe set OsAffx.32348.1.S1_at while SFP number 9, 28 and 65 came from the probe set OsAffx.32348.1.A1_x_at respectively. These two probe sets aligned overlapping on the rice plastid genome (Os_Plas_orf100). When PCR fragments containing these SFPs were amplified from genomic DNA of KD and RH, it was found that the KD fragment was smaller than the RH fragment (data not shown) and sequence comparison revealed 69-bp deletion in KD plastid genome (Figure 6). This 69-bp deletion in KD's plastid genome caused hybridization intensity of these features/probes in KD arrays less than hybridization intensity of ILs arrays with the hybridization fold change from 65 to 666 times (Table 2).

3.2 Identification of potential deletion in KD/RH genome

To validate the predicted SFPs, PCR amplification of DNA fragments harboring 25-bp feature/probe region of predicted SFPs was performed. However it was found that in some amplification cases the fragments were unable to be amplified from KD or RH genomes (data not shown). This may be due to there are deletion events at those feature/probe region in KD or RH genome. However since these deletion events were not proofed by sequence comparison yet they were called as “potential deletion”. In this study, 34 and 15 potential deletions were identified from KD and RH genome respectively (Table 3 and 4). These potential deletions caused hybridization intensity in respective genome less than another. The potential deletion in the KD genome caused hybridization fold change from 10 to 340 times and the potential deletion in the RH genome caused hybridization fold change from 14 to 114 times respectively.

3.3 Identification of sequence polymorphism in predicted SFPs

Twenty-four DNA fragments harboring predicted SFPs were amplified from KD and RH genomic DNA. DNA sequences of these fragments were compared together with the feature/probe sequences of Nip variety which was used as sequence of the features/probes on the array and sequence polymorphisms were identified (Table 5). These polymorphisms including single nucleotide polymorphism (SNP), insertion and deletion (indel) in which SNP caused less hybridization fold change than indel polymorphism.

```
                         anti_SFP65:TGTTCTTATACCCATGCAATAGAGA
                            anti_SFP28:TCTTATACCCATGCAATAGAGAGCG
                               anti_SFP09:TATACCCATGCAATAGAGAGCGAGT
                                     SFP120:TACCCATGCAATAGAGAGCGAGTGG
                           SFP82:TTTTGTTCTTATACCCATGCAATAG
                                    SFP72:TATACCCATGCAATAGAGAGCGAGT
                         SFP49:ATTTTTGTTCTTATACCCATGCAAT
                              SFP34:TGTTCTTATACCCATGCAATAGAGA
                                  SFP15:CTTATACCCATGCAATAGAGAGCGA
                               SFP06:GTTCTTATACCCATGCAATAGAGAG
                     SFP05:TCCTATTTTTGTTCTTATACCCATG
                    SFP04:ATCCTATTTTTGTTCTTATACCCAT
                       SFP01:CTATTTTTGTTCTTATACCCATGCA
KD          TACTTTTTTTCA---------------------------------------------------------------------TTTTTCCCTTAA
Rathu       TACTTTTTTTCAGAATCCTATTTTTGTTCTTATACCCATGCAATAGAGAGCGAGTGGGAAAAGGGAGGTTACTTTTTTTCATTTTTCCCTTAA
            ************                                                                     ************
```

Figure 6 Identification of 69-bp deletion I plastid genome of KD

Table 2 SFP validation by comparison of hybridization fold change and polymorphism detected in the probe region between KD and RH: identification of 69-bp deletion in KD’s plastid genome; ILs = hybridization intensity of ILs, KD = hybridization intensity of KD, FC = hybridization fold change

SFP no.	AffyInfo	ILs	KD	FC	polymorphism
1	OsAffx.32348.1.S1_at3	1,524.6	4.2	362.8	deletion
4	OsAffx.32348.1.S1_at1	813.7	4.4	184.1	deletion
5	OsAffx.32348.1.S1_at2	621.0	4.7	131.2	deletion
6	OsAffx.32348.1.S1_at7	3,464.6	5.2	666.1	deletion
9	OsAffx.32348.1.A1_x_at1	4,521.0	13.4	336.7	deletion
15	OsAffx.32348.1.S1_at8	7,660.5	11.5	666.9	deletion
28	OsAffx.32348.1.A1_x_at2	1,943.7	17.3	112.5	deletion
34	OsAffx.32348.1.S1_at6	2,751.2	9.0	307.3	deletion
49	OsAffx.32348.1.S1_at4	1,532.0	6.7	229.2	deletion
65	OsAffx.32348.1.A1_x_at3	838.0	8.8	95.4	deletion
72	OsAffx.32348.1.S1_at9	5,981.7	26.4	226.5	deletion
82	OsAffx.32348.1.S1_at5	1,154.8	6.0	193.4	deletion
120	OsAffx.32348.1.S1_at10	5,674.6	38.2	148.7	deletion

Table 3 SFP validation by comparison of hybridization fold change and polymorphism detected in the probe region between KD and RH: identification of potential deletion in KD's genome.

SFP no.	AffyInfo	ILs	KD	FC	polymorphism
2	OsAffx.10840.1.S1_at2	2,678.6	8.2	326.5	KD potential deletion
7	OsAffx.19957.1.S1_at6	2,354.1	21.0	111.8	KD potential deletion
8	OsAffx.10840.1.S1_at5	315.1	4.8	65.3	KD potential deletion
10	OsAffx.19957.1.S1_at10	2,175.7	6.4	340.6	KD potential deletion
11	OsAffx.32252.1.A1_at3	753.7	5.2	145.7	KD potential deletion
12	OsAffx.32252.1.S1_at3	2,042.7	6.0	340.1	KD potential deletion
13	OsAffx.19957.1.S1_at11	1,485.3	10.7	139.1	KD potential deletion
16	OsAffx.32252.1.S1_at4	2,366.6	31.1	76.2	KD potential deletion
18	OsAffx.32252.1.S1_at9	4,134.0	106.8	38.7	KD potential deletion
20	OsAffx.10840.1.S1_at10	148.5	4.3	34.7	KD potential deletion
24	OsAffx.32252.1.A1_at11	1,524.7	29.5	51.7	KD potential deletion
27	OsAffx.32252.1.S1_at10	3,622.8	71.7	50.5	KD potential deletion
31	OsAffx.13680.1.S1_at10	337.8	18.4	18.4	KD potential deletion
37	OsAffx.32252.1.A1_at9	1,209.3	38.7	31.2	KD potential deletion
50	OsAffx.3783.1.S1_at10	159.2	4.3	36.8	KD potential deletion
55	Os.11685.1.S1_at11	339.0	12.7	26.7	KD potential deletion
56	OsAffx.17249.1.S1_at9	617.1	22.5	27.4	KD potential deletion
60	OsAffx.32252.1.A1_at4	2,907.2	28.7	101.4	KD potential deletion
66	Os.11685.1.S1_at10	215.0	4.7	45.4	KD potential deletion
75	OsAffx.32252.1.S1_at11	4,784.7	144.6	33.1	KD potential deletion
78	OsAffx.32252.1.A1_at7	2,618.5	69.2	37.8	KD potential deletion
83	OsAffx.29436.1.S1_at1	151.6	5.5	27.7	KD potential deletion
84	OsAffx.3783.1.S1_at7	130.6	5.6	23.3	KD potential deletion
95	OsAffx.32252.1.S1_at5	1,364.5	106.8	12.8	KD potential deletion
96	Os.8888.1.S1_at11	60.3	5.6	10.8	KD potential deletion
110	OsAffx.10840.1.S1_at7	671.0	9.6	70.1	KD potential deletion
118	OsAffx.10840.1.S1_at1	922.8	14.5	63.8	KD potential deletion
121	OsAffx.32252.1.S1_at2	2,976.7	79.3	37.5	KD potential deletion
133	OsAffx.29436.1.S1_at9	110.7	10.7	10.4	KD potential deletion
134	OsAffx.29436.1.S1_at3	113.2	6.2	18.1	KD potential deletion
137	OsAffx.32252.1.S1_at6	1,096.1	38.9	28.2	KD potential deletion
141	Os.8888.1.S1_at8	98.2	5.3	18.6	KD potential deletion
145	OsAffx.32252.1.A1_at1	1,817.0	27.6	65.8	KD potential deletion
150	OsAffx.32252.1.S1_at8	1,552.3	150.8	10.3	KD potential deletion

Table 4 SFP validation by comparison of hybridization fold change and polymorphism detected in the probe region between KD and RH: identification of potential deletion in RH’s genome. ILs = hybridization intensity of ILs, KD = hybridization intensity of KD, FC = hybridization fold change

SFP no.	AffyInfo	ILs	KD	FC	polymorphism
17	Os.18164.1.S1_at11	5.4	255.8	47.5	RH potential deletion
21	Os.52198.3.S1_at9	5.8	669.6	114.8	RH potential deletion
26	OsAffx.25899.1.S1_at4	5.0	116.7	23.3	RH potential deletion
29	Os.51195.1.S1_at11	5.2	227.0	43.9	RH potential deletion
33	Os.54321.1.S1_at8	9.1	412.5	45.3	RH potential deletion
42	Os.27327.1.S1_at8	9.4	243.2	25.8	RH potential deletion
47	OsAffx.25981.1.S1_at7	5.6	115.1	20.7	RH potential deletion
52	OsAffx.13771.1.S1_x_at10	6.0	150.1	25.2	RH potential deletion
53	Os.53255.1.S1_at2	17.3	439.2	25.4	RH potential deletion
58	Os.26445.2.S1_at1	8.8	135.6	15.4	RH potential deletion
59	Os.53255.1.S1_at1	7.4	246.0	33.5	RH potential deletion
61	Os.27327.1.S1_at11	5.4	488.5	90.2	RH potential deletion
73	OsAffx.3920.1.S1_at9	4.6	108.5	23.6	RH potential deletion
77	OsAffx.3920.1.S1_at8	7.5	105.6	14.0	RH potential deletion
89	Os.52198.3.S1_at8	6.4	272.2	42.8	RH potential deletion

Table 5 SFP validation by comparison of hybridization fold change and polymorphism detected in the probe region between KD and RH: identification of sequence polymorphism between KD, RH and Nip

SFP	AffyInfo	ILs	KD	FC	polymorphism
22	OsAffx.32291.1.A1_at10	2,189.7	33.2	66.0	TTTTGCAGCGAGTCAAGCGTAATAT RH TTTTGCAGCGAGTCAAGCGTAATAC KD TTTTGCAGCG---------TAATAC ********** *****
29	Os.51195.1.S1_at11	5.2	227.0	43.9	GCATGCCAGGGTTCATCGGTAACGT RH GCATGCCAGGGTTCATCGGCAACGT KD GCATGCCAGGGTTCATCGGTAACGT ******************* *****
32	OsAffx.32326.1.S1_x_at10	140.0	4.8	28.9	CTTAAAATATCAGAAACGGTTCTTG RH CTTAAAATATCAGAAACGGTTCTTG KD CTTAAAATATCAGAAACTGTTCTTG ***************** *******
38	OsAffx.3839.1.S1_at11	7.4	191.3	26.0	CCCTCGTAAATGTCCCTATCAG--AAG RH CCCATGTAAATTCCCCTGCAATTCAAG KD CCCTCATAAATGTCCCTATCAG--AAG *** ***** **** * ***
46	OsAffx.3768.1.S1_at5	464.0	29.7	15.6	GTACCTATGTTTAGACCCAGACCAA RH GTACCTATGTTTAGACCCAGACCAA KD GTACCTATGTTTAGATCCAGACCAA *************** *********
48	Os.50641.2.S1_a_at11	197.6	8.8	22.4	GCGACGAGCCGACAGGTGACATTCT RH GCGACGAGCCGACAGGTGACATTCT KD GCGACGAGCCGACAGGTGACATTCT *************************
51	OsAffx.27426.2.S1_at3	4.8	162.8	33.9	CAGAACCTTTTACCTTCATGCATTG RH CAGAACCTTTTACCTTGATGCATTG KD CCTAACCTTTTACCTTCATGCATTG * ************* ********
55	Os.11685.1.S1_at11	339.0	12.7	26.7	GAAGATGCTCCCACAATAGATCATG RH GAAGATGCTCCCACAGTAGATCATG KD TGTGCTCCAACCACTATTGATCTTG * * * **** * **** **

Table 5 (continued)

SFP	AffyInfo	ILs	KD	FC	polymorphism
63	OsAffx.26123.1.S1_at8	6.4	155.5	24.4	GTTGACTTATCATATACCCTCTGTA RH GTTGATTTATCCTATACCCTCTGTA KD GTTGACTTATCATATACCCTCTGTA ***** ***** *************
64	Os.27099.1.S1_at10	20.1	260.7	13.0	TTTCCAGTTCTGTGCTAGGCCATGA RH TTTCCAGTTCTATTCTACGCCATGA KD TTTCCAGTTCTGTGCTAGGCCATGA *********** * *** *******
66	Os.11685.1.S1_at10	215.0	4.7	45.4	GGACTCAAGCTCAGTTCTAGGCACT RH GGACTCAAGCTCAGCTCTAGGCACT KD GGACTCAAGTTCAGTTCTTGGTACT ********* **** *** ** ***
68	OsAffx.13994.1.S1_at7	317.7	22.5	14.1	GAACCAGGCTCATTTCGAGGATCAT RH GAACCAGGCTCATTTCGAGGATCAT KD GAACCAGGCTCTCTTCGAGGATCGT *********** ********** *
71	Os.6048.1.A1_at11	6.5	129.8	19.8	ACTGTATTTAACCTCATCACGTGTA RH ACTGTATTTAACATCATCATGTGTA KD ACTGTATTTAACCTCATCACGTGTA ************ ****** *****
81	OsAffx.26013.1.S1_at6	206.6	18.3	11.3	GTTCGCTGATAGGTACAAGGCCACG RH GTTCGCTGATAGGTACAAGGCCACG KD GTTCGTTGATAGGTACAAGGCCACG ***** *******************
90	Os.49625.1.S1_at8	4.8	120.6	25.1	TTTCCCACTGAGTTACTTGTCTCTT RH TTTCCCACTGAGTTACATGTCTTTT KD TTTCCCACTGAGTTACTTGTCTCTT **************** ***** **
107	Os.44812.1.S1_at4	8.9	320.1	35.9	TGCTCTCTAAATTACAGCCGTTAGC RH TGCTCTCTAAGTTACAGCCGTTAGC KD TGCTCTCTAAATTACAGCCGTTAGC ********** **************
115	Os.10277.1.S1_at4	8.1	180.7	22.3	ACTATGCTCTCTCAGACAATGGGAA RH -----------------AATGGAA KD ACTATGCTCTCTCAGACAATGGGAA ********

Table 5 (continued)

SFP	AffyInfo	ILs	KD	FC	polymorphism
117	OsAffx.26190.1.S1_at2	5.2	111.5	21.5	TGAACTTTACCGTTTCCATGGCACT RH TGAACTTTACCGTTTCCATGGTACT KD TGAACTTTACCGTTTCCATGGCACT ********************* ***
125	OsAffx.18129.2.A1_at5	84.7	6.4	13.2	CAAAGGTGCGAATGATCTGGTAAAT RH CGAAGGTGCGAATGATCTGGTAAAT KD CGAAGGTGCGAATGATCTGGTAAAT * ***********************
127	OsAffx.13716.1.S1_at2	288.7	25.6	11.3	ATGAAGACCTTGCTCTCATCGTCAA RH ATGAAGACCTTGCTCTCATCGTCAA KD ATGAAGACCTTGCTCTCATCGTCAA *************************
129	Os.25535.1.S1_at8	80.5	10.9	7.3	TGGT-TCTTTTGTCATGCTGTGTTTG RH TGGT-TCTTTTGTCATGCTGTGTTTG KD TGGTGTCTTTTGTCATGCTGAGTTTG **** *************** *****
142	Os.12770.2.S1_x_at7	790.7	43.1	18.3	ATCCCATAT---ACATACACTGCACTAC RH ATCCCATAT---ACATACACTGCACTAC KD ATCCCATATTATACATACACTGCACTAC ********* ****************
148	Os.44984.1.S1_at11	34.9	4.9	7.1	TATTTGCCACTATTCATTTCTGATG RH TATTTGCCACTATTCATTTGTGATG KD TATTTGCCATTATTCATTTGTGATG ********* ********* *****
151	OsAffx.1869.2.S1_at9	2,779.3	313.1	8.9	GTCCGGGTCTAATTGGGCCATGCGA RH GTCCGGGTCTAATTGGGCCATGCGA KD GTCCGGG--------GGCCATGCGA ******* **********

4. Expression analysis of SFP-containing genes

A total of 15 genes were chosen from 24 validated SFP-containing genes in which an SNP or a small indel between KD and RH was identified (Table 5). RT-PCR expression analyses and the functional ontologies of 15 SFP-containing genes are shown (Figure 7). The Os08g31970 and Os04g27430 genes clearly exhibited differential expression under normal conditions, with no expression in the susceptible KD rice variety. In contrast, the remaining genes exhibited constitutive expression between KD and RH. The differential expression of Os08g31970 and Os04g27430, an NHL repeat-containing protein that plays a role in signal transduction and a *TPS* responsible for the biosynthesis of volatile compounds, respectively, was further verified. Os04g27670 and Os04g27720 (two SFP-containing *TPS* genes, Appendix Table A4) were also selected for a total of four genes that were evaluated in a two-day BPH feeding test using two-week-old seedlings and the UBN-BPH (Jairin et al. 2009). Interestingly, only Os04g27430 exhibited differential expression between the control and the BPH feeding condition in IL162; however, no change in expression levels was observed for Os08g31970 in IL162 (Figure 8). No expression was detected for the remaining two TPS genes in the rice plants, perhaps because the genes were not functional during the seedling stage or because they may be pseudogenes. The role of Os04g27430 in response to BPH attack was investigated in greater detail.

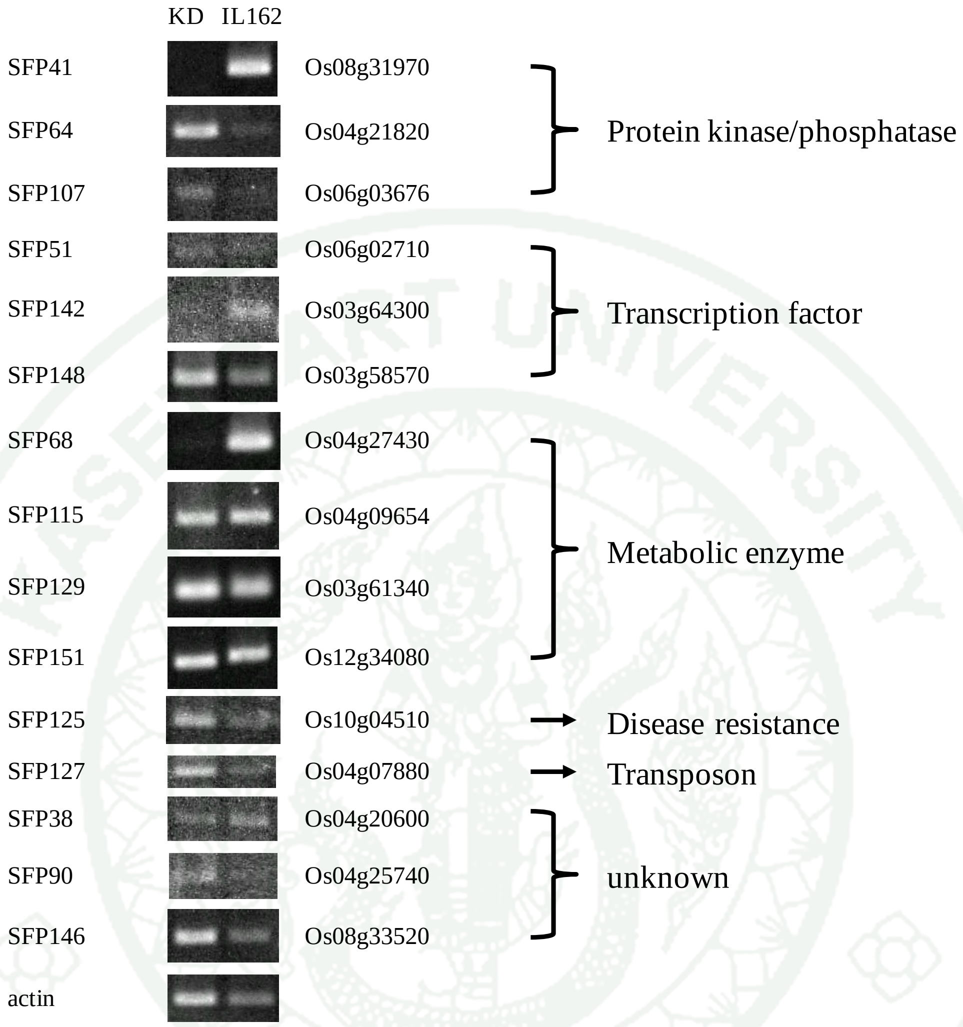


Figure 7 Expression analyses of SFP-containing genes by RT-PCR of rice variety KD and IL162. Actin was used as a positive control. The functional classes of these genes refer to the functional classes in Appendix Table A5.

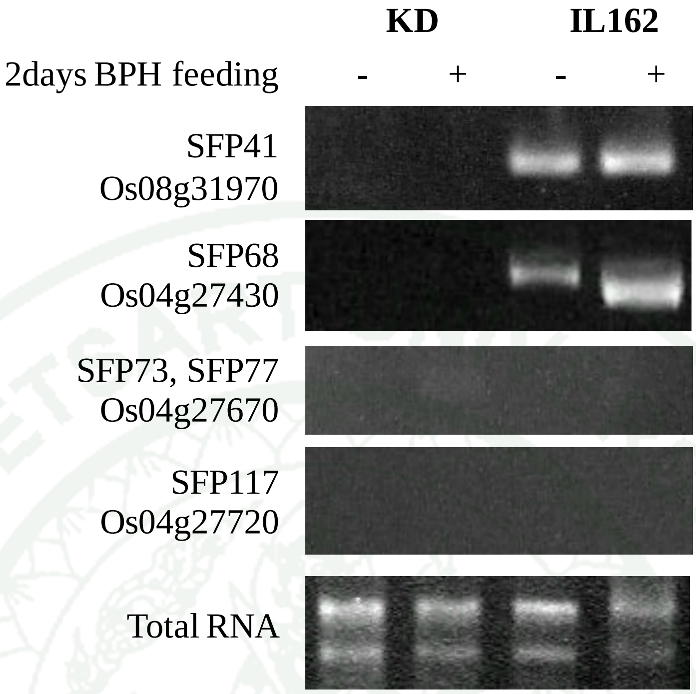


Figure 8 Expression analysis of rice plants following two days of BPH infestation (+) and control plants (-). The total RNA in each lane is represented by the amount of RNA used in the reaction.

Part II: Genomic and functional analysis of rice *TPS* gene, Os04g27430

1. Genomic, cDNA and amino acid sequence analysis of Os04g27430

The genomic region of Os04g27430 in RH, KD, and IL162 was sequenced and compared (accession nos. KC511049 – KC511051), and major polymorphisms were identified (Figure 9). Three indels were found to be located in intron 1 (645 bp) and intron 3 (679 and 32 bp, respectively). In the coding sequence, an in-frame 6-bp deletion was present in exon 2 in RH and IL162. More significantly, a 2-bp SNP was present in KD exon 5 with a strong correlation to a 21-bp deletion in its cDNA. Comparisons between the genomic DNA, cDNA, and predicted amino acid sequences (accession nos. KC511027 – KC511029) revealed a 21-bp deletion in exon 5 of the KD cDNA allele that resulted in the deletion of seven residues from the amino acid sequence in KD. The deduced amino acid sequences of the KD and RH alleles were translated, yielding protein sequences containing 500 and 505 residues, respectively. The conserved DDXXD motif that functions as a substrate binding site was present in both the KD and RH alleles (Figure 10A). The seven-amino-acid deletion was WxHQxxx, a signature motif in several plant *TPS* genes (Figure 10B). Based on the presence of this important deletion in KD, Os04g27430 mRNA may be subjected to post-transcriptional degradation, and the absence of this mRNA may responsible for the BPH susceptibility observed in the KD parent.

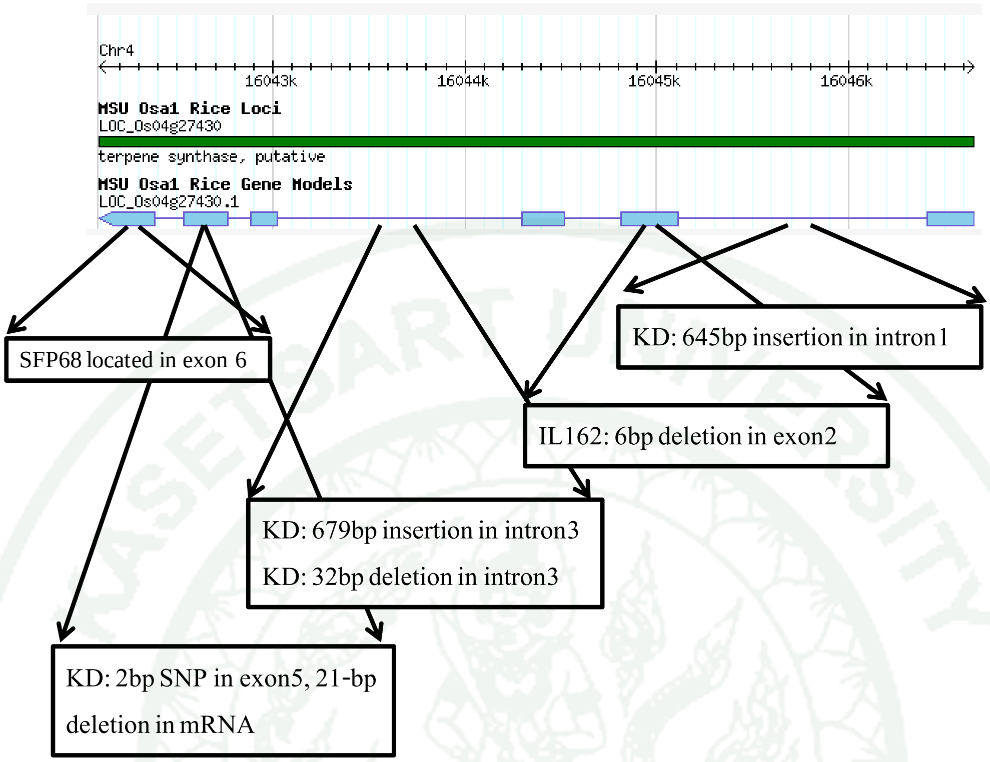
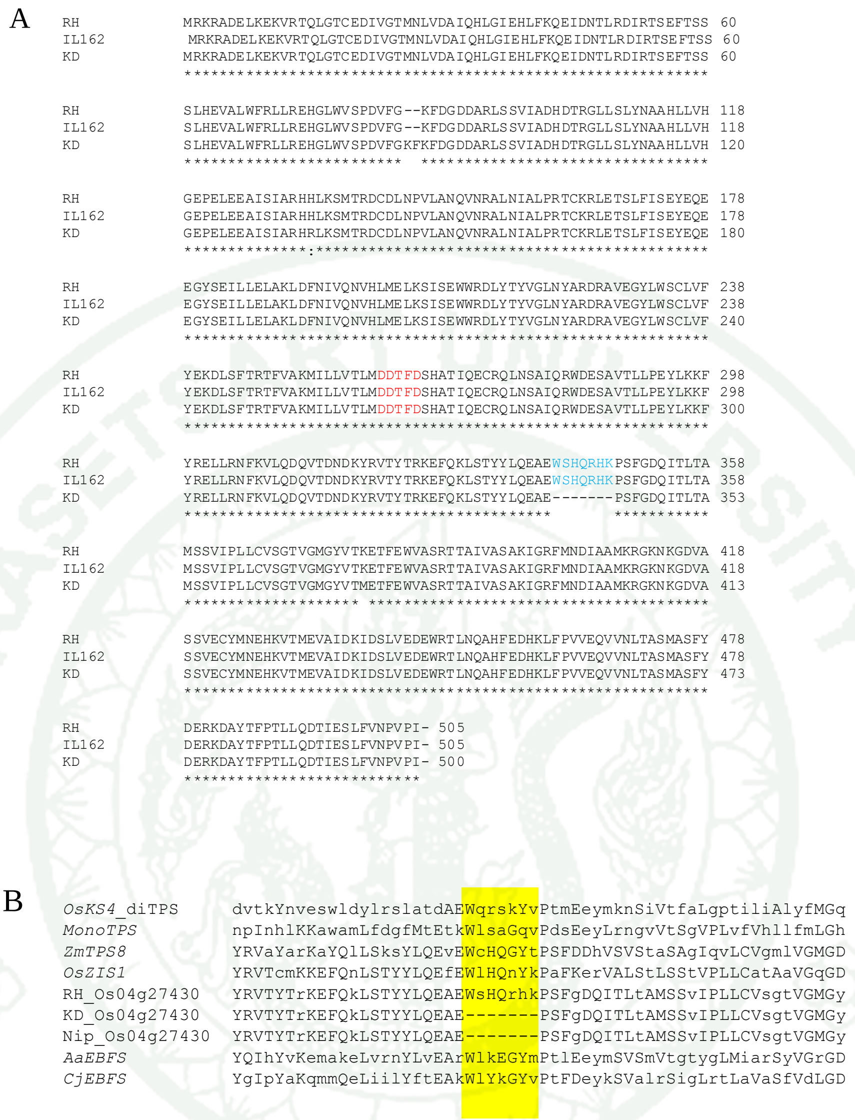


Figure 9 Gene construct of Os04g27430 from the Rice Genome Annotation Database (http://rice.plantbiology.msu.edu). Major polymorphisms between the KD and RH alleles (SNP or insertion/ deletion) are marked in horizontal boxes below the construct.



```
RH          MRKRADELKEKVRTQLGTCEDIVGTMNLVDAIQHLGIEHLFKQEIDNTLRDIRTSEFTSS 60
IL162       MRKRADELKEKVRTQLGTCEDIVGTMNLVDAIQHLGIEHLFKQEIDNTLRDIRTSEFTSS 60
KD          MRKRADELKEKVRTQLGTCEDIVGTMNLVDAIQHLGIEHLFKQEIDNTLRDIRTSEFTSS 60
            ************************************************************

RH          SLHEVALWFRLLREHGLWVSPDVFG--KFDGDDARLSSVIADHDTRGLLSLYNAAHLLVH 118
IL162       SLHEVALWFRLLREHGLWVSPDVFG--KFDGDDARLSSVIADHDTRGLLSLYNAAHLLVH 118
KD          SLHEVALWFRLLREHGLWVSPDVFGKFKFDGDDARLSSVIADHDTRGLLSLYNAAHLLVH 120
            *************************  *********************************

RH          GEPELEEAISIARHHLKSMTRDCDLNPVLANQVNRALNIALPRTCKRLETSLFISEYEQE 178
IL162       GEPELEEAISIARHHLKSMTRDCDLNPVLANQVNRALNIALPRTCKRLETSLFISEYEQE 178
KD          GEPELEEAISIARHRLKSMTRDCDLNPVLANQVNRALNIALPRTCKRLETSLFISEYEQE 180
            **************:*********************************************

RH          EGYSEILLELAKLDFNIVQNVHLMELKSISEWWRDLYTYVGLNYARDRAVEGYLWSCLVF 238
IL162       EGYSEILLELAKLDFNIVQNVHLMELKSISEWWRDLYTYVGLNYARDRAVEGYLWSCLVF 238
KD          EGYSEILLELAKLDFNIVQNVHLMELKSISEWWRDLYTYVGLNYARDRAVEGYLWSCLVF 240
            ************************************************************

RH          YEKDLSFTRTFVAKMILLVTLMDDTFDSHATIQECRQLNSAIQRWDESAVTLLPEYLKKF 298
IL162       YEKDLSFTRTFVAKMILLVTLMDDTFDSHATIQECRQLNSAIQRWDESAVTLLPEYLKKF 298
KD          YEKDLSFTRTFVAKMILLVTLMDDTFDSHATIQECRQLNSAIQRWDESAVTLLPEYLKKF 300
            ************************************************************

RH          YRELLRNFKVLQDQVTDNDKYRVTYTRKEFQKLSTYYLQEAEWSHQRHKPSFGDQITLTA 358
IL162       YRELLRNFKVLQDQVTDNDKYRVTYTRKEFQKLSTYYLQEAEWSHQRHKPSFGDQITLTA 358
KD          YRELLRNFKVLQDQVTDNDKYRVTYTRKEFQKLSTYYLQEAE-------PSFGDQITLTA 353
            ******************************************       ***********

RH          MSSVIPLLCVSGTVGMGYVTKETFEWVASRTTAIVASAKIGRFMNDIAAMKRGKNKGDVA 418
IL162       MSSVIPLLCVSGTVGMGYVTKETFEWVASRTTAIVASAKIGRFMNDIAAMKRGKNKGDVA 418
KD          MSSVIPLLCVSGTVGMGYVTMETFEWVASRTTAIVASAKIGRFMNDIAAMKRGKNKGDVA 413
            ******************** ***************************************

RH          SSVECYMNEHKVTMEVAIDKIDSLVEDEWRTLNQAHFEDHKLFPVVEQVVNLTASMASFY 478
IL162       SSVECYMNEHKVTMEVAIDKIDSLVEDEWRTLNQAHFEDHKLFPVVEQVVNLTASMASFY 478
KD          SSVECYMNEHKVTMEVAIDKIDSLVEDEWRTLNQAHFEDHKLFPVVEQVVNLTASMASFY 473
            ************************************************************

RH          DERKDAYTFPTLLQDTIESLFVNPVPI- 505
IL162       DERKDAYTFPTLLQDTIESLFVNPVPI- 505
KD          DERKDAYTFPTLLQDTIESLFVNPVPI- 500
            ***************************
```

```
OsKS4_diTPS      dvtkYnveswldylrslatdAEWqrskYvPtmEeymknSiVtfaLgptiliAlyfMGq
MonoTPS          npInhlKKawamLfdgfMtEtkWlsaGqvPdsEeyLrngvVtSgVPLvfVhllfmLGh
ZmTPS8           YRVaYarKaYQlLSksYLQEvEWcHQGYtPSFDDhVSVStaSAgIqvLCVgmlVGMGD
OsZIS1           YRVTcmKKEFQnLSTYYLQEfEWlHQnYkPaFKerVALStLSStVPLLCatAaVGqGD
RH_Os04g27430    YRVTYTrKEFQkLSTYYLQEAEWsHQrhkPSFgDQITLtAMSSvIPLLCVsgtVGMGy
KD_Os04g27430    YRVTYTrKEFQkLSTYYLQEAE-------PSFgDQITLtAMSSvIPLLCVsgtVGMGy
Nip_Os04g27430   YRVTYTrKEFQkLSTYYLQEAE-------PSFgDQITLtAMSSvIPLLCVsgtVGMGy
AaEBFS           YQIhYvKemakeLvrnYLvEArWlkEGYmPtlEeymSVSmVtgtygLMiarSyVGrGD
CjEBFS           YgIpYaKqmmQeLiilYftEAkWlYkGYvPtFDeykSValrSigLrtLaVaSfVdLGD
```

Figure 10 Amino acid sequence analysis. (A) Amino acid sequence alignment of the KD, RH, and IL162 alleles of Os04g27430. The conserved DDXXD domain found in the plant *TPS* genes is labeled in red, and the seven-amino-acid deletion in the KD allele is labeled in blue. (B) Consensus sequence detected in Os04g27430 and the *TPS* genes of rice and other plant species. *OsKS4* and *MTPS* are rice *TPS*s, *ZmTPS8* is a terpene synthase from *Zea mays* (NP_001105912), *OsZIS1* is a rice putative *zingiberene synthase I* (ACM41835), *AaEBFS* is the *(E)-β-farnesene synthase* from *Artemisia annua* (AAX39387), and *CjEBFS* is the *(E)-β-farnesene synthase* from *Citrus junos* (AAK54279).

2. Analysis of 5'upstream region of Os04g27430

The 5' upstream region of Os04g27430 in RH, KD, and IL162 was sequenced (accession nos. KC511031, KC527594, KC511035), and searching for transcription factor (TF) binding sites was performed (http://www.cbrc.jp/research/db/TFSEARCH.html). Three consensus elements for the transcription factors ATHB-1 (*Arabidopsis thaliana* homeobox protein 1), SBF-1 (silencer-binding factor 1), and P (maize activator P) were identified in this promoter region. Interestingly, the KD allele contained one SNP in each element. These SNPs led to the non-recognition of the ATHB-1 element (score = 0) and a decreased TF search score for the SBF-1 and P elements (scores = 92.5 and 86.5) (Figure 11). These three SNPs, particularly the SNP in the ATHB-1 element, may be responsible for the low expression levels of this gene in KD because the transcription factor cannot bind at the target site to enhance gene expression. Therefore, a sequence comparison of other BPH-resistant rice varieties is needed to further explore the understanding of how this gene is controlled.

```
RH      TTTCAGCAATTCTATATCTTTTATAATGTAGGTAGGGTACGTATATCATAGTTTTATTAT 60
IL162   TTTCAGCAATTCTATATCTTTTATAATGTAGGTAGGGTACGTATATCATAGTTTTATTAT 60
KD      TTTCAGCAATTCTATATCGTTTATAATGTAGGTAGGGTACGTATATCATAGTTTTATTAT 60
        ****************** *****************************************

RH      TTGCGTAGCCTAAATATTTGATGGACTTCCTACGTGATGAGATGATCACAAAAATTAACA 120
IL162   TTGCGTAGCCTAAATATTTGATGGACTTCCTACGTGATGAGATGATCACAAAAATTAACA 120
KD      TTGCGTAGCCTAAATATTTGATGGACTTCCTACGTGATGAGATGATCACAAAAATTAACA 120
        ************************************************************

RH      ATCAACATGTTCAGATTTGAATAAGCGCTTATATTCAAGCAGAATTAATGTGTACATATA 180
IL162   ATCAACATGTTCAGATTTGAATAAGCGCTTATATTCAAGCAGAATTAATGTGTACATATA 180
KD      ATCAACATGTTCAGATTTGAATAAGCGCTTATATTCAAGCAGAATTAATGTGTACATATA 180
        ************************************************************

RH      TTTCTGATGTTTTTCTTGCACATGGTATTAGTTCATGTAAGATTATCTTTCCTTTTTTTT 240
IL162   TTTCTGATGTTTTTCTTGCACATGGTATTAGTTCATGTAAGATTATCTTTCCTTTTTTTT 240
KD      TTTCTGATGTTTTTCTTGCACATGGTATTAGTTCATGTAAGATTATCTTTCCTTTTTTTT 240
        ************************************************************      TF search
                                                                          score
                                        ATHB-1
RH      CATAATTTCTGCATGTACTACTTTTGACAAATTAATTATTGCTAGAACTGTTTCATGAAT 300     87.2
IL162   CATAATTTCTGCATGTACTACTTTTGACAAATTAATTATTGCTAGAACTGTTTCATGAAT 300     87.2
KD      CATAATTTCTGCATGTACTACTTTTGACAAATTCATTATTGCTAGAACTGTTTCATGAAT 300     0
        *********************************.**************************

                                      SBF-1
RH      ATTGTAATGTTATTTCACAAAGCTTTGTGTGGTTAATAATCTCTTTTTGATAACTATAAT 360     98.1
IL162   ATTGTAATGTTATTTCACAAAGCTTTGTGTGGTTAATAATCTCTTTTTGATAACTATAAT 360     98.1
KD      ATTGTAATGTTATTTCACAAAGCTTTGCATGGTTAATAATCTCTTTTTGATAACTATAAT 360     92.5
        *************************** .*******************************

                               P
RH      AAATCAAACTCGTGAATCCAACCAACCAGAGATCGGAAGAATGG 404                     98.2
IL162   AAATCAAACTCGTGAATCCAACCAACCAGAGATCGGAAGAATGG 404                     98.2
KD      AAATCAAACTCGTGAATCCAACTAACCAGAGATCGGAAGAATGG 404                     86.5
        ********************** *********************
```

Figure 11 Genomic sequence alignment of the 5’ region of Os04g27430. The 3 TF elements are marked in red, and the SNPs of the KD allele are marked in blue. The TF search score for each allele is indicated at the far right of the alignment.

3. Phylogenetic analysis

The amino acid sequences of 10 rice *TPS*s (Cheng et al. 2007, Prisic et al. 2004, Sakamoto et al. 2004, Xu at al. 2004, Yuan et al. 2008) and *TPS*s from other plant species were included in the phylogenetic analysis of the RH allele of Os04g27430, which revealed that Os04g27430 clustered with the *sesquiterpene synthase* (*STPS*) group (Figure 12). Os04g27430 was most similar to *OsZIS1*, *OsZIS2,* and *OsTPS13*. *OsZIS1* and *OsZIS2* are putative *zingiberene synthase* genes whose function has not been confirmed experimentally.

OsTPS13 is an *STPS* that catalyzes the formation of the sesquiterpene alcohol (E, E) farnesol (Cheng et al. 2007). The gene was identified from methyl jasmonate (MeJA)-treated rice seedlings. However, this gene was constitutively expressed in two-week-old KD and IL308 seedlings under both control and BPH feeding conditions for 1, 2, 3, 4, and 8 days (Figure 13). In addition to BPH feeding, 24 and 72 hr of MeJA and wound stress also induced Os04g27430 expression (Figure 14). This discovery suggests that *OsTPS13* is not a BPH feeding-inducible *STPS* and that Os04g27430 expression is induced by both BPH feeding and other stresses, such as MeJA and wounding.

Another *STPS* gene (Os08g07100) that is reportedly induced by BPH (Cho et al. 2005) was not polymorphic between KD and IL308 at the expression level in the present study (Figure 13). This gene was induced by BPH feeding in both rice strains. Moreover, the gene was induced by BPH feeding and by the fall army worm (Yuan et al. 2008), suggesting that the gene plays a common role in the response to herbivore attacks on rice plants.

Os04g27430 is likely the *STPS* that functions as *zingiberene synthase*, which catalyzes the formation of a number of sesquiterpene products (Iijima et al. 2004). Sesquiterpene volatile compounds are the potential products of this gene and may play a role in BPH resistance mechanisms in RH and ILs.

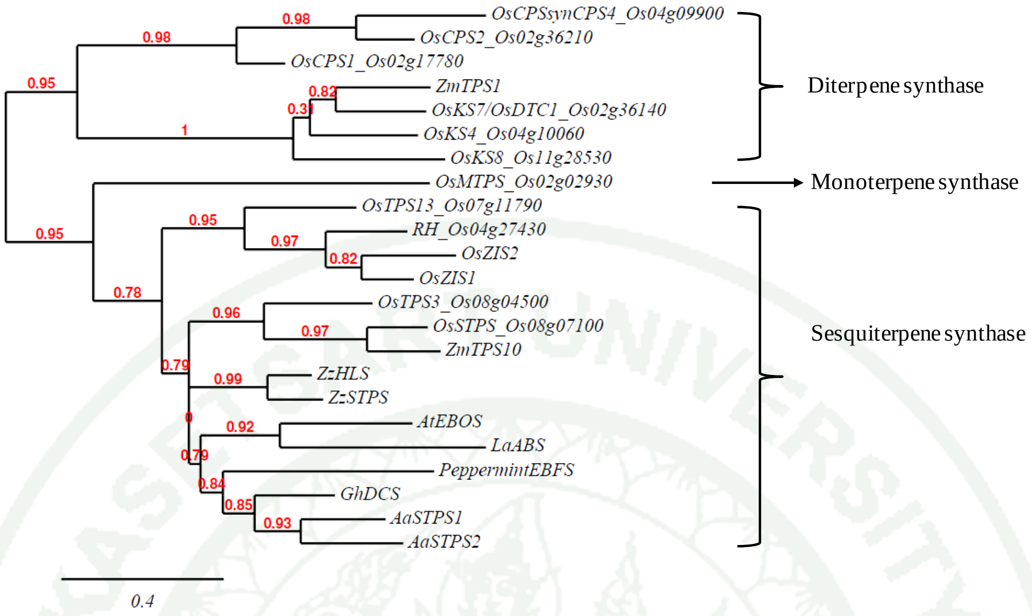


Figure 12 Phylogenetic analysis based on the degree of sequence similarity between Os04g27430 and other rice *TPS* genes. Rice gene identities are based on the gene name in the reference and the rice genome database; the prefix 'LOC_' is omitted. *ZmTPS1* and *ZmTPS10* are *TPS* from *Zea mays* (AAO18435, AAX99146), *AaSTPS1* and *2* are *sesquiterpene cyclases* from *Artemisia annua* (CAC12732, AAG24640), *OsZIS1* and *2* are rice putative *zingiberene synthases* (ACM41835, ACM41834), *ZzAHS* and *ZzSTPS* are *α-humulene synthase* and *sesquiterpene synthase* 4 from *Zingiber zerumbet* (BAG12020, BAG50434), PeppermintEBFS is *(E)-β-farnesene synthase* from peppermint tree (Mentha x piperita) (AAB95209), *GhDCS* is a *δ-cadinene synthase* from *Gossypium hirsutum* (AF270425), *AtEBOS* is *(E)-β-ocimene synthase* from *Arabidopsis thaliana* (NP_567511.3), and *LaABS* is *α-bergamotene synthase* from *Lavandula angustifolia* (ABB73046).

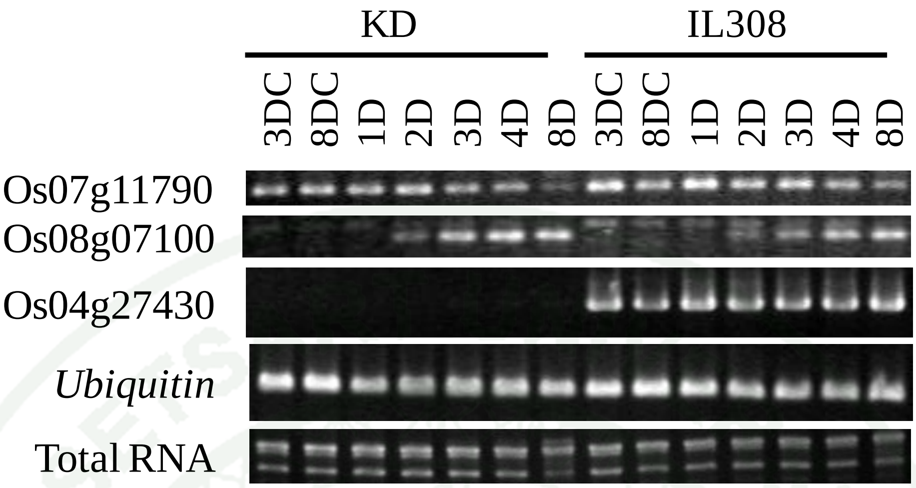


Figure 13 Expression analysis of *STPS* genes. The expression of three *STPS*s in KD and IL308 under control (3DC and 8DC) and BPH feeding (1D, 2D, 3D, 4D, and 8D) conditions; D = day(s). *Ubiquitin* and total RNA were used as positive controls for RT-PCR.

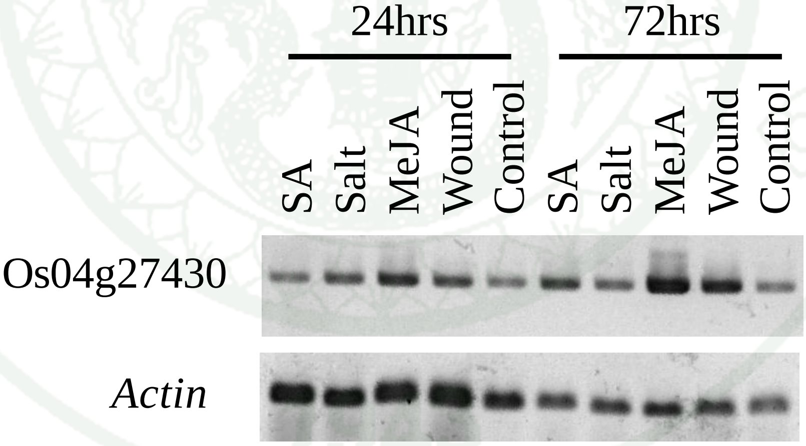


Figure 14 Expression of Os04g27430 in response to stress conditions by RT-PCR analysis. *Actin* was used as a positive control. Two-week-old RH seedlings were subjected to several stresses. For SA and MeJA, the seedlings were sprayed with 1 mM and 250 µM salicylic acid and methyl jasmonate, respectively; for the salt treatment, the seedlings were transplanted into 150 mM NaCl solution; and for wounding, the seedlings were cut into small pieces in distilled water.

4. Expression of LOC_Os04g27430 is correlated with 2 SNP at transcription factor (TF) binding sites.

Correlation between the SNPs at three TF binding elements; ATHB-1 (Arabidopsis thaliana homeobox protein 1), SBF1 (silencer-binding factor 1) and P (maize activator P) factor and the expression of the Os04g27430 was investigated in 17 rice varieties/ lines. The genomic regions at the 5’upstream portion of the gene in 17 rice varieties/ lines were sequenced (accession numbers KC511030 to KC511047) and it was found that the rice varieties PK2, PK3, HCS and RBR which have A > C (Figure 15) and C > T (Figure 16) alteration at ATHB-1 and P elements, respectively, did not express the gene under both BPH-feeding and control conditions while the other 13 rice cultivars which didn’t have base changes at both elements clearly expressed the gene under control conditions and expression of the gene was induced under the BPH-feeding condition (Figure 17). On the other hand, SNP at the SBF-1 element (Figure 18) did not show consistency with expression of the gene. The correlation between 2 SNPs at consensus elements for the transcription factor ATHB-1 and the P-factor binding site and expression of the Os04g27430 gene suggested that expression of the gene may be controlled at transcription level by the binding of transcription factors at these two cis-acting DNA elements.

```
                                     ATHB-1
JHN                    TTTGACAAATTAATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 358
ARC10550               TTTGACAAATTAATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 353
ASD7                   TTTGACAAATTAATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 353
TN1                    TTTGACAAATTAATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 356
PTB33                  TTTGACAAATTAATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 353
RH                     TTTGACAAATTAATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 355
IL162                  TTTGACAAATTAATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 356
IL302                  TTTGACAAATTAATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 347
IL308                  TTTGACAAATTAATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 357
Mudgo                  TTTGACAAATTAATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 358
Pokkali                TTTGACAAATTAATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 340
Nipponbare             TTTGACAAATTAATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 343
IL143                  TTTGACAAATTAATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 357
PK3                    TTTGACAAATTCATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 356
RBR                    TTTGACAAATTCATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 346
HCS                    TTTGACAAATTCATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 358
PK2                    TTTGACAAATTCATTATTGCTAGAACTGTTTCATGAATATTGTAATGTTATTTCACAAAG 355
                       *********** ************************************************
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Figure 15 Sequence alignments at ATHB-1 transcription factor binding site, consensus sequence of the elements is labeled in red color. The SNP found in the element are labeled in blue color.

```
                             P
JHN                    CAACCAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 478
ARC10550               CAACCAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 473
ASD7                   CAACCAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 473
TN1                    CAACCAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 476
PTB33                  CAACCAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 473
RH                     CAACCAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 475
IL162                  CAACCAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 476
IL302                  CAACCAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 467
IL308                  CAACCAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 477
Mudgo                  CAACCAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 478
Pokkali                CAACCAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 460
Nipponbare             CAACCAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 462
IL143                  CAACCAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 477
PK3                    CAACTAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 476
RBR                    CAACTAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 466
HCS                    CAACTAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 478
PK2                    CAACTAACCAGAGATCGGAAGAATGGATGAGGAAGAGAGCTGACGAGCTCAAGGAGAAAGTCCG 475
                       **** ***********************************************************
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Figure 16 Sequence alignments at P-factor transcription factor binding site, consensus sequence of the elements is labeled in red color. The SNP found in the element are labeled in blue color.

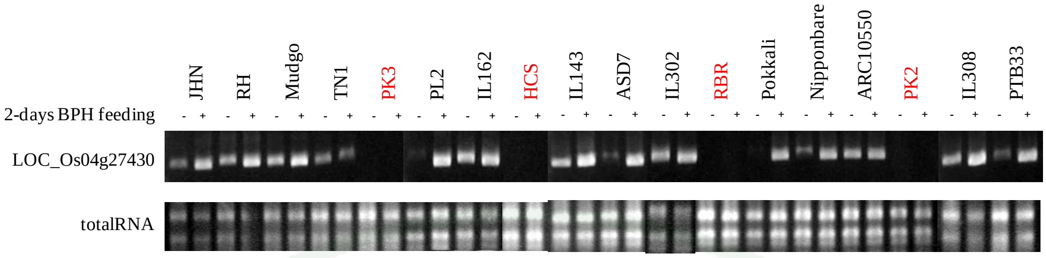

Figure 17 Expression pattern *of OS04G27430* gene in control (-) and 2-days BPH feeding (+) condition, rice varieties with undetectable expression of the gene are labeled in red.

```
                                  SBF-1
JHN                  CTTTGCGTGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 418
ARC10550             CTTTGCGTGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 413
ASD7                 CTTTGCGTGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 413
TN1                  CTTTGCGTGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 416
PTB33                CTTTGTGTGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 413
RH                   CTTTGTGTGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 415
IL162                CTTTGTGTGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 416
IL302                CTTTGTGTGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 407
IL308                CTTTGTGTGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 417
Mudgo                CTTTGCGTGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 418
Pokkali              CTTTGCGTGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 400
Nipponbare           CTT-GCGTGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 402
IL143                CTTTGTGTGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 417
PK3                  CTTTGCATGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 416
RBR                  CTTTGCATGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 406
HCS                  CTTTGCATGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 418
PK2                  CTTTGCATGGTTAATAATCTCTTTTTGATAACTATAATAAATCAAACTCGTGAATCCAAC 415
                     *** *  *****************************************************
```

Figure 18 Sequence alignments at SBF-1 transcription factor binding site, consensus sequence of the elements is labeled in red color. The SNP found in the element are labeled in blue color.

5. Seven-amino-acid-deletionl in the Os04g27430 gene is natural variation found in several rice genotypes

The KD allele of the Os04g27430 gene contained two SNPs, TG > CA in exon 5, leading to 21-bp deletion and 7aa-del in mRNA and protein sequence, respectively. To investigate whether this 21-bp deletion occurred in other rice varieties, cDNA fragments harboring this region of the gene were amplified from several rice varieties. The expressed Os04g27430 genes from nine rice cultivars and three ILs were sequenced (accession numbers KC511057 to KC511068). Interestingly, it was found that not only KD but also JHN, TN1, Mudgo, Pokkali, ARC10550, ASD7 and Nip have this 21-bp deletion in their allele of the gene (Figure 19A). On the other hand, the 21-bp deletion was not found in the allele of BPH-resistant varieties, such as PTH33, PL2 and all ILs tested. As expected, the deduced amino acid sequences of these cultivars were translated and 7aa-del was found in JHN, TN1, Mudgo, Pokkali, ARC10550, ASD7 and Nip (Figure 19B). This finding suggested that not only KD but also other rice varieties each contained the 7aa-del lesion in the Os04g27430 gene. This finding suggested that not only transcription level, but also translation level that may alter the function of this Os04g27430 gene since some rice genotypes were induced to expressed the gene as they contained the cis-acting DNA elements at the promoter region but the 7aa-del lesion occurred in the resulted protein.

(A)

```
KD               AAGCTGAA---------------------CCAAGCTTCGGTGACCAGATTACTTT
JHN              AAGCTGAA---------------------CCAAGCTTCGGTGACCAGATTACTTT
TN1              AAGCTGAA---------------------CCAAGCTTCGGTGACCAGATTACTTT
Mudgo            AAGCTGAA---------------------CCAAGCTTCGGTGACCAGATTACTTT
Pokkali          AAGCTGAA---------------------CCAAGCTTCGGTGACCAGATTACTTT
ARC10550         AAGCTGAA---------------------CCAAGCTTCGGTGACCAGATTACTTT
ASD7             AAGCTGAA---------------------CCAAGCTTCGGTGACCAGATTACTTT
Nipponbare       AAGCTGAA---------------------CCAAGCTTCGGTGACCAGATTACTTT
IL143            AAGCTGAATGGTCACACCAGAGGCATAAACCAAGCTTCGGTGACCAGATTACTTT
IL302            AAGCTGAATGGTCACACCAGAGGCATAAACCAAGCTTCGGTGACCAGATTACTTT
IL162            AAGCTGAATGGTCACACCAGAGGCATAAACCAAGCTTCGGTGACCAGATTACTTT
PTB33            AAGCTGAATGGTCACACCAGAGGCATAAACCAAGCTTCGGTGACCAGATTACTTT
PL2              AAGCTGAATGGTCACACCAGAGGCATAAACCAAGCTTCGGTGACCAGATTACTTT
IL308            AAGCTGAATGGTCACACCAGAGGCATAAACCAAGCTTCGGTGACCAGATTACTTT
RH               AAGCTGAATGGTCACACCAGAGGCATAAACCAAGCTTCGGTGACCAGATTACTTT
                 ********                     **************************
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(B)

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JHN           EFQKLSTYYLQEAE-------PSFGDQITLTAMSSVIPL
TN1           EFQKLSTYYLQEAE-------PSFGDQITLTAMSSVIPL
Mudgo         EFQKLSTYYLQEAE-------PSFGDQITLTAMSSVIPL
Pokkali       EFQKLSTYYLQEAE-------PSFGDQITLTAMSSVIPL
ARC10550      EFQKLSTYYLQEAE-------PSFGDQITLTAMSSVIPL
ASD7          EFQKLSTYYLQEAE-------PSFGDQITLTAMSSVIPL
Nipponbare    EFQKLSTYYLQEAE-------PSFGDQITLTAMSSVIPL
IL143         EFQKLSTYYLQEAEWSHQRHKPSFGDQITLTAMSSVIPL
IL302         EFQKLSTYYLQEAEWSHQRHKPSFGDQITLTAMSSVIPL
IL162         EFQKLSTYYLQEAEWSHQRHKPSFGDQITLTAMSSVIPL
PTB33         EFQKLSTYYLQEAEWSHQRHKPSFGDQITLTAMSSVIPL
PL2           EFQKLSTYYLQEAEWSHQRHKPSFGDQITLTAMSSVIPL
IL308         EFQKLSTYYLQEAEWSHQRHKPSFGDQITLTAMSSVIPL
RH            EFQKLSTYYLQEAEWSHQRHKPSFGDQITLTAMSSVIPL
              **************       ******************
```

Figure 19 Sequence alignment analyses. cDNA (A), amino acid (B) sequence alignments of 2SNPs, 21-bp, and 7-amino acid deletion respectively in several rice varieties. The rice varieties with 21-bp and 7aa-del are labeled.

6. Os04g27430 may play a more important role than Bph3 in the antixenosis mechanism

Three ILs were selected to elucidate the epistasis of Os04g27430 and the BPH 3 major gene within the BPH resistance mechanism. UBN03078-101-342-4-7(IL7), UBN03078-101-342-4-72 (IL72), and UBN03078-101-342-6-82 (IL82) contain a *Bph3* critical region, Os04g27430, and both, respectively. The antixenosis of the BPH feeding preference (AFP) was compared between each IL with KD. Within the first 8 hr, UNB-BPHs randomly landed on each tested plant and consistently moved to KD. At 120 hr, the majority of the UBN-BPH population (> 80%) had settled on KD instead of the IL plants (Figure 20A-20C). This result as well as an AFP test between IL7 and IL72 suggest that either *Bph3* or Os04g27430 is sufficient to confer protection against BPH landing within the first 120 hr of attack (Figure 20D). In a comparison between IL82, which contains both Bph3 and Os04g27430, with IL7 and IL72 (Figure 20E-20F), UBN-BPH was found to prefer IL7 over both IL72 and IL82. This result suggests that Os04g27430 is more important than *Bph3* in determining BPH landing preference.

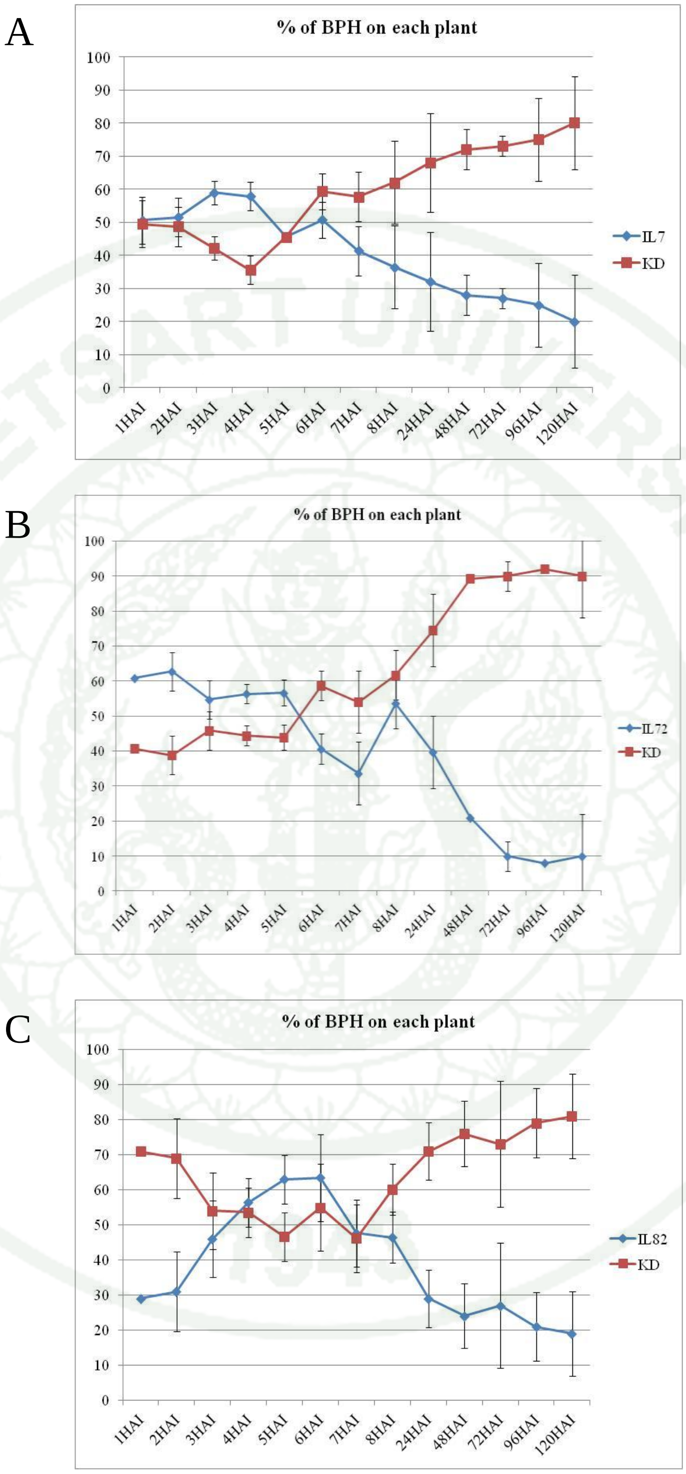


Figure 20 Antixenosis of BPH feeding preference (AFP) test showing comparisons between KD and the ILs (A to C) and between the ILs (D to F). The y-axis indicates the number of BPH that settled on each plant as a percentage, and the x-axis shows the progress between 1 and 120 hr after BPH infestation.

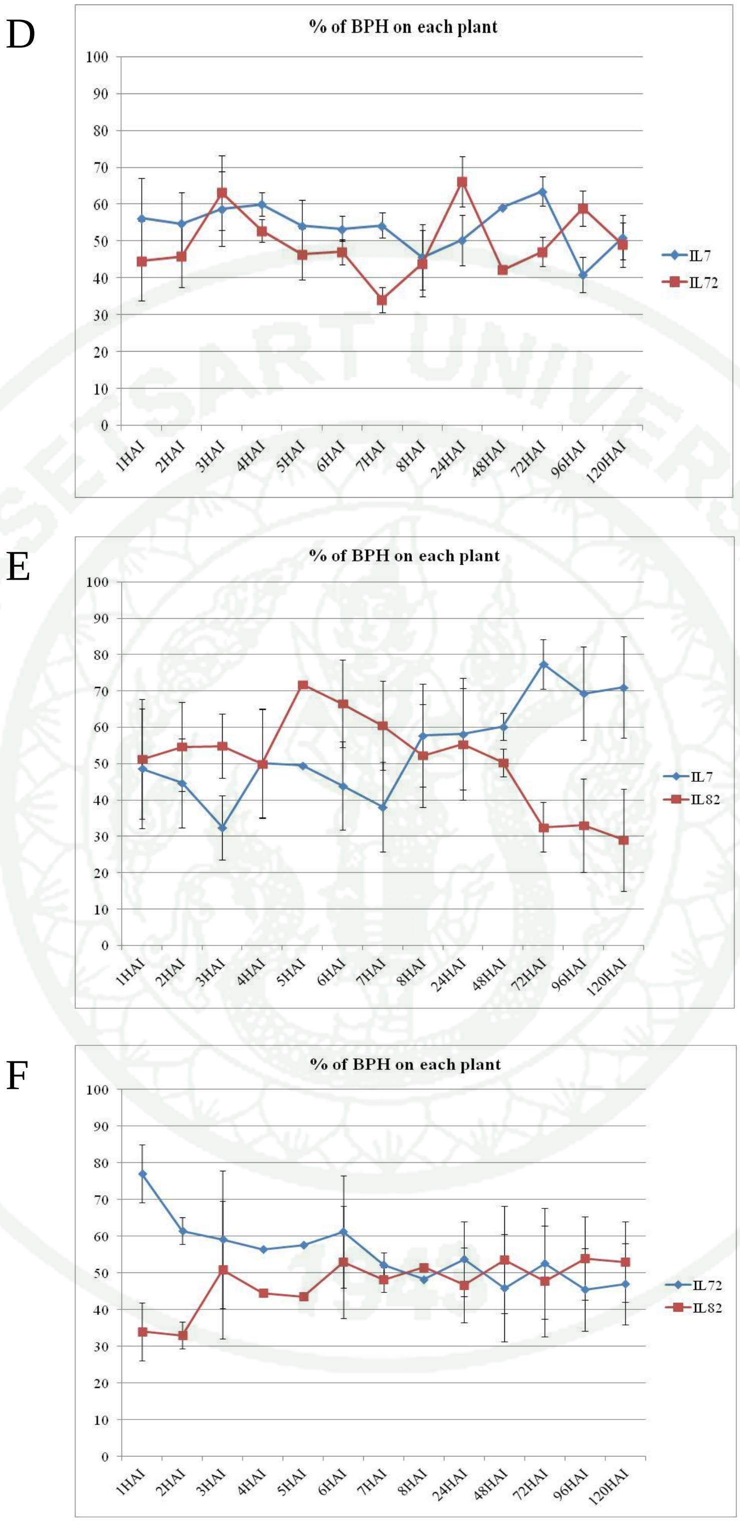


Figure 20 (continued)

7. The antixenosis mechanism may not function in rice varieties harboring 7aa-del in the Os04g27430 gene

To elucidate if the 7aa-del in the deduced protein sequence of the Os04g27430 gene affect its putative function in the AFP mechanism or not, 6 rice varieties/ lines were selected; JHN (BPH susceptible, 7aa-del Os04g27430) , Mudgo (Bph1, 7aa-del Os04g27430), ASD7 (bph2, 7aa-del Os04g27430), Pokkali (Bph9, 7aa-del Os04g27430) and IL308 (Bph3, active Os04g27430) for AFP study. BPH damage score using UBN-BPH showed that IL308 and Pokkali were BPH resistance, Mudgo and ASD7 were BPH moderate susceptible and JHN was BPH susceptible (Appendix Figure 1). The AFP comparison between IL308 and other rice varieties revealed that in all combinations, BPH preferred to settle on Mudgo, Pokkali, JHN and ASD7, respectively, than IL308 (Figure 21A – 21D). Both IL308 and Pokkali were BPH resistances (score 0.7 and 1.7, respectively), but the AFP test clearly demonstrated that in the 96-h period of BPH infestation BPHs more preferred Pokkali than IL308. This difference may be due to the fact that Os04g27430 in Pokkali contained 7aa-del lesion in its protein chain.

The AFP comparisons between JHN, the BPH susceptible variety, and other rice varieties were shown in Figure 21E – 21G. Interestingly, the AFP comparison revealed that even JHN was the most susceptible rice variety in this group but BPHs were randomly settled on the tested plants in the 96-h period of BPH settling choice test. This finding could be explained as all the rice varieties test had the common Os04g27430 gene with 7aa-del lesion leading to non functional gene that unable to initiate repellent volatile compound production.

The AFP comparison between the rice genotypes contained difference BPH resistant genes but expressed the same 7aa-del Os04g27430 gene, Mudgo, ASD7 and Pokkali, were shown in Figure 21H – 21J. In all combinations, it seem like BPH population were confusing in host plant selection. There are forward and backward selection behaviors between the tested plants in 96-hr test period and finally random settled on the tested plant almost equally at the end. This could be explained as follow:- since Mudgo, ASD7 and Pokkali were all contained 7aa-del in Os04g27430, this deletion affected the ability of volatile compound production of all varieties leading to non-function antixenosis mechanism of rice against BPH infestation.

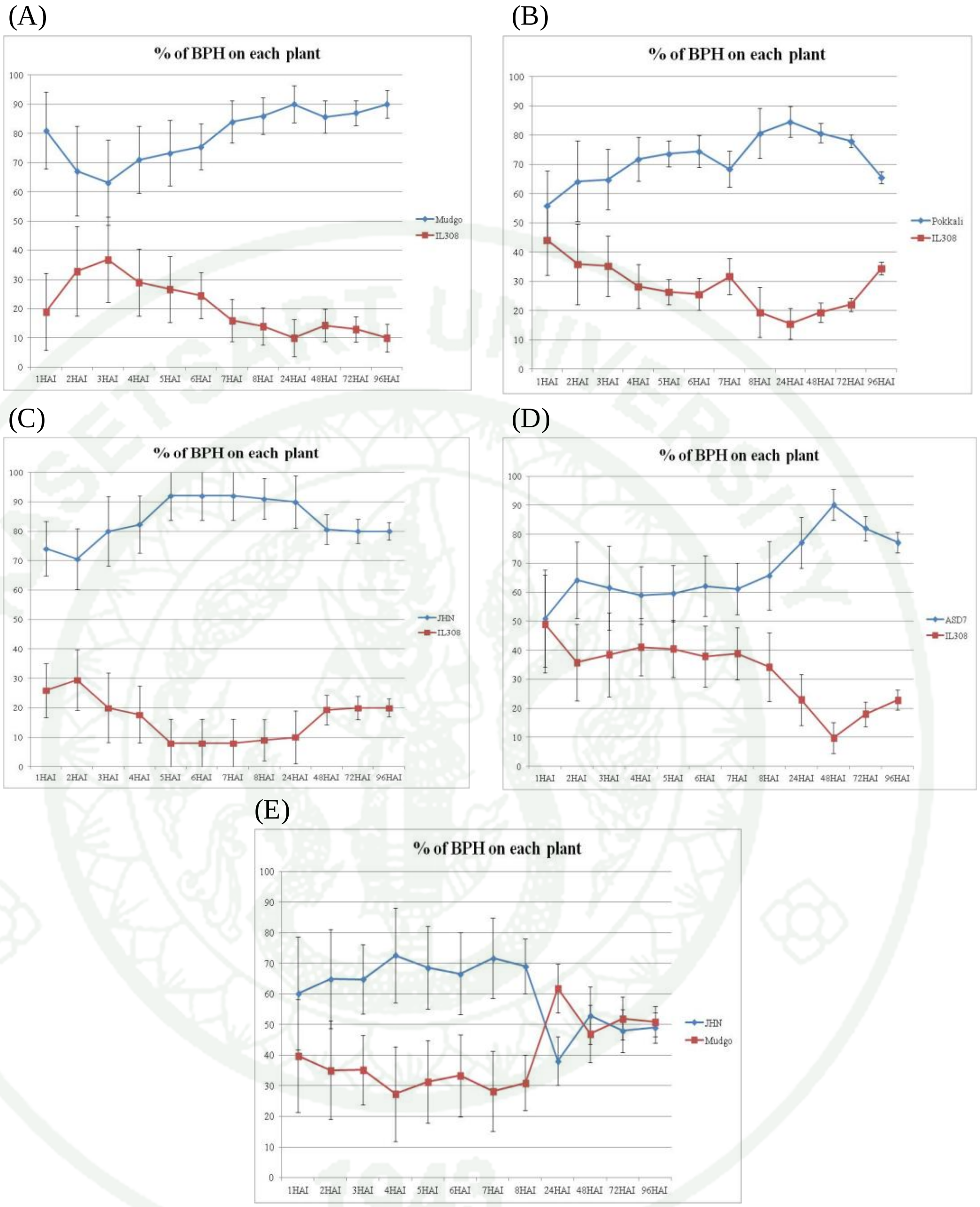


Figure 21 Antixenosis of BPH feeding preference (AFP) test of rice varieties differ in 7-aa-del in Os04g27430. The y-axis indicated the number of BPH that settled on each plant, and the x-axis showed the progress from 1 to 96 h after BPH infestation.

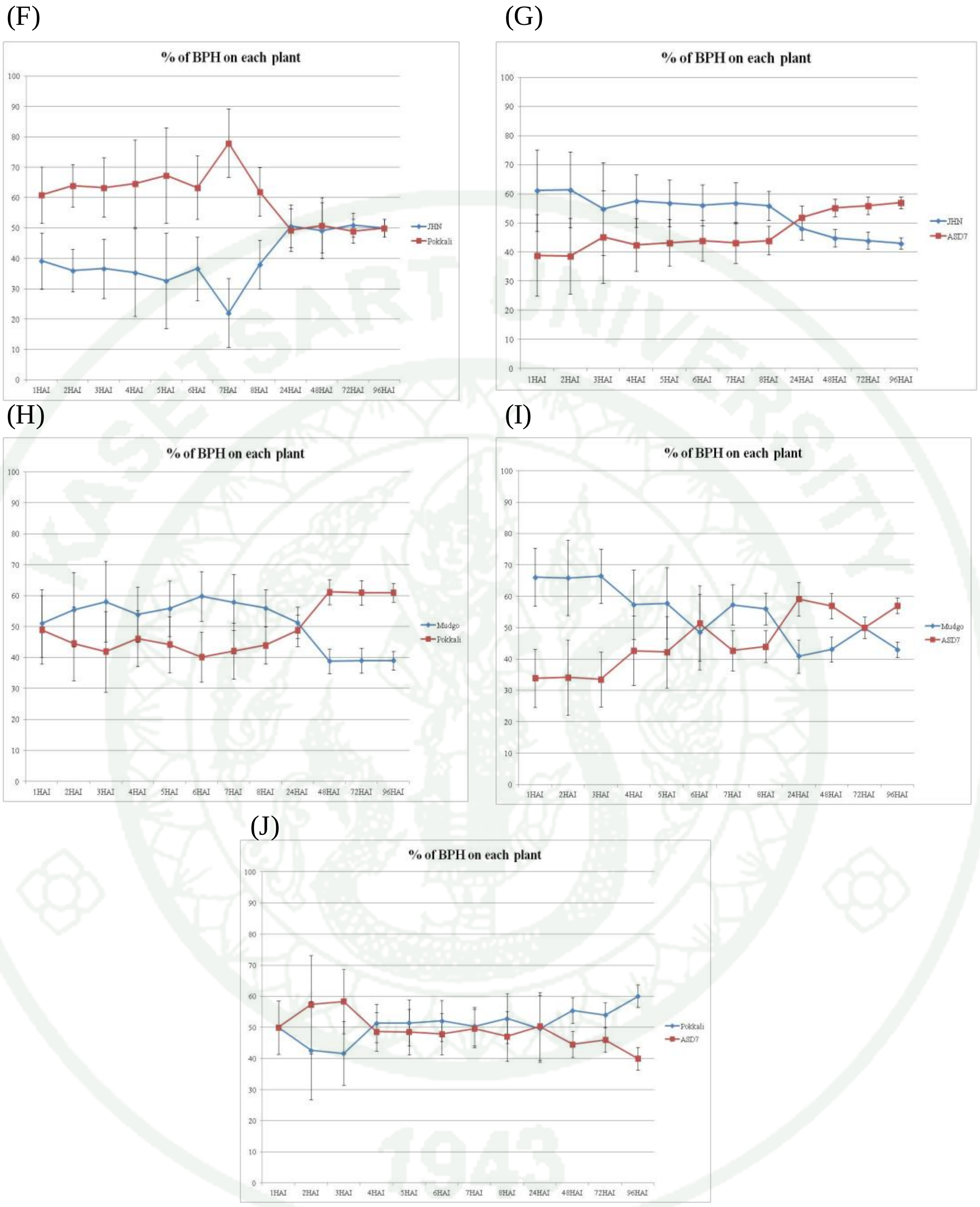


Figure 21 (continued)

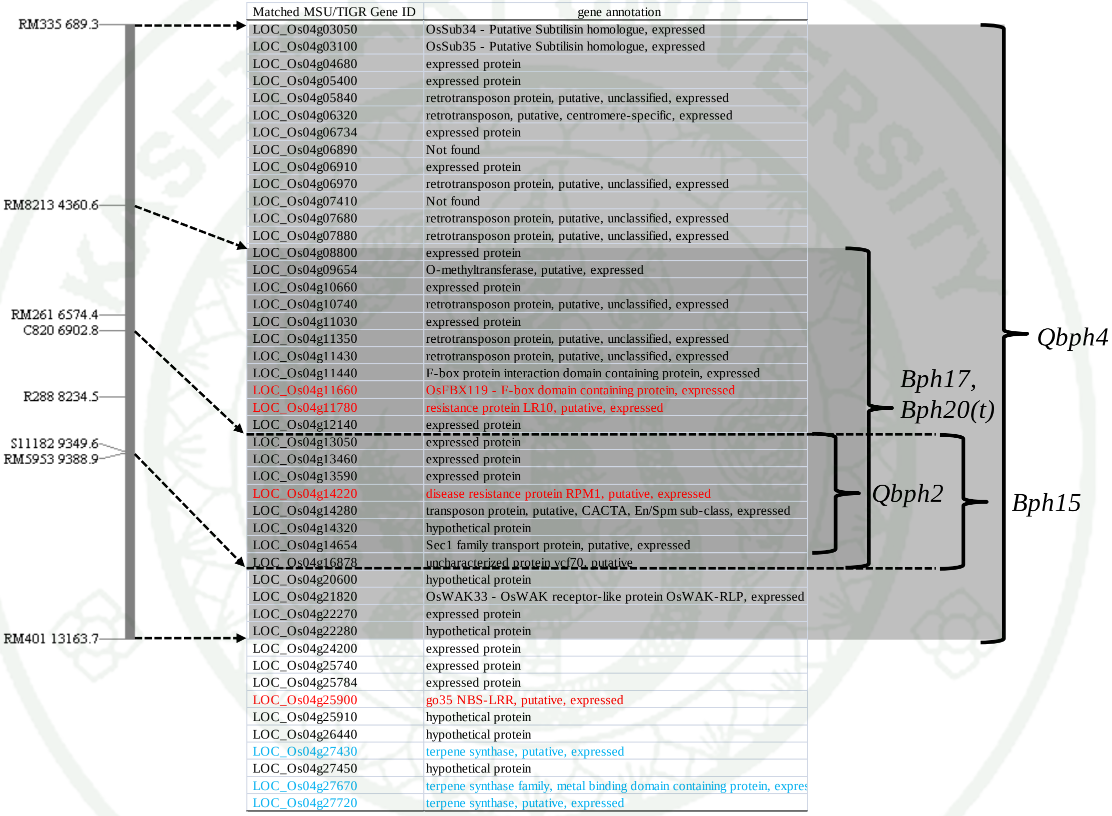
Discussion

SFP mapping identified several minor QTLs that may complement BPH resistance

In this study, SFP mapping was used to identify the gene(s) responsible for BPH resistance of rice cultivar RH from Sri Lanka which was mapped on rice chromosome 6 (Jairin et al. 2007a). Surprisingly, only five SFPs were identified on chromosome 6 and were all outside the critically mapped location. This observation was consistent with the expression analysis of BPH-infested RH conducted by another research group that did not identify any gene in the *Bph3* candidate region (Wang et al. 2012). However half of identified genes were located on rice chromosome 4 (Figure 35) where several BPH resistance genes have been mapped (Sun et al. 2005, Yang et al. 2002, Yang et al. 2004, Rahman et al. 2009, Huang et al. 2001, Liu et al. 2009).

Qbph4, *Bph17*, and *Bph20(t)* were mapped to the intervals RM335 – RM401 and RM8213 – RM5953 and with the linked marker RM5953, respectively. The Qbph4 region encompassed a position from 689,354 to 13,163,724 bp, and *Bph17* encompassed 4,360,621 to 9,388,937 bp on pseudomolecule 4 (Os-Nipponbare-Reference-IRGSP-1.0). A total of 36 SFP-containing genes (Os04g03050 – Os04g22280) were located in the Qbph4 region (Figure 35). Nineteen of these genes (Os04g08800 – Os04g16878) were also specifically associated with the *Bph17* region. The Qbph2 and *Bph15* genes were mapped in the same region by the RFLP markers C820-R288 and C820-S11182. The region located between 6,902,846 and 9,349,627 bp on pseudomolecule 4 contained eight SFP-containing genes (Os04g13050 – Os04g16878). The linked marker of *Bph12(t)*, RM261, was adjacent to Os04g11780, the resistance protein LR10, with a physical distance of 130.5 kb. Moreover, another NBS-LRR resistance-related protein, Os04g25900, also contained an SFP.

Compared to the expression analysis of BPH-infested RH studied by Wang et al. (2012), three BPH resistance-related genes, two putative resistance proteins, Os04g11780 (LR10) and Os04g14220 (RPM1), and an F-box-containing protein, Os04g11660, were found to contain SFP (Figure 35) in the present study. In contrast, no candidate BPH resistance gene on chromosome 3, 6, and 10 identified in the study by Wang et al. (2012) was found to contain an SFP in our study. This difference may be due to the different BPH-susceptible rice cultivars, KD and TN1, used for the RH comparison in the two studies.



Matched MSU/TIGR Gene ID	gene annotation
LOC_Os04g03050	OsSub34 - Putative Subtilisin homologue, expressed
LOC_Os04g03100	OsSub35 - Putative Subtilisin homologue, expressed
LOC_Os04g04680	expressed protein
LOC_Os04g05400	expressed protein
LOC_Os04g05840	retrotransposon protein, putative, unclassified, expressed
LOC_Os04g06320	retrotransposon, putative, centromere-specific, expressed
LOC_Os04g06734	expressed protein
LOC_Os04g06890	Not found
LOC_Os04g06910	expressed protein
LOC_Os04g06970	retrotransposon protein, putative, unclassified, expressed
LOC_Os04g07410	Not found
LOC_Os04g07680	retrotransposon protein, putative, unclassified, expressed
LOC_Os04g07880	retrotransposon protein, putative, unclassified, expressed
LOC_Os04g08800	expressed protein
LOC_Os04g09654	O-methyltransferase, putative, expressed
LOC_Os04g10660	expressed protein
LOC_Os04g10740	retrotransposon protein, putative, unclassified, expressed
LOC_Os04g11030	expressed protein
LOC_Os04g11350	retrotransposon protein, putative, unclassified, expressed
LOC_Os04g11430	retrotransposon protein, putative, unclassified, expressed
LOC_Os04g11440	F-box protein interaction domain containing protein, expressed
LOC_Os04g11660	OsFBX119 - F-box domain containing protein, expressed
LOC_Os04g11780	resistance protein LR10, putative, expressed
LOC_Os04g12140	expressed protein
LOC_Os04g13050	expressed protein
LOC_Os04g13460	expressed protein
LOC_Os04g13590	expressed protein
LOC_Os04g14220	disease resistance protein RPM1, putative, expressed
LOC_Os04g14280	transposon protein, putative, CACTA, En/Spm sub-class, expressed
LOC_Os04g14320	hypothetical protein
LOC_Os04g14654	Sec1 family transport protein, putative, expressed
LOC_Os04g16878	uncharacterized protein ycf70, putative
LOC_Os04g20600	hypothetical protein
LOC_Os04g21820	OsWAK33 - OsWAK receptor-like protein OsWAK-RLP, expressed
LOC_Os04g22270	expressed protein
LOC_Os04g22280	hypothetical protein
LOC_Os04g24200	expressed protein
LOC_Os04g25740	expressed protein
LOC_Os04g25784	expressed protein
LOC_Os04g25900	go35 NBS-LRR, putative, expressed
LOC_Os04g25910	hypothetical protein
LOC_Os04g26440	hypothetical protein
LOC_Os04g27430	terpene synthase, putative, expressed
LOC_Os04g27450	hypothetical protein
LOC_Os04g27670	terpene synthase family, metal binding domain containing protein, expres
LOC_Os04g27720	terpene synthase, putative, expressed

Figure 22 SFP-containing genes located on chromosome 4 and the location of BPH resistance genes mapped to chromosome 4. The vertical bar represents the map location on chromosome 4. RM markers and their physical location on the pseudomolecule are shown at the left. The SFP-containing genes located in the *Qbph2*, *Qbph4*, *Bph15*, and *Bph17*regions are highlighted. The three SFP-containing *TPS* genes are labeled in blue. The SFP-containing resistance related genes are labeled in red.

Identification of BPH resistance genes in rice

The resistance in rice germplasm to BPH has been explored since 1969, resulting in the detection of 24 resistance genes in cultivated *O. sativa* and wild species of rice including *O. australiensis, O. eichingeri, O. latifolia, O. officinalis, O. rufipogon, O. glaberrina,* and *O. minuta* (Khush, 1979; Deen et al., 2010; Jena and Kim, 2010; Huang et al., 2012). To date, 20 BPH resistance genes have been located on the rice chromosomes. Following a long search for BPH resistance genes in rice, *Bph14,* the first BPH resistance gene identified using map-based cloning, providing a means for understanding the molecular basis for rice resistance to BPHs (Du et al., 2009). The *Bph14* gene encodes a coiled-coil, nucleotide-binding, and leucine-rich repeat (CC–NB–LRR) protein of the NB–LRR family, which is an immune receptor type similar to R proteins functioning in disease resistance. *Bph14* confers antibiosis resistance by inhibiting BPH feeding and reducing BPH growth rate and longevity. The discovery of *Bph14* is beginning to shed a light on the complicated interactions between BPHs and the rice immune system. The mechanism of *Bph14* resistance indicates that *Bph14* is an innate immune receptor, which may be activated by specific insect factors (Du et al 2009).

Host selection behavior of BPH and *TPS* genes

Host searching behavior is an important process by which BPH seek resources to find food and settle down (Bell, 1990). It can be divided into several phases including habitat location and host location, acceptance, suitability and regulation. Volatile compounds play important role in the location of hosts by BPHs (Liu et al. 2002, Qi et al. 2011). Qi et al. (2011) reported the cloning of two chloroplast-localized phospholipase D (PLD) genes from rice (Oryza sativa), *OsPLDa4* and *OsPLDa5* respectively. These two genes involved in the oxylipin pathway, the central importance for plant defensive response. Antisense expression of these two genes resulted in reducing volatile compounds production of rice plant infested by striped stem borer (*Chilo suppressalis*). Qiu et al. (2012) developed the pyramiding rice lines containing *Bph6* and *Bph12* and showed the developed lines were more repellent to BPH than those having only *Bph6* or *Bph12*.

The volatile compounds are reported to involve in the indirect defense mechanisms of plant against herbivorous (Yuan et al 2008). Terpenoid, the secondary metabolites of plants, are often the most common and diverse group among insect-induced plant volatiles (Pare and Tumlinson, 1999). Plants produced this large group of compounds by the expression of *TPS* genes. In rice, more than forty *TPS* genes have been annotated in the genome, however, only few of them were intensively studied (Cheng et al. 2007; Wilderman et al. 2004; Xu et al. 2004; Yuan et al. 2008). All reported rice *TPS* genes are not specific with the infestation of BPH on rice plants. On the other hand, this present study is the first *TPS* identified that play role in rice response to BPH feeding.

The anticipation of the gene Os04g27430 in BPH resistance mechanism

In this study, Os04g27430 was identified as the BPH feeding-induced *STPS* gene from the BPH-resistant rice cultivar RH. A BPH-feeding test choice experiment of ILs revealed that the gene may play a role in the antixenosis mechanism against BPH infestation. This *STPS* was found to be defective at both the genomic and translated protein levels in the KD rice cultivar, which is highly susceptible to BPH attack. These damages were three SNPs at transcription factor binding sites in the promoter region and 21-bp deletion in cDNA leading to 7aa-del in the protein chain.

Several investigations confirmed that the expressions of *TPS* genes were regulated at the transcription level (Chen et al. 2003, Herde et al. 2008, Schnee et al. 2002, Wilderman et al. 2004, Xu et al. 2004). However, to our knowledge, only one cis-acting DNA element, the W-box, has been identified as the regulatory site for *TPS* expression (Xu et al. 2004). The element, (T)TGAC(C/T), was found to be the target site for the binding of the transcription factor protein, GaWRKY1, leading to the induction of *δ-cadinene synthase* (*CAD1-A*) gene expression which catalyzes the formation of the cotton phytoalexin, gossypol. Our finding that the expression of the

Os04g27430 gene is associated with the cis-acting elements, ATHB-1 and P-factor, may pave the way to understanding how this *STPS* gene were regulated in more detail.

Allelic variation in *TPS* genes leading to a volatile compound composition difference has been reported in maize (Köllner et al. 2004). In rice, several rice varieties have been shown to release different volatile blends (Lou et al. 2006); however, the gene(s) that controls these differences has not yet been identified. In our preliminary study, a total of 25 sesquiterpenes were identified by GC-MS in the mixture of volatile compounds emitted by KD and RH rice plants infested by BPH. The major sesquiterpene product that KD emitted at a lower level than RH is E-β-farnesene (Figure 36), the common constituent of the aphid alarm pheromone (Bowers et al. 1972, Edwards et al. 1973, Pickett and Griffiths. 1980) and the aphid repellent of wild potato (Gibson and Pickett. 1983). This variation may be due to a defect in the KD allele of Os04g27430 at both the genomic and the protein sequence levels.

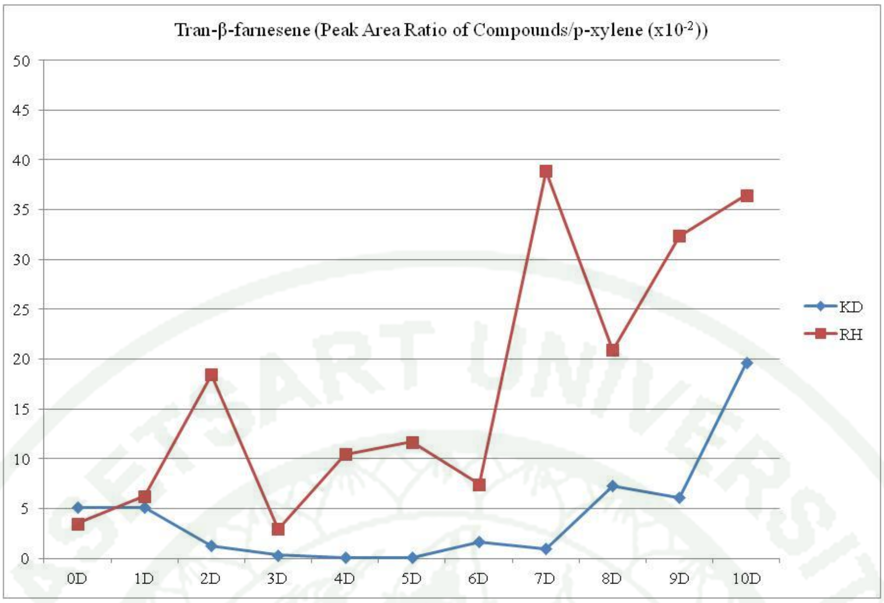


Figure 23 Changing of trans -β-farnesene emission after BPH feeding for 0, 1, 2, …, 10 days (0D to 10D) in RH (red line), and KD (blue line). KD and RH plants were attacked by BPH for 10 days, volatile compound were extracted and analyzed by Solid Phase Microextraction (SPME) and Gas chromatography-mass spectrometry (GC-MS). Twenty-five sesquiterpene compounds were identified (data not shown). The most significant compound that RH emitted at higher level than KD is trans-β-farnesene

CONCLUSION

Single-feature polymorphism (SFP) mapping was used to localize 84 candidate genes for BPH resistance related genes on chromosomes 1, 2, 3, 4, 6, 8, 10, and 12. Os04g27430 was identified to encode a putative *sesquiterpene synthase* (*STPS*) gene that was induced by BPH feeding in RH and BPH-resistance ILs. Os04g27430 is the second known rice *STPS* induced by BPH. The gene may involve an antixenosis BPH resistance mechanism. The combination of the *STPS* and the *Bph3* locus was more effective than *Bph3* alone in the tested ILs. These findings may facilitate the breeding of new rice cultivars that express BPH resistance more stably and with longer duration in the most intensive rice cultivation region in the world.

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APPENDIX

Appendix Table A1 List of PCR primers used for SFP validation, re-sequencing of Os04g27430

primer	**forward sequence (5'--->3')**	**reverse sequence (5'--->3')**
SFP1	CAACCCACCCCATAAAATTG	CGAGGTCGTGGTAAATCCAT
SFP2	TACTTCCCGATGCCAACTTC	GCTGGACGATCGAGAGAAAG
SFP3	ATGTAGCACCGGGGTCATAG	GGCCGGAGTAAATAGATCTGG
SFP7	cactggcgattgctttcttt	ccgtaattcctccgctgtta
SFP9	CAACCCACCCCATAAAATTG	CGAGGTCGTGGTAAATCCAT
SFP12	ggatcctggattggagttca	gtgcaggatctccttcccta
SFP14	TGAGGGCCAAGTAAAACCAG	TAAACAGAAAGGCCGGAAAA
SFP17	gaaacaacctatcacatgcaaagac	gatctggtgacccaaagctc
SFP19	ACTGATCCCCCAAATGATGA	GTGCCCCTTCAGATGACAGT
SFP21	ggggatggatagccaaatct	tctggtgctcacattcgttc
SFP26	ATTTCTTCCGGCGTTAAGGT	AGCGAAGCAGCATATCCACT
SFP29	ggcgcacaaacagtgaaca	tcatcggtaacgttcagcag
SFP31	ACGAAATTAAGGGCAACCAA	GGCGTCGTAATGTTGGACTT
SFP32	cttatgttggattggcacga	cgttgcaattgatgttcgat
SFP33	GGGGCAAGAGCTCCTAAGAT	CCAGATCCCAAAAGCACAGT
SFP35	GCCTGCTCAAGAAGTTCCAC	CGGTGTCTCCTGGTGTAGGT
SFP36	TGTTGAGATCCGAATGGTGA	AACCTGTTTCCATTGGTTGG
SFP38	ACCCACTGGCAAGTCTTCAC	TTGCAGGGGAATTTACATGG
SFP39	CGATTTTTCCGGAATTGATG	GAAATACGGCAGCATGTTCA
SFP40	tcatatgccaccacttatcca	ccagttgaagtgtaaattgatgacc
SFP41	CCTGCATCTTATGGCAAGGT	TTTGACACGAGAATAGCAGCA
SFP42	TGGATATGGAACACGCATTG	CATGCCAAAAGAGAGTGAGACTT
SFP44	CCACCCAATGAAGGATCAAG	CTCTTAACGGGCCAACAAGC
SFP45	TCTCGTGATGGAGATGGACA	TGCTCACAAGACCCTTGAAA
SFP46	GCCTGCAATCTCCATGATCT	ATGTCGCTGATGGCAATAGA
SFP47	TATTAGTGCTCCGCCATCCT	AACCTGCTGATGCCATTTTC
SFP48	TGGGGAGAGGGAAGATAGAGA	TGTGGGAGTGAAGCAAAGAA
SFP50	tcgaacgtgaagtgttctgg	caggtgaccttgaccagtgt
SFP51	AGCAGCCGTTGATAGGCTAA	ATTTGGCCTCGTTGTGAAGT
SFP52	GCGATAGAAACGCCTGCTAC	CTACAAATGTCGCACCATCG
SFP53	GTTCATACCCTGGCTGGATG	CTTGCAGTGGGAGATCATCA
SFP55	aggcgcgattcaaacaaaat	acgatgatggtttggttggt
SFP56	GGAGGCACGTAAACTCTACCC	TGCAGATTCCTCGTGAGATG
SFP57	GGCGAGGACGACACATAGAC	TTTTCCGGTGATCTCCACTC
SFP58	AGGGGATTTGCCAAGTAGGT	CACCATCATGGATAGCATCG

Appendix Table A1 (continued)

primer	forward sequence (5'--->3')	reverse sequence (5'--->3')
SFP62	AGGCAGAAGACGATCCTGAA	GATATCGGCACCTGAGTGGT
SFP63	GACCCGAGCTGCAGATAGAC	AGAGCATGCGATGAATCTGT
SFP64	TCTCTTGCGGAAGATTGCTT	TCCATTCCACCGGAATAACT
SFP67	CGCCACTGAGACAAGATGAA	GCATGTTTGGCTGAGTTCAA
SFP68	TCAAGGTGCTTCAGGATCAA	TTACATAACCCATGCCTACCG
SFP69	CCGACTTATGGCGAAGAAAG	TTGTTTGTTGATGCCTTCCA
SFP70	gccaagaagcaggtgagagt	acgagaagctgagtgccaat
SFP71	gatgcagcttacccagctct	cgaagaactccccaaatgag
SFP73	TCATGATTTTGTGGCTCGTC	GGCATCATTCCTGTCATCGT
SFP74	TCGATTTCCATCTCGGATTC	TGATGTCGTGCACTGAAGCTA
SFP79	aaggcggcttacaagaggta	gaggcaaaaccagaaccaaa
SFP80	actgatgctttcgtgtgtcg	accgctttagtcgggttagg
SFP81	ATCGTGGCATACACGTTCAA	TCTTAGTTCTTCCGCCAGGA
SFP83	GGTCAGCCAGACTGAGGAAG	TCTTGGAGCTCTTTGCCCTA
SFP85	CATCAGGATCACCGGAAACT	CTACAGCATCGCCAACTTCA
SFP88	TACCCTTAGTCGCGGTTTGT	CGCCTGCTTACCTGTGAAA
SFP90	GCTTCTAGACGGTTCGTTCC	ACAATACTGGGCTGCCAAAA
SFP91	GGGTTGGACATCGTTCTCTG	AAAAAGCCACAATGCGAATC
SFP92	GGCCAATTATTTGGGAGGTT	GCGAGTCTTGCACATCTTGA
SFP93	TCATCCGATCCACTCTCTTTC	GAAGACGCTCCTAGGGTCAA
SFP96	cagcaagcaggagtagcaga	caaaaccaaagaactcccaca
SFP97	CTGGCCTTGCTCAAAATAGG	GGTTGGCCGCATAGACTAGA
SFP99	AAAATGAGCAGGATCGGGTA	AGGTTCCATTCGATCAGGTG
SFP100	CGCTACATGATGCCATGAAA	CATTGAAAGCAAAGCAAGGA
SFP101	ATTGACGAAGTCACCACAGG	GAATCACCGCTACCAAAAGC
SFP102	TGTGGAGGCTACAAGGCTCT	GGAAGCCAGCGAACTAGAGA
SFP103	CAACAACAACTGCGTGGCTA	TGCATAATGCCGTCCATCTA
SFP104	TGGGATCCTGGTGGTTTATG	CCAATGGGTTCCTGACAATC
SFP105	GTCCGACTCACCTCCCTCTT	CCATCATCAATTCATCCAATCC
SFP106	CGAAGCTCTCAAGGCAGTCT	CGGAGGGAGTATATGAGAGCA
SFP107	CAGAAGTGACGAGAGCAACG	CACACGCCTTTTCTTTTCAG
SFP108	TTTTCTGCTCGTTTGTGGTG	GCCAAGTATGCACAGCGTAA
SFP109	CTGCACTCCACCTGAAGGAC	TGAACATCATGTGCCGGTAA
SFP111	GAAGGCATCGAGTTTCTTCG	TCCCAAGAGGGACTTTCTCA
SFP113	CAGTTCATTGGGTGGAGCTT	TTTGATCCAGTGTCGGTGTT
SFP114	GAAACCCCATGCAAAGAAAA	GCTCAGGGTTTCCAGATGAG
SFP115	GTTGTTGGCCCACTCTTCAT	GGGGTTTCAGGGAGAACAAT
SFP116	CCTTGCTTTAACCCCCTTCT	TCGGGTCCCAAAACTAATGA

Appendix Table A1 (continued)

primer	forward sequence (5'--->3')	reverse sequence (5'--->3')
SFP117	ATACACGTGAAGCGTGGAAA	AGATGGGAACAGGCTTGACA
SFP119	AACCGACAAGAGCGATGTTT	TGGGGGAATATCCAGTGAAA
SFP125	TGGCTACCATGTGCTGGATA	TTTCTTCCCAACTCCACGAC
SFP126	CAATGTTATCCTGAGAGTGCTTG	GCGAATCAGGAATATGTTGGA
SFP127	AGGACTTGGGAGCAATGAGA	ATCGAAGCAGGTTTCCTTCA
SFP129	CCATCTCCCAGGAGTCAAAA	TGGCATTACAAAAGCTCTGC
SFP131	AGTATTGGGCATCCTTGCTG	TGCCTTGAGCTTCTGCTGTA
SFP132	TGCTTCAACAGGCTCAGATG	AGGTCATGGCCTAGAAGCAA
SFP136	ACAACGCTAGGGTCGCTTTA	GCAACATGTTTACAAGCACATAGG
SFP142	CATCATCTGCCACCTCTCCT	TACATGGTTGGGGCAAAAAT
SFP144	ggacaaaactcgagggcata	atggagttcatgggctgaag
SFP146	GACAGCAAAACGAGGAGGAA	GACGGTTAGACCGGTGACAT
SFP148	gcagtgtggcaaatgtggt	ttcagggattaagggttgga
SFP149	GAAGGAGCTATGCCCAACCT	TGTGCATCTCGTCCAAATTC
SFP151	ggtccttttgctcattccag	cccgttttgacccttattca
SFP152	CGGGTATGTCGTTCGTTTTT	CCTCCATCTTTGGCTCAGTG
SFP153	AGATGTCACGGAGGAAAAGC	TGATGCCGGTTAGTTCAAAA
SFP154	GTGAATGCTTCGCCTTTCAT	AGTTCCAGATGGCTTCCAGA
SFP157	TCCAGAAAATGCTGCTTGTG	AGATGCCTGGAGCCTGTCTA

Appendix Table A2 A list of 157 predicted SFPs

SFP	d.value	stdev	rawp	q.value	R.fold	Name
1	-15.89	0.05	5.30E-07	0.312	2.76E-03	OsAffx.32348.1.S1_at3
2	-13.73	0.12	1.06E-06	0.312	3.06E-03	OsAffx.10840.1.S1_at2
3	-12.91	0.13	1.59E-06	0.312	4.03E-03	OsAffx.23064.1.S1_at7
4	-12.15	0.13	2.12E-06	0.312	5.43E-03	OsAffx.32348.1.S1_at1
5	-12.03	0.10	2.65E-06	0.312	7.62E-03	OsAffx.32348.1.S1_at2
6	-11.99	0.30	3.18E-06	0.312	1.50E-03	OsAffx.32348.1.S1_at7
7	-11.53	0.11	3.71E-06	0.312	8.94E-03	OsAffx.19957.1.S1_at6
8	-11.15	0.06	4.24E-06	0.312	1.53E-02	OsAffx.10840.1.S1_at5
9	-10.89	0.29	4.77E-06	0.312	2.97E-03	OsAffx.32348.1.A1_x_at1
10	-10.54	0.31	5.30E-06	0.312	2.94E-03	OsAffx.19957.1.S1_at10
11	-10.41	0.20	5.83E-06	0.312	6.86E-03	OsAffx.32252.1.A1_at3
12	-10.3	0.33	6.36E-06	0.312	2.94E-03	OsAffx.32252.1.S1_at3
13	-10.16	0.21	6.89E-06	0.312	7.19E-03	OsAffx.19957.1.S1_at11
14	-9.95	0.04	7.42E-06	0.312	2.71E-02	OsAffx.13758.1.S1_at2
15	-9.81	0.48	7.95E-06	0.312	1.50E-03	OsAffx.32348.1.S1_at8
16	-9.59	0.17	8.48E-06	0.312	1.31E-02	OsAffx.32252.1.S1_at4
17	9.34	0.11	9.01E-06	0.312	4.75E+01	Os.18164.1.S1_at11
18	-9.16	0.09	9.54E-06	0.312	2.58E-02	OsAffx.32252.1.S1_at9
19	-9.09	0.13	1.01E-05	0.312	2.03E-02	Os.45357.1.S1_at5
20	-9.07	0.08	1.06E-05	0.312	2.88E-02	OsAffx.10840.1.S1_at10
21	9.03	0.27	1.11E-05	0.312	1.15E+02	Os.52198.3.S1_at9
22	-8.82	0.20	1.17E-05	0.312	1.51E-02	OsAffx.32291.1.A1_at10
23	-8.68	0.27	1.22E-05	0.312	1.07E-02	OsAffx.23064.1.S1_at11
24	-8.44	0.19	1.27E-05	0.312	1.93E-02	OsAffx.32252.1.A1_at11
25	-8.33	0.16	1.33E-05	0.312	2.42E-02	OsAffx.23064.1.S1_at10
26	8.3	0.06	1.38E-05	0.312	2.33E+01	OsAffx.25899.1.S1_at4
27	-8.26	0.20	1.43E-05	0.312	1.98E-02	OsAffx.32252.1.S1_at10
28	-8.15	0.36	1.48E-05	0.312	8.89E-03	OsAffx.32348.1.A1_x_at2
29	8.04	0.19	1.54E-05	0.312	4.39E+01	Os.51195.1.S1_at11
30	-8.02	0.03	1.59E-05	0.312	5.70E-02	OsAffx.29272.4.S1_at6
31	-7.98	0.04	1.64E-05	0.312	5.44E-02	OsAffx.13680.1.S1_at10
32	-7.98	0.12	1.70E-05	0.312	3.46E-02	OsAffx.32326.1.S1_x_at10
33	7.98	0.21	1.75E-05	0.312	4.53E+01	Os.54321.1.S1_at8
34	-7.82	0.58	1.80E-05	0.312	3.25E-03	OsAffx.32348.1.S1_at6
35	-7.74	0.08	1.86E-05	0.312	4.86E-02	Os.50401.1.S1_at11
36	7.69	0.02	1.91E-05	0.312	1.50E+01	Os.50131.1.S1_at2
37	-7.68	0.16	1.96E-05	0.312	3.20E-02	OsAffx.32252.1.A1_at9
38	7.67	0.13	2.01E-05	0.312	2.60E+01	OsAffx.3839.1.S1_at11

Appendix Table A2 (continued)

SFP	d.value	stdev	rawp	q.value	R.fold	Name
39	7.66	0.14	2.07E-05	0.312	2.75E+01	OsAffx.13701.1.S1_at10
40	7.63	0.14	2.12E-05	0.312	2.70E+01	Os.27163.1.A1_at5
41	-7.61	0.23	2.17E-05	0.312	2.32E-02	Os.17983.1.S1_at9
42	7.61	0.13	2.23E-05	0.312	2.58E+01	Os.27327.1.S1_at8
43	-7.55	0.40	2.28E-05	0.312	9.95E-03	OsAffx.23064.1.S1_at6
44	7.5	0.07	2.33E-05	0.312	1.82E+01	OsAffx.13709.1.S1_at9
45	-7.46	0.05	2.39E-05	0.312	6.30E-02	OsAffx.25995.1.S1_at11
46	-7.45	0.05	2.44E-05	0.312	6.40E-02	OsAffx.3768.1.S1_at5
47	7.36	0.11	2.49E-05	0.312	2.07E+01	OsAffx.25981.1.S1_at7
48	-7.35	0.13	2.55E-05	0.312	4.46E-02	Os.50641.2.S1_a_at11
49	-7.32	0.60	2.60E-05	0.312	4.36E-03	OsAffx.32348.1.S1_at4
50	-7.3	0.23	2.65E-05	0.312	2.72E-02	OsAffx.3783.1.S1_at10
51	7.28	0.21	2.70E-05	0.312	3.39E+01	OsAffx.27426.2.S1_at3
52	7.25	0.16	2.76E-05	0.312	2.52E+01	OsAffx.13771.1.S1_x_at10
53	7.22	0.16	2.81E-05	0.312	2.54E+01	Os.53255.1.S1_at2
54	-7.21	0.22	2.86E-05	0.312	2.99E-02	OsAffx.13813.1.S1_at6
55	-7.2	0.17	2.92E-05	0.312	3.75E-02	Os.11685.1.S1_at11
56	-7.18	0.18	2.97E-05	0.312	3.65E-02	OsAffx.17249.1.S1_at9
57	7.17	0.19	3.02E-05	0.312	2.85E+01	OsAffx.13995.1.S1_at1
58	7.11	0.07	3.08E-05	0.312	1.54E+01	Os.26445.2.S1_at1
59	7.09	0.23	3.13E-05	0.312	3.35E+01	Os.53255.1.S1_at1
60	-7.07	0.47	3.18E-05	0.312	9.86E-03	OsAffx.32252.1.A1_at4
61	7.06	0.43	3.23E-05	0.312	9.02E+01	Os.27327.1.S1_at11
62	-6.99	0.24	3.29E-05	0.312	3.07E-02	OsAffx.25882.1.S1_at11
63	6.95	0.18	3.34E-05	0.312	2.44E+01	OsAffx.26123.1.S1_at8
64	6.95	0.05	3.39E-05	0.312	1.30E+01	Os.27099.1.S1_at10
65	-6.94	0.47	3.45E-05	0.312	1.05E-02	OsAffx.32348.1.A1_x_at3
66	-6.93	0.31	3.50E-05	0.312	2.20E-02	Os.11685.1.S1_at10
67	-6.92	0.24	3.55E-05	0.312	3.12E-02	Os.24417.1.S2_at11
68	-6.92	0.07	3.61E-05	0.312	7.08E-02	OsAffx.13994.1.S1_at7
69	6.92	0.07	3.66E-05	0.312	1.41E+01	OsAffx.13696.1.S1_at2
70	6.9	0.20	3.71E-05	0.312	2.61E+01	Os.11090.1.S1_at11
71	6.8	0.15	3.76E-05	0.312	1.98E+01	Os.6048.1.A1_at11
72	-6.79	0.67	3.82E-05	0.312	4.42E-03	OsAffx.32348.1.S1_at9
73	6.77	0.19	3.87E-05	0.312	2.36E+01	OsAffx.3920.1.S1_at9
74	6.77	0.18	3.92E-05	0.312	2.30E+01	Os.36056.1.A1_at8
75	-6.77	0.26	3.98E-05	0.312	3.02E-02	OsAffx.32252.1.S1_at11
76	-6.75	0.33	4.03E-05	0.312	2.23E-02	OsAffx.32190.1.S1_x_at7

Appendix Table A2 (continued)

SFP	d.value	stdev	rawp	q.value	R.fold	Name
77	6.73	0.08	4.08E-05	0.312	1.40E+01	OsAffx.3920.1.S1_at8
78	-6.72	0.29	4.14E-05	0.312	2.64E-02	OsAffx.32252.1.A1_at7
79	-6.71	0.07	4.19E-05	0.312	7.49E-02	Os.35787.2.S1_at2
80	6.7	0.08	4.24E-05	0.312	1.40E+01	OsAffx.13667.1.S1_at8
81	-6.68	0.04	4.30E-05	0.312	8.85E-02	OsAffx.26013.1.S1_at6
82	-6.67	0.64	4.35E-05	0.312	5.17E-03	OsAffx.32348.1.S1_at5
83	-6.63	0.24	4.40E-05	0.312	3.62E-02	OsAffx.29436.1.S1_at1
84	-6.62	0.20	4.45E-05	0.312	4.29E-02	OsAffx.3783.1.S1_at7
85	-6.51	0.04	4.51E-05	0.312	9.33E-02	Os.48315.1.S1_at5
86	-6.51	0.40	4.56E-05	0.312	1.87E-02	OsAffx.32251.1.A1_at11
87	-6.51	0.31	4.61E-05	0.312	2.83E-02	OsAffx.23064.1.S1_at2
88	6.49	0.03	4.67E-05	0.312	1.03E+01	OsAffx.25887.1.S1_at7
89	6.43	0.36	4.72E-05	0.312	4.28E+01	Os.52198.3.S1_at8
90	6.42	0.24	4.77E-05	0.312	2.51E+01	Os.49625.1.S1_at8
91	6.42	0.04	4.83E-05	0.312	1.02E+01	Os.52443.1.S1_at7
92	-6.34	0.10	4.88E-05	0.312	7.81E-02	OsAffx.29439.1.S1_at3
93	6.32	0.10	4.93E-05	0.312	1.29E+01	OsAffx.26152.1.S1_at9
94	-6.32	0.24	4.98E-05	0.312	4.19E-02	OsAffx.32251.1.S1_at6
95	-6.31	0.10	5.04E-05	0.312	7.83E-02	OsAffx.32252.1.S1_at5
96	-6.3	0.06	5.09E-05	0.312	9.22E-02	Os.8888.1.S1_at11
97	6.27	0.07	5.14E-05	0.312	1.09E+01	OsAffx.25900.1.S1_at9
98	6.27	0.28	5.20E-05	0.312	2.72E+01	OsAffx.26152.1.S1_at7
99	6.26	0.18	5.25E-05	0.312	1.81E+01	OsAffx.27426.2.S1_at5
100	6.25	0.16	5.30E-05	0.312	1.63E+01	Os.52198.1.S1_at10
101	-6.21	0.26	5.36E-05	0.312	4.07E-02	OsAffx.17249.1.S1_at7
102	-6.2	0.11	5.41E-05	0.312	7.76E-02	OsAffx.3789.1.S1_at11
103	6.19	0.28	5.46E-05	0.312	2.64E+01	OsAffx.26162.1.S1_at1
104	-6.19	0.09	5.51E-05	0.312	8.45E-02	Os.14086.1.S1_s_at3
105	-6.16	0.15	5.57E-05	0.312	6.55E-02	Os.50414.1.S1_at5
106	-6.13	0.10	5.62E-05	0.312	8.32E-02	OsAffx.25847.1.S1_at1
107	6.11	0.36	5.67E-05	0.312	3.59E+01	Os.44812.1.S1_at4
108	6.09	0.11	5.73E-05	0.312	1.22E+01	OsAffx.29467.1.S1_at4
109	-6.08	0.20	5.78E-05	0.312	5.73E-02	Os.51283.1.S1_at1
110	-6.06	0.53	5.83E-05	0.312	1.43E-02	OsAffx.10840.1.S1_at7
111	-6.06	0.16	5.89E-05	0.312	6.60E-02	Os.27666.1.A1_at7
112	-6.06	0.15	5.94E-05	0.312	7.01E-02	Os.35787.2.S1_at3
113	-6.05	0.19	5.99E-05	0.312	5.91E-02	Os.38286.1.S1_at5

Appendix Table A2 (continued)

SFP	d.value	stdev	rawp	q.value	R.fold	Name
114	6.04	0.22	6.04E-05	0.312	1.94E+01	OsAffx.27424.1.S1_at8
115	6.04	0.26	6.10E-05	0.312	2.23E+01	Os.10277.1.S1_at4
116	6.03	0.20	6.15E-05	0.312	1.74E+01	Os.18078.1.S1_at7
117	6.01	0.25	6.20E-05	0.312	2.15E+01	OsAffx.26190.1.S1_at2
118	-6.01	0.53	6.26E-05	0.312	1.57E-02	OsAffx.10840.1.S1_at1
119	-5.96	0.06	6.31E-05	0.312	1.05E-01	Os.54429.1.S1_at2
120	-5.94	0.76	6.36E-05	0.312	6.72E-03	OsAffx.32348.1.S1_at10
121	-5.94	0.40	6.42E-05	0.312	2.67E-02	OsAffx.32252.1.S1_at2
122	-5.91	0.44	6.47E-05	0.312	2.30E-02	OsAffx.23064.1.S1_at8
123	-5.88	0.25	6.63E-05	0.312	5.10E-02	OsAffx.29439.1.S1_at4
124	-5.87	0.13	6.73E-05	0.312	8.23E-02	OsAffx.19957.1.S1_at7
125	-5.86	0.15	6.79E-05	0.312	7.57E-02	OsAffx.18129.2.A1_at5
126	-5.86	0.50	6.84E-05	0.312	1.97E-02	OsAffx.23064.1.S1_at1
127	-5.8	0.12	7.00E-05	0.312	8.87E-02	OsAffx.13716.1.S1_at2
128	-5.79	0.56	7.05E-05	0.312	1.41E-02	OsAffx.19957.1.S1_at9
129	-5.75	0.02	7.26E-05	0.312	1.36E-01	Os.25535.1.S1_at8
130	-5.74	0.12	7.32E-05	0.312	9.03E-02	OsAffx.2364.1.S1_at11
131	-5.73	0.25	7.37E-05	0.312	5.34E-02	OsAffx.18201.1.S1_at3
132	-5.71	0.15	7.42E-05	0.312	8.17E-02	OsAffx.30398.1.S1_at4
133	-5.65	0.11	7.69E-05	0.312	9.64E-02	OsAffx.29436.1.S1_at9
134	-5.61	0.26	7.79E-05	0.312	5.51E-02	OsAffx.29436.1.S1_at3
135	-5.6	0.11	7.85E-05	0.312	1.01E-01	OsAffx.26002.1.S1_at11
136	-5.6	0.06	7.90E-05	0.312	1.20E-01	Os.24417.3.S1_at7
137	-5.59	0.38	7.95E-05	0.312	3.55E-02	OsAffx.32252.1.S1_at6
138	-5.57	0.16	8.11E-05	0.312	8.20E-02	OsAffx.32251.1.S1_at3
139	-5.57	0.37	8.17E-05	0.312	3.82E-02	OsAffx.26031.1.S1_at10
140	-5.57	0.47	8.22E-05	0.312	2.36E-02	OsAffx.25806.1.S1_at11
141	-5.54	0.28	8.27E-05	0.312	5.38E-02	Os.8888.1.S1_at8
142	-5.53	0.28	8.32E-05	0.312	5.45E-02	Os.12770.2.S1_x_at7
143	-5.53	0.06	8.38E-05	0.312	1.23E-01	Os.24417.3.S1_at11
144	-5.52	0.07	8.43E-05	0.312	1.20E-01	OsAffx.3857.1.S1_x_at8
145	-5.47	0.64	8.75E-05	0.312	1.52E-02	OsAffx.32252.1.A1_at1
146	-5.45	0.18	8.80E-05	0.312	8.26E-02	OsAffx.29492.1.S1_at5
147	-5.45	0.21	8.86E-05	0.312	7.29E-02	OsAffx.20519.2.S1_at3
148	-5.44	0.03	8.91E-05	0.312	1.41E-01	Os.44984.1.S1_at11
149	-5.43	0.11	8.96E-05	0.312	1.05E-01	Os.24417.2.S1_at1
150	-5.41	0.14	9.07E-05	0.312	9.72E-02	OsAffx.32252.1.S1_at8

Appendix Table A2 (continued)

SFP	d.value	stdev	rawp	q.value	R.fold	Name
151	-5.4	0.10	9.12E-05	0.312	1.13E-01	OsAffx.1869.2.S1_at9
152	-5.34	0.19	9.49E-05	0.312	8.10E-02	OsAffx.25911.1.S1_at9
153	-5.33	0.14	9.60E-05	0.312	9.84E-02	Os.11326.1.S1_at2
154	-5.33	0.10	9.65E-05	0.312	1.16E-01	Os.54805.2.S1_at6
155	-5.33	0.18	9.70E-05	0.312	8.79E-02	OsAffx.30405.1.S1_x_at11
156	-5.32	0.14	9.76E-05	0.312	9.99E-02	Os.35787.2.S1_at5
157	-5.32	0.45	9.81E-05	0.312	3.19E-02	OsAffx.25965.1.S1_at10

Appendix Table A3 SFPs without LOC_number

Array Element ID	Matched MSU/TIGR Gene ID	Matched KOME cDNA ID	SFP No.
Os.18078.1.S1_at	multiple	AK241601	116
Os.18164.1.S1_at	intergenic	N/A	17
Os.27163.1.A1_at	multiple	AK289041	40
Os.36056.1.A1_at	multiple	AK064569	74
Os.52198.3.S1_at	intergenic	AK065570	21, 89
Os.8888.1.S1_at	N/A	AK241453	96, 141
OsAffx.10840.1.S1_at	multiple	AK064483	2, 8, 20, 110, 118
OsAffx.13813.1.S1_at	N/A	N/A	54
OsAffx.20519.2.S1_at	N/A	N/A	147
OsAffx.23064.1.S1_at	N/A	AK063354	3, 23, 25, 43, 87, 122, 126
OsAffx.2364.1.S1_at	multiple	N/A	130
OsAffx.26002.1.S1_at	multiple	N/A	135
OsAffx.26031.1.S1_at	intergenic	N/A	139
OsAffx.29272.4.S1_at	N/A	N/A	30
OsAffx.30405.1.S1_x_at	intergenic	N/A	155
OsAffx.32251.1.A1_at	Os_Mito_orf288	N/A	86
OsAffx.32251.1.S1_at	Os_Mito_orf288	N/A	94, 138
OsAffx.32252.1.S1_at	Os_Mito_orf194	N/A	12, 16, 18, 27, 75, 95, 121, 137, 150
OsAffx.32252.1.A1_at	Os_Mito_orf194	N/A	11, 24, 37, 60, 78, 145
OsAffx.32348.1.A1_x_at	Os_Plas_orf100	AK288615	9, 28, 65
OsAffx.32348.1.S1_at	Os_Plas_orf100	AK288615	1, 4, 5, 6, 15, 34, 49, 72, 82, 120

Appendix Table A4 SFP-containing genes

Rice Gene ID	gene annotation	array element id	SFP
LOC_Os01g58039.1	uncharacterized protein ycf70	OsAffx.32190.1.S1_x_at	76
LOC_Os01g05930.1	Not found	Os.45357.1.S1_at	19
LOC_Os02g06170.1	expressed protein	Os.38286.1.S1_at	113
LOC_Os02g24626.1	uncharacterized protein ycf70	OsAffx.32190.1.S1_x_at	76
LOC_Os03g41200.1	retrotransposon protein	Os.11685.1.S1_at	55, 66
LOC_Os03g58570.1	WD domain, G-beta repeat domain containing protein	Os.44984.1.S1_at	148
LOC_Os03g61340.1	hydrolase, alpha/beta fold family domain containing protein	Os.25535.1.S1_at	129
LOC_Os03g61800.1	polygalacturonase	Os.48315.1.S1_at	85
LOC_Os03g63150.2	powdery mildew resistance protein PM3b	Os.35787.2.S1_at	79, 112, 156
LOC_Os03g63160.1	expressed protein	OsAffx.25806.1.S1_at	140
LOC_Os03g64300.1	THION30 - Plant thionin family protein precursor	Os.12770.2.S1_x_at	142
LOC_Os03g64310.1	expressed protein	Os.12770.2.S1_x_at	142
LOC_Os04g03050.1	OsSub34 - Putative Subtilisin homologue	OsAffx.25847.1.S1_at	106
LOC_Os04g03100.1	OsSub35 - Putative Subtilisin homologue	Os.26445.2.S1_at	58
LOC_Os04g04680.1	expressed protein	OsAffx.13667.1.S1_at	80
LOC_Os04g05400.1	expressed protein	OsAffx.13680.1.S1_at	31
LOC_Os04g05840.1	retrotransposon protein	OsAffx.25882.1.S1_at	62
LOC_Os04g06320.1	retrotransposon	OsAffx.25887.1.S1_at	88
LOC_Os04g06734.1	expressed protein	OsAffx.13696.1.S1_at	69
LOC_Os04g06890.1	Not found	OsAffx.25899.1.S1_at	26
LOC_Os04g06910.1	expressed protein	OsAffx.25900.1.S1_at	97
LOC_Os04g06970.1	retrotransposon protein	OsAffx.13701.1.S1_at	39
LOC_Os04g07410.1	Not found	OsAffx.13709.1.S1_at	44
LOC_Os04g07680.1	retrotransposon protein	OsAffx.25911.1.S1_at	152
LOC_Os04g07880.1	retrotransposon protein	OsAffx.13716.1.S1_at	127
LOC_Os04g08800.1	expressed protein	Os.54321.1.S1_at	33
LOC_Os04g09654.1	O-methyltransferase, putative	Os.10277.1.S1_at	115
LOC_Os04g10660.1	expressed protein	OsAffx.13758.1.S1_at	14
LOC_Os04g10740.1	retrotransposon protein	OsAffx.25965.1.S1_at	157
LOC_Os04g11030.1	expressed protein	Os.6048.1.A1_at	71

Appendix Table A4 (continued)

Rice Gene ID	gene annotation	array element id	SFP
LOC_Os04g11350.1	retrotransposon protein	Os.50401.1.S1_at	35
LOC_Os04g11430.1	disease resistance RPP13-like protein 1	OsAffx.25981.1.S1_at	47
LOC_Os04g11440.1	F-box protein interaction domain containing protein	Os.52198.1.S1_at	100
		OsAffx.13771.1.S1_x_at	52
LOC_Os04g11660.2	OsFBX119 - F-box domain containing protein	Os.54805.2.S1_at	154
LOC_Os04g11780.1	resistance protein LR10	OsAffx.3768.1.S1_at	46
LOC_Os04g12140.1	expressed protein	OsAffx.25995.1.S1_at	45
LOC_Os04g13050.1	expressed protein	OsAffx.26013.1.S1_at	81
LOC_Os04g13460.1	expressed protein	Os.50131.1.S1_at	36
LOC_Os04g13590.1	expressed protein	OsAffx.3783.1.S1_at	50, 84
LOC_Os04g14220.1	disease resistance protein RPM1	Os.27666.1.A1_at	111
LOC_Os04g14280.1	transposon protein, putative, CACTA, En/Spm sub-class	Os.51283.1.S1_at	109
LOC_Os04g14320.1	hypothetical protein	OsAffx.3789.1.S1_at	102
LOC_Os04g14654.1	Sec1 family transport protein	Os.52443.1.S1_at	91
LOC_Os04g16878.1	uncharacterized protein ycf70	OsAffx.32190.1.S1_x_at	76
LOC_Os04g20600.1	hypothetical protein	OsAffx.3839.1.S1_at	38
LOC_Os04g21820.1	OsWAK33 - OsWAK receptor-like protein OsWAK-RLP	Os.27099.1.S1_at	64
LOC_Os04g22270.1	expressed protein	Os.38286.1.S1_at	113
LOC_Os04g22280.1	hypothetical protein	OsAffx.3857.1.S1_x_at	144
LOC_Os04g24200.1	expressed protein	OsAffx.26123.1.S1_at	63
LOC_Os04g25740.1	expressed protein	Os.49625.1.S1_at	90
LOC_Os04g25784.1	expressed protein	Os.27327.1.S1_at	42, 61
LOC_Os04g25900.1	go35 NBS-LRR	Os.53255.1.S1_at	53, 59
LOC_Os04g25910.1	hypothetical protein	OsAffx.26152.1.S1_at	93, 98
LOC_Os04g26440.1	hypothetical protein	OsAffx.26162.1.S1_at	103
LOC_Os04g27430.1	terpene synthase	OsAffx.13994.1.S1_at	68
LOC_Os04g27450.1	hypothetical protein	OsAffx.13995.1.S1_at	57
LOC_Os04g27670.1	terpene synthase family, metal binding domain containing protein	OsAffx.3920.1.S1_at	73, 77
LOC_Os04g27720.1	terpene synthase	OsAffx.26190.1.S1_at	117

Appendix Table A4 (continued)

Rice Gene ID	gene annotation	array element id	SFP
LOC_Os06g02660.1	hypothetical protein	OsAffx.27424.1.S1_at	114
LOC_Os06g02710.1	expressed protein	OsAffx.27426.2.S1_at	51, 99
LOC_Os06g02730.1	aspartic proteinase nepenthesin-2 precursor	Os.51195.1.S1_at	29
LOC_Os06g03676.1	CAMK_CAMK_like.6 - CAMK includes calcium/calmodulin depedent protein kinases	Os.44812.1.S1_at	107
LOC_Os08g30470.1	expressed protein	OsAffx.29436.1.S1_at	83, 133, 134
LOC_Os08g30510.1	expressed protein	Os.11326.1.S1_at	153
LOC_Os08g30554.1	expressed protein	Os.50414.1.S1_at	105
LOC_Os08g30634.1	DC1 domain containing protein	Os.24417.1.S2_at	67
LOC_Os08g30634.2	DC1 domain containing protein	Os.24417.3.S1_at	136, 143
LOC_Os08g30634.3	DC1 domain containing protein	Os.24417.2.S1_at	149
LOC_Os08g30650.1	expressed protein	OsAffx.17249.1.S1_at	56, 101
LOC_Os08g30660.1	NB-ARC domain containing protein	OsAffx.29439.1.S1_at	92, 123
LOC_Os08g31700.1	UNE2, putative, expressed (putative carbohydrate transporter)	Os.11090.1.S1_at	70
LOC_Os08g31970.1	NHL repeat-containing protein	Os.17983.1.S1_at	41
LOC_Os08g32340.1	expressed protein	OsAffx.29467.1.S1_at	108
LOC_Os08g33488.1	OsMADS23 - MADS-box family gene with MIKCc type-box	Os.50641.2.S1_a_at	48
LOC_Os08g33520.1	expressed protein	OsAffx.29492.1.S1_at	146
LOC_Os09g17049.1	expressed protein	Os.14086.1.S1_s_at	104
LOC_Os10g04074.1	retrotransposon protein, putative	Os.14086.1.S1_s_at	104
LOC_Os10g04510.1	expressed protein	OsAffx.18129.2.A1_at	125
LOC_Os10g07548.1	wall-associated receptor kinase-like 4 precursor	Os.54429.1.S1_at	119
LOC_Os10g10140.1	hypothetical protein	OsAffx.18201.1.S1_at	131
LOC_Os10g12190.1	nodulin	OsAffx.30398.1.S1_at	132
LOC_Os10g21196.1	chloroplast 30S ribosomal protein S16	OsAffx.32326.1.S1_x_at	32

Appendix Table A4 (continued)

Rice Gene ID	gene annotation	array element id	SFP
LOC_Os10g21216.1	uncharacterized protein ycf70	OsAffx.32190.1.S1_x_at	76
LOC_Os12g34104.1	hypothetical protein	OsAffx.32291.1.A1_at	22
LOC_Os12g34120.1	hypothetical protein	OsAffx.19957.1.S1_at	7, 10, 13, 124, 128
LOC_Os12g34148.1	expressed protein	OsAffx.1869.2.S1_at	151

Appendix Table A5 Functional classification of predicted SFPs

Rice Gene ID	gene annotation
unknown	
LOC_Os02g06170.1	expressed protein
LOC_Os04g04680.1	expressed protein
LOC_Os04g05400.1	expressed protein
LOC_Os04g06734.1	expressed protein
LOC_Os04g06910.1	expressed protein
LOC_Os04g10660.1	expressed protein
LOC_Os04g11030.1	expressed protein
LOC_Os04g12140.1	expressed protein
LOC_Os04g13460.1	expressed protein
LOC_Os04g22270.1	expressed protein
LOC_Os04g24200.1	expressed protein
LOC_Os04g25784.1	expressed protein
LOC_Os08g30510.1	expressed protein
LOC_Os08g30554.1	expressed protein
LOC_Os08g30650.1	expressed protein
LOC_Os08g33520.1	hypothetical protein
LOC_Os04g14320.1	hypothetical protein
LOC_Os04g20600.1	hypothetical protein
LOC_Os04g25910.1	hypothetical protein
LOC_Os04g26440.1	hypothetical protein
LOC_Os12g34120.1	hypothetical protein
LOC_Os04g06890.1	hypothetical protein OsI_14744
LOC_Os04g25740.1	hypothetical protein OsJ_14256
LOC_Os08g32340.1	hypothetical protein OsJ_27331
LOC_Os09g17049.1	AC092553_18Unknown protein
LOC_Os10g10140.1	hypothetical protein_ac093181_6 polyprotein
LOC_Os01g05930.1	Not found
LOC_Os02g24626.1	uncharacterized protein ycf70, putative
LOC_Os04g16878.1	uncharacterized protein ycf70, putative
LOC_Os10g21216.1	uncharacterized protein ycf70, putative
LOC_Os01g58039.1	uncharacterized protein ycf70, putative, expressed
transposon and retrotransposon	
LOC_Os04g27450.1	retrotransposon line subclass
LOC_Os06g02660.1	retrotransposon line subclass
LOC_Os03g41200.1	retrotransposon protein, putative, unclassified, expressed
LOC_Os04g05840.1	retrotransposon protein, putative, unclassified, expressed
LOC_Os04g06970.1	retrotransposon protein, putative, unclassified, expressed
LOC_Os04g07680.1	retrotransposon protein, putative, unclassified, expressed
LOC_Os04g07880.1	retrotransposon protein, putative, unclassified, expressed

Appendix Table A5 (continued)

Rice Gene ID	gene annotation
LOC_Os04g10740.1	retrotransposon protein, putative, unclassified, expressed
LOC_Os04g11350.1	retrotransposon protein, putative, unclassified, expressed
LOC_Os10g04074.1	retrotransposon protein, putative, unclassified, expressed
LOC_Os04g08800.1	retrotransposon unclassified
LOC_Os08g30470.1	retrotransposon unclassified
LOC_Os12g34104.1	retrotransposon unclassified
LOC_Os04g07410.1	retrotransposon unclassified
LOC_Os04g06320.1	retrotransposon, putative, centromere-specific, expressed
LOC_Os04g13590.1	transposon en spm sub-class
LOC_Os04g22280.1	transposon en spm sub-class
LOC_Os04g13050.1	transposon mutator sub-class
LOC_Os04g14280.1	transposon protein, putative, CACTA, En/Spm sub-class, expressed
disease resistance protein	
LOC_Os04g14220.1	disease resistance protein RPM1, putative, expressed
LOC_Os04g11430.1	disease resistance rpp13-like protein 1-like
LOC_Os10g04510.1	nb-arc domain containing protein
LOC_Os08g30660.1	NB-ARC domain containing protein, expressed
LOC_Os04g25900.1	NBS-LRR, putative, expressed
LOC_Os03g63150.2	powdery mildew resistance protein PM3b, putative, expressed
LOC_Os04g11780.1	resistance protein LR10, putative, expressed
metabolic enzyme	
LOC_Os12g34148.1	atpase subunit 1
LOC_Os03g61340.1	hydrolase, alpha/beta fold family domain containing protein, expressed
LOC_Os10g12190.1	nodulin, putative, expressed
LOC_Os04g09654.1	O-methyltransferase, putative, expressed
LOC_Os04g03050.1	OsSub34 - Putative Subtilisin homologue, expressed
LOC_Os04g03100.1	OsSub35 - Putative Subtilisin homologue, expressed
LOC_Os03g61800.1	polygalacturonase, putative, expressed
LOC_Os04g27670.1	terpene synthase family, metal binding domain containing protein, expressed
LOC_Os04g27430.1	terpene synthase, putative, expressed
LOC_Os04g27720.1	terpene synthase, putative, expressed
protein kinase and phosphatase	
LOC_Os06g03676.1	CAMK_CAMK_like, protein kinases, expressed
LOC_Os08g31970.1	NHL repeat-containing protein, putative, expressed
LOC_Os04g21820.1	OsWAK33 - OsWAK receptor-like protein OsWAK-RLP, expressed
LOC_Os10g07548.1	wall-associated receptor kinase-like 4 precursor, putative, expressed

Appendix Table A5 (continued)

Rice Gene ID	gene annotation
transcription factor	
LOC_Os08g30634.1	DC1 domain containing protein, expressed
LOC_Os06g02710.1	transcription factor_nam-like protein
LOC_Os08g33488.1	OsMADS23 - MADS-box family gene with MIKCc type-box, expressed
LOC_Os03g64310.1	transcriptional corepressor leunig
LOC_Os03g64300.1	transcriptional corepressor leunig
LOC_Os03g58570.1	WD domain, G-beta repeat domain containing protein, expressed
transporter	
LOC_Os04g14654.1	Sec1 family transport protein, putative, expressed
LOC_Os08g31700.1	UNE2, putative, expressed (putative carbohydrate transporter)
other	
LOC_Os10g21196.1	chloroplast 30S ribosomal protein S16, putative, expressed
LOC_Os04g11660.2	F-box domain containing protein, expressed
LOC_Os04g11440.1	F-box protein interaction domain containing protein, expressed
LOC_Os03g63160.1	nitrate-induced noi protein
LOC_Os06g02730.1	aspartic proteinase nepenthesin-2 precursor, putative, expressed

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