Proteomic analysis of isogenic rice lines under brown planthopper infestation

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ABSTRACT: The brown planthopper (BPH) is a major rice pest in Thailand. In this study, comparative proteomic analysis was applied to investigate total protein expression of rice plants under BPH infestation. Proteomic profiles of 2 contrasts BC_3F_5 isogenic lines, including BIL15 (resistant-harboring QTL from Chr6) and BIL7 (susceptible-without any QTL) derived from Rathu Heenati (RH, BPH-resistant-donor) and KDML105 (Thai Jasmine rice-BPH-susceptible-recipient) were compared. The total expressed proteins were extracted from all the above ground portion of 25 day-old seeding, with and without BPH infestation at 3 and 24 hours after infestation (HAI). Proteomic analysis could define some interested candidate BPH responsive proteins specifically expressed in the resistant rice line (PSER). For early response investigation, the PSER under BPH treatment at 3 hours after infestation (PSER3) were focused and identified some interesting proteins such as wall-associated receptor kinase 2 (WAK2), serine/threonine-protein kinase OXI1 (OXI1) and TPR domain containing protein. WAK2 could perceive free calcium ion or pectin leaked from wounding cell wall following transfer signaling to MAPK cascade by its kinase activity. OXI1 contained kinase activity might play a role in MAPK signaling pathway. TPR domain containing protein could facilitate BPH defense through wax production which perturb insect-plant physical interaction. In conclusion, the identified PSER3 might initiate plant immune system through MAPK cascades. MAPK signaling might regulate expression of some defense related-functional proteins and related-transcription factors. The information obtained could further contribute for selecting potential candidate genes to develop effective functional markers. The potential resistance genes could facilitate for BPH resistant rice improvement using marker-assisted selection or genetic engineering.
Keywords: BPH; expressed protein; WAK2; TPR; OXI1

Introduction

Rice is an important cereal and a source of energy for nearly half of the world population (Fairhurst and Dobermann, 2002). Rice productivity is decreased by several biotic and abiotic factors (Anami et al., 2020). An approximate one-fifth of the global rice production is lost due to attack of insect pests annually (Sharma et al., 2017).

One of the most important rice pests is brown planthopper (*Nilaparvata lugen* (Stål), BPH) which can cause significant yield losses by destruction of rice plants and transmission of rice viruses such as ragged stunt virus and grassy stunt virus (Huang et al., 2015).

BPH is a typical vascular feeder mainly sucking phloem sap. Both of nymphs and adults of BPH can attack the rice plants at all stages of plant growth and prefer staying at the lower part of the plants and suck the plant sap from stem and leaf sheath. The damage caused by BPH is popularly known as 'hopper burn' symptom (Sogawa and Cheng, 1979). BPH infestations have increased in Asian rice growing countries since 2003 (Watanabe et al., 2009; Hu et al., 2014). The largest outbreak of BPH in Thailand has occurred during 2009-2010 covered around 380,000 hectare in central and lower northern regions. BPH outbreak has recurrence and damaged around 268,000 hectare in 2011(Sriratanasak et al., 2020).

Conventional approaches for controlling BPH are mainly use of chemical. However, extensive use of chemical insecticides has generated several problems including safety risks for human and domestic animals. Furthermore, their repeated use has led to the development of resistance in BPH populations (Wu et al., 2018). According to disadvantages of using chemical insecticides, the application of host plant resistance is considered to be an alternative and effective way for controlling BPH. Therefore, developments of resistant rice cultivars through host plant resistance become a key approach for the BPH management (Stout, 2014).

Up to now, several BPH resistance loci have been identified and reported (Jena and Kim, 2010; Hu et al., 2016). Liu et al. (2014) have successfully cloned a cluster of 3 BPH resistance genes located in chromosome 4 of Rathu Heenati, a strong BPH resistance cultivar from Sri Lanka, and identified as *Bph3*. The identified *Bph3* on chromosome 4, encoding lectin receptor kinases (OsLecRK1-3), are transmembrane proteins. The OsLecRK proteins may play roles in priming the pattern-triggered immunity response to BPH feeding (Liu et al., 2014). Recently, a novel BPH resistance gene *Bph32*, located in chromosome 6 has been successfully cloned from PTB33 rice variety. *Bph32* gene encoded a short consensus repeat (SCR) domain-containing protein is localized in plasma membrane of the leaf sheath cells. The plasma membrane-localized *Bph32* may play a critical role in resistant rice by inhibiting BPH infestation (Ren et al., 2016). Several BPH resistance genes have been discovered and localized in rice genome. However, understanding of rice plant response to the BPH has still discussed.

In Thailand, 4 of BPH resistance genes, which are *Bph1, bph2, Bph3* and *bph4* have been widely used for decades in rice breeding programs. However, some widely cultivated rice varieties containing BPH resistance loci such as *Bph1* and *bph2* are rapidly broken down in just a few years (Jairin, 2008). Interestingly, Rathu Heenati carrying BPH resistance gene *Bph3* used as a donor in Thai rice breeding programs for more than 30 years are still maintained resistance to BPH (Jairin et al., 2007; Jairin, 2008). The BPH resistant introgression lines developed from the backcross between Rathu Heenati and KDML105 have been utilized for BPH resistant rice improvements and rice-BPH defense response investigation (Jairin et al., 2009; Kamolsukyunyong et al., 2013; Kusumawati et al., 2018; Kamolsukyunyong et al., 2019; Uawisetwathana et al., 2019). The observed BPH resistant phenotypes of this introgressed population have not been as strong as that of donor Rathu Heenati and likely to be normal distribution. The observations suggested

other BPH resistant loci existing in a donor Rathu Heenati and the effective BPH resistance regulated by many genes or QTLs (Jairin et al., 2009; Kamolsukyunyong et al., 2013; Kusumawati et al., 2018).

Mode of molecular mechanism of BPH resistance in rice has been discussed. When BPH feed on rice, rice pattern-recognition receptors (PRRs) such as Bph15 and Bph32 primarily recognize herbivore-associated molecular patterns (HAMPs) or damage-associated molecular patterns (DAMPs) are activated. Some BPH resistant proteins together with transcription factors stimulate the salicylic acid (SA) signaling pathway, up-regulate SA-responsive defense gene expression, up-regulate trypsin protease inhibitors (TryPIs) and phytoalexin, and induce callose deposition (Du et al., 2020). SA and JA defense mechanisms have synergism in rice plants carrying the BPH6 resistance gene (Guo et al., 2018). However, molecular mechanisms of rice plants initiate BPH resistance and downstream events have remained under investigation.

Proteomic analysis is the large-scale and systematic technique to investigate a complete set of proteins expressed by a genome in a given time (Wilkins et al., 1996). Proteomics has served as a powerful tool to determine physiological changes at cellular level, analyze biological processes and compare proteomes changes under different stresses (Agrawal et al., 2009). Proteomic analysis is growing considerably and widely applied for investigation of various features of plant protein function. BPH responsive proteins in rice has been investigated by proteomics using isogenic lines originated from the susceptible variety *Taichung Native* 1 and the resistant variety containing the BPH resistance gene *Bph15*. *BPH15* gene was a major BPH resistance gene mapped on chromosome 4 (Yang et al., 2004; Wei et al., 2009). Their results showed the significant changes in expressed proteins involved in multiple pathways including jasmonic acid synthesis proteins, oxidative stress response proteins, beta-glucanases protein, kinases, clathrin protein, glycine cleavage system protein, photosynthesis proteins and aquaporins (Wei et al., 2009).

This research was performed to investigate response in terms of alterations of protein expression in 2 contrasts isogenic lines, resistant-harboring QTL from Chr6 and susceptible-without any QTL, during BPH infestation by proteomics analysis. The proteomic analysis provided important clues to the function and responsiveness closely reflected biological events going on at an assay time point in a system. The obtained results provided insight information on molecular basis and protein networks of BPH resistance in rice contributed to BPH resistant QTL in Chromosome 6. The understanding of molecular interaction between BPH and rice could facilitate BPH management. Some BPH responsive proteins might have potential to be candidates for molecular marker development and genetic engineering for the development of rice lines resistant to BPH.

Materials and Methods

Materials

Plant materials

Plant materials used for proteomics analysis were the 2 BC_3F_5 isogenic lines (BILs) from backcrossing between a BPH susceptible KDML105 and a BPH resistant Rathu Heenati (Jairin, 2008). These 2 BILs were UBN03078-80-354-7 (BIL7, without any QTL, BIL7^{-6}) and UBN03078-80-354-15 (BIL15, contained QTL from Chromosome 6, BIL15^{+6}). BIL7 showed susceptible reaction to BPH collected from Ubon Ratchathani province (UBN-BPH), while BIL15 showed

resistance against UBN-BPH (Jairin et al., 2009; Kusumawati et al., 2018; Kamolsukyeunyong et al., 2019). The genetic background of the BIL7 and BIL15 was analyzed by single-feature polymorphisms included BPH resistance gene markers at chromosome 4 and 6. BIL7 contained approximately 100% recovery of KDML105 recurrent recipient genome. BIL15 recovered approximately 96% with the KDML105 genetic background (Kamolsukyunyong et al., 2013; Kusumawati et al., 2018). Apart from the BPH resistance introgressed traits, the other traits were very similar. These plant materials were obtained from Rice Gene Discovery Laboratory, Kasetsart University.

Insect materials

The BPH used for infestation was collected from Ubon Ratchathani province and maintained on the susceptible cultivar *Taichung Native* 1 (TN1). Insect multiplication was done by mass rearing on tillering stage of TN1 inside a wire screen cage at a natural greenhouse (Jairin et al., 2005; Jairin, 2008).

Methods

BPH treatment and sample collection

Rice planting and BPH treatments were modified from two previous reports with 3 biological replications (Wei et al., 2009; Uawisetwathana et al., 2019). Germinating seeds of each rice variety were grown in an individual seedboxes with 2 plants per pot (2 pots per replication) covered with fine nylon mesh in a greenhouse with natural light condition. Approximately one week before BPH inoculation, each entry was divided into 2 groups (with and without BPH treatment). Each treatment entry was separately moved into each new fine mesh cage. The control groups without BPH treatment were separated maintained in another fine mesh cage. For UBN-BPH infestation, 25-day-old seedling rice was consistently inoculated with 2nd-3rd instar of UBN-BPH nymphs at a density of approximately 20 insects per seedling.

For protein extraction and analysis, all above the ground portion of rice seedling were harvested at 2 time points after BPH infestation, 3 hours after infestation (HAI) and 24 HAI. The corresponding parts of control plants were collected at the same time points. All of collected samples were washed, dried and immediately stored in liquid nitrogen. The phenotypic variation of tested plants was observed every other day up to 11 days after infestation (DAI) or until all of susceptible BIL7 under BPH treatment was dead.

Proteomic analysis by LC MS/MS

Total proteins in rice seedling were extracted according to TCA/acetone precipitation protocol (Wu et al., 2014). The 12.5% gel SDS-PAGE was performed to pre-fractionate 15 μg of total extracted rice proteins (Laemmli, 1970). Tryptic digestion was performed to digest total in-gel proteins into peptides combination. The digested peptides were subjected to LC-MS/MS analysis (Shevchenko et al., 2007; Abere et al., 2012). LC-MS/MS analysis of the peptides were executed for 3 replication into an Ultimate 3000 Nano and Capillary LC System (Thermo Fisher Scientific) coupled to a Q-Tof impact II™ (Bruker Daltonics) equipped with a Nano-captive spray ion source (Turathum et al., 2020). The mass spectra were acquired using Bruker Compass DataAnalysis 4.4 software (Bruker Daltonics).

Proteins identification and characterization

Differential Analysis Software (DeCyderMS), the automated image analysis software provided by GE Healthcare, was used to analyze the raw mass spectra obtained from LC-MS/MS analysis (Johansson et al., 2006; Thorsell et al., 2007). The DeCyderMS resolved data were further submitted to the Mascot software provided by Matrix Science for protein identification against protein database (Perkins et al., 1999). The identified proteins were confidence when at least one peptide showed an individual mascot score corresponding to $P < 0.05$. The number of expressed proteins between BPH treatment and control conditions were filtered using Venn diagram software available online at http://bioinformatics.psb. ugent.be/webtools/Venn/ (Ruskey and Weston, 2012). Protein functions and classifications were determined through the Universal Protein Resource (UniProt) available at https://www.uniprot.org/ which the databases are the UniProt Knowledgebase (UniProtKB), the UniProt Reference Clusters (UniRef), and the UniProt Archive (UniParc) (The UniProt Consortium, 2018). The predicted protein networks were conducted by Search Tool for Interacting Chemicals (STITCH) accessible at http://stitch.embl.de/ (Szklarczyk et al., 2016). Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis available at https://www.genome.jp/kegg/tool/map_pathway1.html was used to predict the biologically pathway (Kanehisa and Goto, 2000).

Results and discussions

Rice phenotypes during BPH infestation used for proteomic analysis

In this study, the difference of total protein expression between a resistant BIL15 carrying BPH resistant QTL in chromosome 6 and a susceptible BIL7 without any BPH resistant QTL was investigated. Phenotypes of the two isogenic lines in treatment and control groups were similar before BPH infestation. No apparent damage was observed on the resistant BIL15 and a susceptible BIL7 at sample collected time points, 3HAI (**Figure 1 (a, b)**) and 24 HAI (**Figure 1 (c, d)**), probably due to too early to see the damage phenotypes. These assay time points were examined to identify proteins involved in early response against BPH attack. After BPH attack for 11 days, the tested resistant BIL15 was slightly damaged whereas a susceptible BIL7 was completely dead (**Figure 1 (e, f)**). This result indicated that QTL in chromosome 6 with about 4% of RH genetic background was highly resistant against BPH feeding whereas a susceptible BIL7 without any BPH resistant QTL was completely sensitive to BPH.
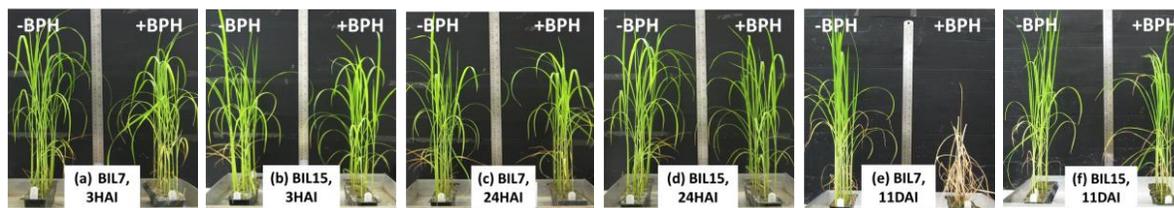

Figure 1 Phenotype observation of BIL7 and BIL15 seedling at 3 HAI (a, b), 24 HAI (c, d) and 11 DAI (e, f)

Proteomic analysis of brown planthopper response in rice seedling

Proteomic analysis was conducted to identify responsive proteins in rice between BPH resistant and susceptible isogenic lines during BPH infestation. A total of 1,687 expressed rice proteins were detected based on one-dimensional gel electrophoresis and LC-MS/MS analysis. All identified proteins were subjected to Venn diagram analysis to visualize common and specifically expressed proteins in each experimental condition. The expressed proteins in comparisons between control and BPH treatment of each rice cultivar were displayed in **Figure 2**. The numbers of protein specifically expressed under BPH treatments were indicated in yellow circles. Most of total expressed proteins of each tested line at each time point were similar under control and treatment conditions except for BIL7 at 3HAI. Approximately 90 BPH responsive proteins were specific for BIL15 at 3 HAI and 24 HAI and for BIL7 at 24 HAI under BPH attack, whereas 249 proteins specific for susceptible BIL7 under BPH treatment at 3 HAI. Expressed proteins presented only under BPH treatment were selected for further analysis.
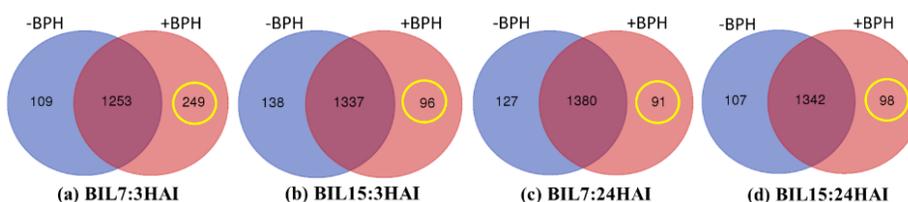

Figure 2 Venn diagrams of total expressed proteins in rice seedling under BPH control and treatment conditions. Blue circle represented total expressed proteins from control experiment (without BPH). Pink circle represented total expressed proteins under BPH infestation condition. Expressed proteins at 3 HAI of BIL7 (a) and BIL15 (b). Expressed proteins at 24 HAI of BIL7 (c) and BIL15 (d). Yellow circles indicated proteins specifically expressed under UBN-BPH attack

The proteins specifically expressed under BPH treatments indicated by yellow circles in **Figure 1** were re-subjected into Venn diagram analysis. All expressed proteins triggered under UBN-BPH treatments were determined as shown in Venn diagram in **Figure 3**. Under BPH treatments, 194 and 49 proteins were specifically expressed in BIL7 at 3 HAI and 24 HAI, respectively. Additionally, 50 and 59 proteins were specifically expressed in BIL15 under BPH infestation at 3 HAI and 24 HAI, respectively. The BPH responsive proteins that specifically expressed in the resistant rice cultivar (BIL15) at 3 HAI indicated by red circle in Figure 3 were chosen as the expressed proteins of interest.
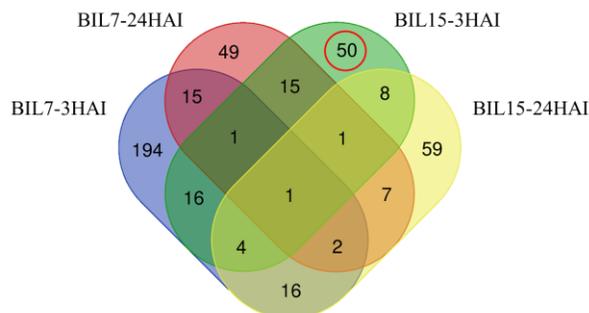

Figure 3 Venn diagram in comparison of expressed proteins under BPH treatment at 3 HAI and 24 HAI

Fifty proteins specifically expressed in a resistant isogenic BIL15 at 3 HAI (PSER3) were chosen as the expressed proteins of interest in this study because these proteins were considered early responsive proteins for BPH resistance. These PSER3 were subjected to functional classification based on Gene Ontology analysis using UniProt database. Classification of PSER3 was shown in **Figure 4**. The predicted Gene Ontology of this protein set included biological process 36%, molecular function 54%, cellular component 46%, metabolic pathway 4% and unknown function 30%. It was found that most PSER3 induced by UBN-BPH attack in a resistant BIL15 played role in molecular function (27 PSER3) and cellular component (23 PSER3). The results showed that most of identified PSER3 had known functions and nearly half of them were cellular components and involved in some molecular functions.
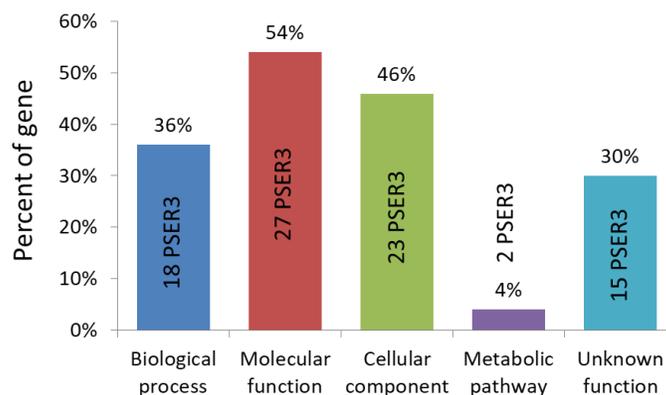

Figure 4 Gene ontology classification of proteins specifically expressed in a resistant BIL15 at 3 HAI (PSER3) under UBN-BPH infestation. The percentages of expressed proteins classified into a group were indicated at top of the bars

The maximum number of PSER3 was the molecular function. Several of them played role more than 1 function. The PSERs were mostly categorized as nucleotide binding proteins such as putative PHD finger family protein (gi|14626277), uncharacterized protein (gi|218197064) and uncharacterized protein (gi|62734281). The predicted molecular function of PSER3 was mainly related to nucleic acid binding activity categorized into DNA binding, RNA binding and nucleic acid binding. Of them, DNA binding category was predominant. Some of them might play role in regulation of transcriptions as well as involved in DNA replication/repair process.

In addition, the protein-protein and protein-ligand networks of some PSER3 nucleotide binding proteins were analyzed using STITCH database. The protein network of a putative PHD finger family protein corresponded to Stitch id. 4334099 was shown in **Figure 5**. A putative PHD finger family protein was predicted to interact with several proteins such as putative acyl carrier protein (4347723), COBRA protein (4338658), and MYB family transcription factor (GAM1). A putative PHD finger family protein might play role in transcription corepressor activity itself or interact with a putative COBRA protein which probably involved in cellulose synthesis and deposition (Ko et al., 2006; Polko and Kieber, 2019). Increasing cellulose synthesis contributed to callose deposition at the BPH-sucking site on cell surface (Gupta et al.,

2019). This predicted protein network of a putative PHD finger family protein supported the proposed BPH resistant mechanism in the resistant rice (Hao et al., 2008).
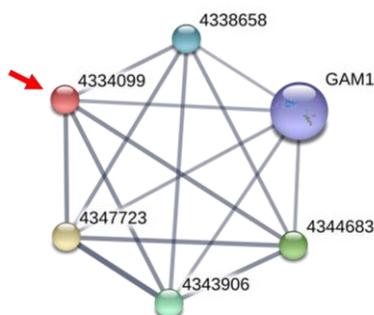

Figure 5 The predicted protein networks of putative PHD finger family protein (4334099). (4338658 = COBRA protein, GAM1 = MYB family transcription factor, 4344683 = transaldolase, 4343906 = 3-oxoacyl-synthase, 4347723 = acyl carrier protein)

The second large group in molecular function category was ATP and ADP binding proteins containing kinase activity such as wall-associated receptor kinase 2 (gi|1002260031, WAK2), serine/threonine-protein kinase OXI1 (gi|1002239884, OXI1) and serine/threonine-protein kinase TIO (gi|1002309783).

WAK2 which contained kinase activity and calcium ion binding activity was membrane-bound protein. WAK2 might played role in cell surface receptor signaling pathway. Previous study suggested that WAK2, in *Arabidopsis thaliana*, played a significant role in signaling pathway responded by the extracellular released pectin. *At*WAK2 stimulated both plant defense and development through MAPK cascade-mediated regulation (Kohorn et al., 2009).

OXI1 was found in cytoplasm, nucleus and plasma membrane. OXI1 which contained kinase activity was defined to play role in MAPK signaling pathway. In *Arabidopsis thaliana*, expression of OXI1 was induced by hydrogen peroxide, which was a widely recognized ROS molecule (Rentel et al., 2004). Respiratory burst NADPH oxidase produced ROS molecule which activated OXI1 expression in *Arabidopsis thaliana* during pathogen infection (Petersen et al., 2009). *At*OXI1 was imperative for MAPK cascade besides activated by hydrogen peroxide or cellulase elicitor (Rentel et al., 2004; Petersen et al., 2009).

The PSER3 classified into oxidoreductase activity category consisted of the cytochrome P450 71D10-like protein (gi|1002306225, CYP 71D10) and aldo-keto reductase family 4 member C10 isoform X2 (gi|1002271571). These 2 enzymes were involved in electron transport chains.

CYP71D10 contained monooxygenase and oxidoreductase activity which catalyzed oxidation-reduction reaction of ROS molecules. CYP71D10 was a member in the largest enzymatic protein superfamily of plants (Xu et al., 2015). Previous phylogenetic study of 727 Cytochrome P450 genes and pseudogenes from a monocot and a dicot classified Cytochrome P450 into 10 clans. The preliminary metabolic pathway of CYP71 clan was consecutive confirmed to be involved in the modification of shikimate products and intermediates (Nelson et al., 2004; Xu et al., 2015). The CYP71D8

and CYP71D9 were reported as elicitor-inducible glyceollin biosynthesis regulators. Glyceollin was commonly acknowledged as a key phytoalexin in soybean (Schopfer and Ebel. 1998). Phytoalexins were plant commonly lipid-derived metabolic compounds. Phytoalexin biosynthesis and accumulation at wounded zones involved in plant defense responses (Kuć and Rush, 1985).

Insight 46% of predicted cellular component proteins, approximately one-third mainly condensed as membrane components. Of them, three of PSER3 membrane proteins might play role in protein phosphorylation process (WAK2, OXI1 and Serine/threonine-protein kinase TIO (gi|1002309783)).

Additionally, the membrane component protein indole-3-acetate beta-glucosyltransferase (gi|1002265065, IAA-Glu) functioned in catalysis the transfer of a glycosyl group from a UDP-sugar to another small hydrophobic molecule. According to previously proposed enzyme-based *Arabidopsis* phytohormone crosstalk network, IAA-Glu could converse UDP-D-glucose and IAA to indole-3-acetyl-beta-1-D-glucose. IAA was the major auxin natural form in plants. This catalysis reaction by IAA-Glu resulted in decreasing auxin and JA levels in the line with IAA conjugate biosynthesis superpathway (Yue et al., 2016). This result indicated that IAA-Glu induced defense response by negative regulation of JA signaling pathway.

Within the biological process category, the PSER3 were mostly involved in regulation of transcription process such as putative PHD finger family protein (gi|14626277) and TPR domain containing protein (gi|110289525). TPR domain containing-protein (gi|110289525) was annotated to involve in positive regulation of wax biosynthetic process. A wax layer, an extracellular lipophilic layer, was commonly known as a cuticle layer in plant. Permeability of wax layer affected defense against pathogen and environmental stress (Lewandowska et al., 2020).

Pathway analysis enriched by KEGG pathway database identified 17 pathways related to 2 PSER3. Serine/threonine-protein kinase OXI1 was played role in MAPK signaling pathway. Aspartate aminotransferase (gi|584706) was classified to participate in several metabolic and biosynthetic pathways included biosynthesis of secondary metabolites, carbon fixation in photosynthetic organisms, biosynthesis of several amino acids (arginine, phenylalanine, tyrosine and tryptophan), metabolism of several amino acids (alanine, aspartate, arginine, cysteine, glutamate, proline, phenylalanine, tyrosine and methionine) and biosynthesis of isoquinoline, tropane, piperidine and pyridine alkaloid.

According to molecular mechanism of BPH resistance in rice proposed by Du et al., (2020), early events of rice response to BPH starting from rice plants perceived BPH infestation at plasma membrane. Our proteomic study identified some candidate PSER3 remarkable for early defense response mechanism. The identified PSER3 might initiate inducible responses through signal transduction pathway (WAK2, CYP71D10, OXI1and IAA-Glu) and transcriptional or translational regulation (TPR domain containing protein and putative PHD finger family protein). Some identified PSER3 (WAK2, CYP71D10 and OXI1) might play role as wound-induced defense response proteins by perceiving receptors for HAMPs or DAMPs. The candidate PSER3s might initiate signaling for downstream resistant mechanisms or play role in plant defense response mechanism through the modulation of phytohormone signaling. Results indicated that effective resistance of a BPH resistant BIL15 carrying BPH resistant QTL in chromosome 6 was not achieved by only *Bph32* but contributed with many genes or QTL.

Conclusions

Proteomic analysis of 2 contrasting BPH responsive isogenic lines, derived from a cross of BPH resistant donor Rathu Heenati and Thai premium rice recurrent recipient KDML105 parents, identified some candidate BPH defense-responsive proteins. The proteins specifically expressed in a resistant rice line under BPH attack at 3 HAI were considered for early defense response investigation. The predicted protein functions and networks provided insight information on molecular mechanism of rice resistance to BPH. Some membrane-bound proteins PSER3 were triggered when BPH attack subsequently activated MAPK cascade by several protein kinases. MAPK signaling activated plant innate immunity system and induced related protein expressions. Some identified PSER3 could be used for development of functional markers used as marker-assisted selection in BPH resistant rice breeding program such as WAK2 and OXI1. In addition, gene expression and molecular functions of the candidate PSER3 should be further verified. Some of PSER3 and other differentially expressed protein coding genes might be potential candidate functional markers used as marker-assisted selection for future BPH resistant rice improvement.

Acknowledgements

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