TRANSCRIPTOMIC AND PROTEOMIC STUDIES TO INVESTIGATE THE BASAL STEM ROT DISEASE IN OIL PALM SEEDLINGS

DAIM, L D J¹; TAN, B A¹; ITHNIN, N¹; OOI, T E K²; NORMAHNANI, M N³; APPLETON, D R¹ and HARIKRISHNA, K⁴

ABSTRACT

When oil palm is exposed to, and infected by Ganoderma boninense, the initial defence system is launched in the roots to reduce the damage caused by the disease. The present work described the transcript and protein profiles in roots of 18 month-old oil palm seedlings that were exposed to the fungal pathogen for 12 months, following artificial inoculation at 6 month-old. Three different phenotypes were observed; control (uninoculated), asymptomatic, and symptomatic (inoculated). It was found that the transcripts from the ubiquitin-mediated proteolysis pathway were common in control and asymptomatic seedlings; while proteins involved in cellular processes, and protein and sugar metabolisms were higher in abundance in asymptomatic seedlings. The transcripts involved in carbon fixation, glycolysis/gluconeogenesis, and pyruvate metabolism together with proteins responsible for stress response were identified in symptomatic seedlings. By integrating these omics data, it was observed that symptomatic seedlings were moving towards generating and storing energy for a possible defence strategy, and at the same time emitting stress signals and responses. This was in contrast with asymptomatic seedlings where regular functions such as cellular processes and carbohydrate metabolisms were found to be active.

Keywords: asymptomatic, disease tolerance, oil palm, omics technologies, symptomatic.

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INTRODUCTION

The basal stem rot (BSR) disease, caused by Ganoderma boninense, is prevalent in Malaysia’s oil palm plantations, especially in the coastal region (Mercière et al., 2017). The transmission and control of the disease are still ambiguous, thus making an accurate diagnosis and carrying out suitable treatments difficult (Hushiarian et al., 2013). The accepted mode of invasion by Ganoderma into the oil palm is through its massive network of roots. The fungal pathogen will attack the root at a point of entry, and slowly reaches the base of the oil palm (Pilotti, 2005; Rees et al., 2009).

There have been many unsuccessful attempts to investigate the thorough infection process at the point of entry at the roots and approaching the bole, up towards the effects of the disease by way of foliar symptoms. Most earlier studies had compared diseased and non-diseased (control vs. treated) young seedlings, following a short exposure time using a specific technology e.g., transcriptome or genome sequencing, where the data are subsequently analysed using a combination of bioinformatic tools (Ho et al., 2019; Qistina Othman et al., 2018). Protein studies related to BSR were also conducted on leaf and root tissues (Daim et al., 2015a; 2015b).

Other than targeting candidates that are frequently studied in plant root-fungal interaction, plant immunisation is an alternative process for studying the defence system induced by biotic or
abiotic stresses. In this case, asymptomatic seedlings, those that do not exhibit foliar symptoms following artificial inoculation with *Ganoderma*, could be considered as ‘immunised’ against the disease. Other scientists classify an asymptomatic host as ‘resistant’ to a specific disease, while a symptomatic host as ‘susceptible’. Nevertheless, asymptomatic or resistant plants could still host the pathogen as the latter grows as a biotroph (Crone et al., 2013).

Omics technologies have long been used in many plant research areas, especially in investigating crop diseases, and understanding how these diseases affect crop health and yield. Previous studies were often conducted using a single method to probe into abiotic or biotic stresses (Bahari et al., 2018; Dai et al., 2019). The findings revolved around either gene expression/regulatory analysis, protein identification, interaction network, or metabolite profiling in the target tissues. A subset of these findings was then routinely validated through various methods. The major findings of omics experiments are the presence and expressions of these specific candidate genes (genomics), proteins (proteomics), or metabolites (metabolomics) in the target tissues.

The research using a multi-omics approach by combining several omics technologies to investigate disease ecology has allowed detailed representations of plant-disease interactions and plant-disease and environment interactions (Crandall et al., 2020; Neik et al., 2020). The plant stress response and defence system can be better understood by integrating data from multi-omics experiments. Specific functions that are expressed in a combined network are far more informative as compared to functions that are obtained using individual omics approaches.

The major problem in the estimation of root disease progression is connected to the fact that data are only collected when the oil palm is destructed. Observations on the changes in the site-specific tissues will then be determined. However, once the plant is destructed, it is practically impossible to track any further changes. Therefore, the present work used omics technologies to simultaneously examine the global transcript and protein levels in the roots of asymptomatic and symptomatic seedlings after prolonged exposure to *Ganoderma*.

**MATERIALS AND METHODS**

**Materials**

*Elaeis guineensis* Jacq. germinated seeds from a single cross were obtained from Sime Darby Plantation Research Sdn. Bhd. A subset of these samples was also used for expression analyses in a separate study (Tan et al., 2016). The parental cross came from Progeny Trial 59 in Dusun Durian Estate, Banting, Selangor planted in May 1979. The palm showed no signs and symptoms of *G. boninense* infection and was still producing bunches at the time of sampling. A total of 790 seedlings were screened in this trial, where 700 of them were subjected to *G. boninense* inoculation, while the remaining 90 seedlings served as control.

**Preparation of Fungal Inoculum**

*Ganoderma boninense* strain PER71 was obtained from the Malaysian Palm Oil Board (MPOB), and maintained at the Crop Protection Unit, Sime Darby Plantation Research Sdn. Bhd. The rubber wood block (RWB) inoculation process using *G. boninense* cultures, Disease Severity Index (DSI) and Disease Severity (Internal) (DI) analyses were conducted by Crop Protection Unit (Breton et al., 2006; Sariah and Zakaria, 2000). A customised RWB with a dimension of 6 × 6 × 6 cm was used as a source of inoculum for *G. boninense*. The RWB was sterilised at 80°C overnight, and then autoclaved at 121°C for 1 hr. A plastic bag containing 120 mL of malt extract agar and one RWB was sealed, autoclaved at 121°C for 15 min, and left to solidify overnight at room temperature. A seven-day-old *G. boninense* culture, maintained on a potato dextrose agar plate, was divided into half, where one part was inoculated onto the sterilised RWB. Inoculated RWBs were sealed in individual plastic bags and incubated in the dark at 25°C-28°C for 150 days, for mycelia to fully colonise all six surfaces of the RWB.

**Artificial Inoculation**

Oil palm germinated seeds (*Dura × Dura*) were sowed in 10” × 12” polybags containing topsoil and sand, at a ratio of 2:1. They were maintained in a shaded nursery for six months, in accordance with the Oil Palm Nursery Technique Good Agricultural Practices (Sime Darby Plantation). Two stringent selection processes were done when the seedlings were three and six months old, where those that were abnormal were culled before the infection process using inoculated RWB. Only those that were normal and healthy were subjected to nursery trials. During transplanting, *Ganoderma*-inoculated RWB was placed in the middle of 15” × 18” polybags that were half-filled with soil. The six month old seedlings were then placed in close contact above the RWB, before more soil was used to fill up the polybags. Control seedlings were prepared in a similar manner using sterilised, uninoculated RWB instead of inoculated ones. Each polybag was positioned above a concrete slab to obstruct the geotropic growth of roots into the soil. All seedlings were maintained in a nursery under the artificial shade with a 50% light cut-off. They were monitored until 18 months old (12 months post-inoculation).
The progression of the disease was also assessed based on DSI (Table 1).

**Sampling**

The boles and roots of three seedlings from three phenotypes were sampled and washed under running water to remove excess soil. These phenotypes were control (uninoculated seedlings), asymptomatic (inoculated seedlings without foliar symptoms), and symptomatic (inoculated seedlings with foliar symptoms). The boles were cut into half, to identify the extent of severity of rotten tissues in inoculated oil palm seedlings (Table 2). Roots were dipped in liquid nitrogen and frozen at -80°C. All samples were pulsed using a mortar and pestle, into fine powder under constant liquid nitrogen, and frozen at -80°C until further analysis. The roots of three biological replicates from each phenotype were used for both transcriptomic and proteomic studies.

**Omics** Technologies

**RNA extraction.** The CTAB method was used to extract total RNA from the roots of oil palm seedlings with several modifications (Asemota and Shah, 2004; Tan et al., 2016). Briefly, 4 mL of extraction buffer (2% CTAB; 0.1 M Tris-HCl, pH 8; 2 M NaCl; 25 mM EDTA; 2% β-mercaptoethanol; 2% PVPP) was added to 2 g of frozen ground material, and mixed well using a vortex. An equal volume of phenol (pH 4.3): chloroform: isoamyl alcohol was added, and the mixture was centrifuged at 10 000 rpm for 15 min at 4°C. The supernatant was transferred into a new tube and precipitated twice in equal volumes of 2 M LiCl. The mixture was centrifuged at 10 000 rpm for 20 min at 4°C, and the pellet was resuspended in RNase-free water. A total of 0.1 vol of 3 M sodium acetate and 2.5 vol of absolute ethanol was added to the solution, and incubated at 80°C for 3 hr. The tube was centrifuged at 12 000 rpm at 4°C for 20 min. The resulting pellet was then washed in 70% ethanol, and thereafter, DNaseI (Epicentre) was applied before the RNA was precipitated in ethanol. Quantification was achieved using a Nanodrop ND-1000 spectrophotometer, where the 260/280 and 260/230 ratios between 1.8-2.0 were accepted. The RNA was run on 1% agarose gels to check the integrity of 18S and 28S rRNA.

**Sequencing library preparation.** The cDNA sequencing libraries were prepared using ScriptSeq™ v2 RNA-Seq Library Preparation Kit (Epicentre, Illumina), according to the manufacturer’s instructions. The RNA was treated with terminator 5'-phosphate dependant exonuclease (Epicentre, Illumina) to deplete the rRNA.

**Bioinformatics analysis.** De novo assembly of raw counts was performed using Trinity software (Broad Institute, Hebrew University of Jerusalem), and unigenes were generated by CAP3 (Huang and Madan, 1999). The NOIseq analysis was used to determine the differential expression between the samples (Tarazona et al., 2011). The RPKM (normalised counts) for each sample was first calculated, and the M value, which is the log 2 fold change, and D value, which is the absolute difference of the comparison, were later determined. With this, the noise distribution was generated for the background, M-D, using the available replicates. The probability value was calculated by comparing the M-D of the gene vs. the noise distribution and was set at >0.75.

**iTRAQ analysis.** The TCA-acetone-phenol method was used to extract the total protein (Daim et al., 2015b). The pellets were air-dried prior to labelling using iTRAQ reagents. The samples were then precipitated using acetone, reduced and alkylated, and finally digested using trypsin. A total of 100 μg of protein was then labelled using the iTRAQ reagents (4-plex). All procedures were conducted according

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**TABLE 1. DISEASE PROGRESSION BASED ON THE DISEASE SEVERITY INDEX (DSI)**

| Class | Symptom                                                                                     |
|-------|--------------------------------------------------------------------------------------------|
| 0     | Healthy (green, healthy leaves)                                                             |
| 1     | Presence of white mycelium, with or without chlorotic/necrotic leaves                       |
| 2     | Presence of basidioma initials at the stem base with 1-3 chlorotic/necrotic leaves          |
| 3     | Formation of basidioma at the stem base with more than 3 chlorotic/necrotic leaves          |
| 4     | Presence of fruiting body with 50% necrotic leaves; plant is dried/dead                      |

**TABLE 2. THE SEVERITY OF BOLE TISSUES IN INOCULATED OIL PALM SEEDLINGS BASED ON INTERNAL DISEASE SEVERITY**

| Class | Symptom                      |
|-------|------------------------------|
| 0     | Healthy (no internal rot)    |
| 1     | Less than 20% of rotten tissues |
| 2     | 20%-50% of rotten tissues    |
| 3     | 51%-90% of rotten tissues    |
| 4     | More than 90% of rotten tissues |
to the manufacturer’s instructions (iTRAQ, AB Sciex). Three separate iTRAQ experiments were conducted to analyse all nine biological samples; one sample from each group (control, symptomatic, and symptomatic) was labelled with a dye, while the fourth dye was labelled with the same control sample.

A pooled sample was prepared by combining all labelled samples. The peptides were desalted using a reversed-phase column (Strata-X 33 μm polymeric, Phenomenex). They were then dissolved in 10 mM KH₂PO₄ (pH 3) containing 10% acetonitrile. The peptides were separated using a strong cation exchange liquid chromatography column (PolySulfoethyl column, 4.6 × 100 mm, 5 μm, 300 A) on an Agilent 1100 HPLC system.

The peptide elution was done with a linear gradient of 0-400 mM KCl. Eight fractions were collected and desalted on Strata-X columns. The fractions were analysed using the 1260 Infinity HPLC system (Agilent) coupled to a 1260 Chipcube Nanospray interface (Agilent) on a QTOF 6540 mass spectrometer (Agilent). The peptides were separated in a ProtID-Chip-150 C₁₈ column with a linear gradient of acetonitrile/0.1% formic acid (v/v). The spectral data were analysed against the NCBI Viridiplantae database, and protein identifications were done with ProteinPilot™ 4.5 Software (AB Sciex). The database was downloaded in July 2013 and contained 2,747,034 sequences.

Proteins with a relative abundance of >5 and with a p-value <0.05 were selected as statistically significant to ensure up- and downregulation. The protein lists were further analysed by the UniProt database. A global FDR was set at <0.1%.

RESULTS AND DISCUSSION

Phenotypic Evaluation

The trial was conducted on 18 month old seedlings to assess the disease severity of one single cross of interest. These seedlings were selected after a final round of culling right before they were exposed to *Ganoderma*-inoculated RWB at the six month old seedling. Results showed that this cross had an estimated disease severity of 5.32% and 10.12% severity of the bole tissues.

Seedlings were chosen for omics studies based on their DSI and disease severity (internal) (Figure 1). They were destructed to confirm the presence of lesions in the boles. Inoculated seedlings that were classified as Class 0 for both DSI and disease severity (internal) were considered asymptomatic, while those that were classified as Class 2 and above for DSI, and Class 1 and above for disease severity (internal) were considered symptomatic. Uninoculated seedlings with Class 0 for both DSI and disease severity (internal) served as control. The roots of three biological replicates that represented the three different phenotypes (control, asymptomatic and symptomatic) were used to construct the libraries for both transcriptomic and proteomic studies.

Destructive sampling was conducted when the seedlings were 18 months old, when there was positive root contact with RWB, in control and treated seedlings (data not shown). This situation (positive root contact with RWB) occurred by using polybags that are suitable for six to nine month old seedlings instead of APM polybags that are bigger in size. Roots of 18 month old seedlings were forced to expand and grow in a confined space within the polybag.

The mode of infection of BSR is through the roots. The progression of the disease is slow and varies for each individual, especially for older seedlings. Once the infection is established in the roots, the disease progresses towards the boles, and the seedlings may or may not show signs of foliar symptoms. Signs and symptoms of infection are prominent and occur early in germinated or younger seedlings. For six month old seedlings, their immune system is more developed than the much younger seedlings. They would have acquired the ability to defend themselves from the same source of fungal infection (the same inoculum source and size).

Asymptomatic seedlings do not show lesions in the boles, but infection is established in the roots (roots that were rotten or starting to rot were visible). DSI and DI data were collected from the foliar and bole, respectively. Control (uninoculated RWB) was put in place to ensure that it was treated in the same manner as inoculated seedlings. In this condition, both control and treated seedlings were treated equally, except for the inoculated RWB in treated (asymptomatic and symptomatic) seedlings. All treated seedlings were exposed to the same fungal pathogen at the same time with the same inoculum size.

The plant’s defence system will entirely dictate its susceptibility (or tolerance) to the disease. Furthermore, infection is more often atopic and might have stopped progressing at the time the seedlings were sampled in the present work. In the case of asymptomatic seedlings, the infection has progressed to the roots but not the boles. Hence, they were identified as asymptomatic (fronds were healthy and green with a few roots that were rotten or started to rot). Symptomatic seedlings showed foliar symptoms but did not present lesions in the boles.

Unigenes in Control, Asymptomatic and Symptomatic Seedlings

Comparisons were made between asymptomatic vs. control, and symptomatic vs. control seedlings.
TRANSCRIPTOMIC AND PROTEOMIC STUDIES TO INVESTIGATE THE BASAL STEM ROT DISEASE IN OIL PALM SEEDLINGS

in terms of differential expression of transcripts. The cDNA libraries were constructed from three biological replicates from the three groups (Table 3). A total of 464,701,946 paired-end reads with an average read length of 150 nucleotides (nt) were obtained. Annotations were performed by using BLAST against the UniProt database at http://www.uniprot.org/. Unigenes from control, asymptomatic, and symptomatic seedlings were identified and categorised into KEGG pathways via https://www.genome.jp/kegg/pathway.html.

A subset of the expressed genes was validated using both real-time quantitative PCR and NanoString in the same samples (data not shown). A part of the present work was also filed for a patent (WO2017155384A1, 2017).

A Venn diagram analysis was performed to group unigenes that appeared only in control, symptomatic, and asymptomatic seedlings (Figure 2). The unigenes were grouped into three different phenotypes based on the KEGG pathways (Table 4). Eight pathways from the unigenes appeared in all three phenotypes. Most of the pathways that appeared in either one or two phenotypes were common in cell metabolism, and carboxylic and amino acid metabolisms.

The ubiquitin-mediated proteolysis pathway was detected in the roots of asymptomatic seedlings and control. It made use of receptors that recognised and deciphered the ubiquitin code to regulate most cellular processes, including host-pathogen interactions (Li et al., 2016). Modifications of the genes that are regulated by the ubiquitin system have been shown to manipulate the host defence system. Increased resistance against the root-infecting fungal pathogen Fusarium oxysporum has

Figure 1. Oil palm seedlings were destructed to observe the severity of rotten tissues in the boles. Seedlings were chosen based on three different categories. (a) control seedling with class 0 DSI, and class 0 DI (internal); (b) asymptomatic seedling with class 0 DSI, and class 0 DI (internal); (c) asymptomatic seedling with class 3 DSI, and class 3 DI (internal), (d) basidiocarp growing at the base of a seedling.

| TABLE 3. SUMMARY OF PRE- AND POST-PROCESSED READS OF RNA-SEQUENCING LIBRARIES |
|---------------------------------------------------------------|
| **RNA-Seq libraries**                                       |
| **Control** | **Asymptomatic** | **Symptomatic** |
| Raw reads     | 221,907,420      | 199,546,208     | 148,818,010    |
| Raw nucleotides| 33,286,113,000  | 29,931,931,200 | 22,322,701,500 |
| Processed reads| 137,203,583     | 197,032,111    | 137,203,582    |
| Processed nucleotides | 30,148,229,470 | 26,032,716,787 | 18,391,549,644 |
| Mapped (%)     | 52              | 57             | 50             |
been displayed by the pub22/23/24 triple mutant. This suggested a role in root-mediated defence by PUB22/23/24 against soil-borne pathogens (Chen et al., 2013; Zhou and Zeng, 2017).

Transcriptional Expression in Roots of Symptomatic and Asymptomatic Seedlings

Most of the transcripts detected in symptomatic and asymptomatic seedlings were linked to cell walls, transporters, disease resistance, and phytohormone signalling pathways. A total of 20 upregulated and 20 downregulated transcripts were found in symptomatic and asymptomatic seedlings (Table 5).

Cell wall-related transcripts were differentially expressed in symptomatic seedlings. Endoglucanase, which is required for secondary cell wall formation in the developing xylem (Glass et al., 2015), was found to be downregulated. Glucanases also belong to the pathogenesis-related protein family (PR-2). They were also found to be involved in the infection of rice roots by Azoarcus sp. strain BH72. The visible sites of expression of the endoglucanase gene eglA were the points where lateral roots and root tips emerged, which are also the primary points of entry into the roots (Reinhold et al., 2006).

The ATP-binding cassette (ABC) proteins transport secondary metabolites and plant hormones that regulate the overall development of plants (Hwang et al., 2016). The ABCI and ABCF transporters which are found down-regulated in asymptomatic seedlings are not directly involved in the transport process but likely function in ribosome recycling and translational control (Kang et al., 2011).

The transcripts of resistance proteins were detected in the roots of inoculated oil palm seedlings. Resistance gene analogues (RGAs) are a large class of potential R-genes (resistance genes) that enable plants to recognise specific races of pathogens and to mount an effective localised defence response (Jones, 1996). Disease resistance and abiotic stress tolerance in Nicotiana benthamiana were enhanced in the overexpressed pathogen-induced grapevine VaRGA1 gene (Li et al., 2017). In strawberries, the Phytophthora cactorum infection in crown rot showed that the gene transcriptional profiles of most RGAs were stronger and quicker in the resistant Bukkamen than in the susceptible FDP821 genotype during the early infection stage (Chen et al., 2016).

Gene expression is regulated by gibberellins by promoting the degradation of transcriptional regulator proteins (Murase et al., 2008). The earliest findings of gibberellic acid (GA) in plant immunity were related to the decreased levels of GA in infected rice caused by Phytooreovirus (Zhu et al., 2005). However, exogenous GA was found to lower the resistance to two rice pathogens Magnaporthe oryzae and Xanthomonas oryzae (Qin et al., 2013; Yang et al., 2008). The rice GA signalling also appears to promote resistance to necrotrophs, and susceptibility to hemibiotrophs (De Bruyne et al., 2014).

Unique Proteins with Different Abundance

The iTRAQ analysis provides a relative quantification and was used for comparing control and diseased roots of oil palm seedlings in the present work. A total of 53 proteins were found to be higher in abundance, while 69 proteins were lower in abundance. The number of proteins that were higher in abundance was found to be lower in symptomatic seedlings, while proteins that were lower in abundance were found in symptomatic seedlings. The activities of an increased number of proteins could be suppressed in the roots of asymptomatic seedlings when the plants are showing signs of disease symptoms. When necrotic tissues in roots of symptomatic seedlings increase with the progression of disease infection, the functions of proteins in healthy tissues will decrease.

These proteins were subsequently grouped based on their functions (Table 6). The number of proteins with the following functions is lower in abundance in the roots of symptomatic seedlings;
TABLE 4. CLASSIFICATION OF UNIGENES BASED ON KEGG PATHWAYS

a) Appear in all phenotypes
(KO00520) Amino sugar and nucleotide sugar metabolisms
(KO00190) Oxidative phosphorylation
(KO04075) Plant hormone signal transduction
(KO04626) Plant-pathogen interaction
(KO04141) Protein processing in the endoplasmic reticulum
(KO03010) Ribosome
(KO03013) RNA transport
(KO00230) Purine metabolism

b) Appear in roots of control and asymptomatic seedlings
(KO04120) Ubiquitin-mediated proteolysis

c) Appear in roots of control and symptomatic seedlings
(KO00710) Carbon fixation in photosynthetic organisms
(KO00010) Glycolysis/gluconeogenesis
(KO00620) Pyruvate metabolism

\[F. \text{oxy}sporum\] revealed that differentially regulated proteins involved in different defence pathways are likely associated with different resistant levels of the three banana cultivars (Li et al., 2013).

Proteins in Roots of Symptomatic and Asymptomatic Seedlings

Many proteins of lower abundance were detected in the roots of symptomatic seedlings (Table 7). Most of these proteins are involved in defence, stress response, and signalling. Unique, 14-3-3 proteins are phosphate-binding proteins
| Contig | Differential expression (log2) | Uniprot |
|-------|------------------------------|---------|
| **Downregulated transcripts** | | |
| Singleton1 | -7.39 | ABC transporter C family member 10 OS=Arabidopsis thaliana |
| Singleton2 | -6.58 | NAC domain-containing protein 68 OS=Oryza sativa subsp. japonica |
| Singleton3 | -6.07 | Adenosine kinase 2 OS=A. thaliana |
| Singleton4 | -6.04 | BII-like protein OS=A. thaliana |
| Singleton5 | -5.57 | DDB1- and CUL4-associated factor homolog 1 OS=A. thaliana |
| Singleton6 | -5.49 | Pentatricopeptide repeat-containing protein At3g63370 OS=A. thaliana |
| Singleton7 | -5.48 | COP9 signalosome complex subunit 6a OS=A. thaliana |
| Singleton8 | -5.30 | Endoglucanase 25 OS=A. thaliana |
| Singleton9 | -5.10 | Putative lipoxygenase 5 OS=O. sativa subsp. japonica |
| Singleton10 | -5.04 | Cleavage and polyadenylation specificity factor CPSF3 OS=A. thaliana |
| Singleton11 | -4.52 | Plasticide glucose transporter 4 OS=A. thaliana |
| Singleton12 | -4.37 | Ammonium transporter 3 member 3 OS=O. sativa subsp. japonica |
| Singleton13 | -4.28 | Chalcone synthase 3 OS=Ruta graveolens |
| Singleton14 | -4.25 | 40S ribosomal protein S4-3 OS=A. thaliana |
| Singleton15 | -4.19 | Probable serine/threonine-protein kinase CPR1 OS=Zea mays |
| Singleton16 | -4.19 | Zinc finger A20 and AN1 domain-containing stress-associated protein 8 OS=O. sativa subsp. japonica |
| Singleton17 | -4.17 | ABC transporter B family member 11 OS=A. thaliana |
| Singleton18 | -4.09 | Putative late blight resistance protein homolog R1B-17 OS=Solanum demissum |
| Singleton19 | -4.02 | Proteasome subunit alpha type-6 OS=Nicotiana tabacum |
| Singleton20 | -4.00 | Phosphoenolpyruvate carboxylase kinase 1 OS=A. thaliana |
| **Upregulated transcripts** | | |
| Singleton21 | 9.18 | Alpha, alpha-trehalose-phosphate synthase _UDP-forming_ 1 OS=A. thaliana |
| Singleton22 | 9.08 | Gibberellic receptor GID1 OS=O. sativa subsp. japonica |
| Singleton23 | 9.07 | Xyloglucan endotransglucosylase/hydrolase protein 22 OS=A. thaliana |
| Singleton24 | 9.07 | Protein GAST1 OS=S. lycopersicum |
| Singleton25 | 8.95 | Sphingosine-1-phosphate lyase OS=O. sativa subsp. japonica |
| Singleton26 | 8.76 | Pentatricopeptide repeat-containing protein At3g02650, mitochondrial OS=A. thaliana |
| Singleton27 | 8.75 | Probable peptide/nitrate transporter At1g33440 OS=A. thaliana |
| Singleton28 | 8.68 | Transcription factor AS1 OS=A. thaliana |
| Singleton29 | 8.64 | Probable receptor-like protein kinase At5g18500 OS=A. thaliana |
| Singleton30 | 8.60 | Long chain acyl-CoA synthetase 4 OS=A. thaliana |
| Singleton31 | 8.40 | Shaggy-related protein kinase beta OS=A. thaliana |
| Singleton32 | 8.40 | Phosphoenolpyruvate carboxylase 1 OS=A. thaliana |
| Singleton33 | 8.35 | Peptide transporter PTR1 OS=A. thaliana |
| Singleton34 | 8.31 | Dehydrin COR410 OS=Triticum aestivum |
| Singleton35 | 8.21 | DNA-directed RNA polymerase D subunit 1 OS=A. thaliana |
| Singleton36 | 8.18 | Malate dehydrogenase, chloroplast OS=A. thaliana |
| Singleton37 | 8.18 | Regulatory protein NPR1 OS=A. thaliana |
| Singleton38 | 8.15 | Putative disease resistance protein RGA1 OS=S. bulbocastanum |
| Singleton39 | 8.10 | 12-oxophytodienoate reductase 3 OS=A. thaliana |
| Singleton40 | 8.09 | Uncharacterised protein At4g10930 OS=A. thaliana |
### TABLE 6. ABUNDANCE OF UNIQUE PROTEINS PERFORMING SPECIFIC FUNCTIONS

| Function                          | Symptomatic |          | Asymptomatic |          |
|----------------------------------|-------------|----------|--------------|----------|
|                                  | Higher      | Lower    | Higher       | Lower    |
| Carbohydrate metabolism          | -           | 4        | 2            | 1        |
| Cell wall-related defence        | 1           | 2        | -            | 1        |
| Cellular processes               | -           | 12       | 9            | 5        |
| Energy                           | 1           | 1        | 5            | -        |
| Lipid metabolism                 | -           | -        | 1            | -        |
| PR protein                       | 1           | 1        | 1            | 2        |
| Protein metabolism               | 2           | 7        | 6            | 1        |
| Signalling                       | 1           | 1        | 2            | 1        |
| Stress response                  | 1           | 11       | 2            |          |
| Sugar metabolism                 | -           | 6        | 6            |          |
| Transporter                      | -           | 2        | 2            |          |

that regulate multiple signalling pathways. These proteins control hormone signalling, cell division, and disease resistance. The 14-3-3 isoform protein family are key targets of bacterial effectors for Xcv disease resistance in tomatoes (Dubrow et al., 2018). The acireductone dioxygenase-like protein contributes to the production of ethylene, a product of the methionine salvage pathway (Friedman et al., 2011). It is important in maintaining the S-adenosylmethionine levels for ethylene production (White and Flashman, 2016). Ethylene is important in the hormonal regulation of plant development, as a virulence factor of fungal and bacterial pathogens, and as a signalling compound in disease resistance (Loon et al., 2006).

Beta-1,3-glucanases are abundant in plants; they work either alone or with chitinases and other antifungal proteins to protect plants against fungal pathogens (Balasubramanian et al., 2012). Beta-1,3-glucanases inhibit the growth of fungal pathogens when they catalyse the hydrolytic cleavage of beta-1,3-glucans, a major structural component present in the fungal cell wall (Su et al., 2016).

Cyclophilins have multiple functions and are highly versatile. Many growth and development processes in plants, particularly hormone signalling and stress responses, are regulated by cyclophilins (Singh et al., 2020). In soybean, the CYP1 transcript level was reduced in response to Phytophthora sojae infection (Mainali et al., 2017). Since isoflavonoids are involved in plant stress resistance against biotic and abiotic factors, the interaction of GmCYP1 with the isoflavonoid regulators GmMYB176 and 14-3-3 protein, suggests its role in the defence system of soybean.

Enolase is essential for the growth and development of plants. Altered ENO2 functions in Arabidopsis plants have reduced cell size and defective cell differentiation with restricted lignification (Eremina et al., 2015).

The 40S ribosomal protein S18-like protein is highly abundant in asymptomatic seedlings (Table 8). Ribosomal proteins are important in maintaining the stability of the ribosomal complex and mediating protein synthesis (Moin et al., 2016). Rice infected with X.oryzae and Rhizoctonia solani have induced the up-regulation of several ribosomal proteins. The transcript levels of RPS4 are also higher during biotic stresses (Saha et al., 2017).

The cellular membrane potential in plants is established by plasma membrane (PM) H+ATPases (Elmore and Coaker, 2011). They interact with RIN4, a negative regulator of disease resistance, to manage stomatal apertures during Pseudomonas syringae invasion of leaf tissues in Arabidopsis (Liu et al., 2009). Furthermore, PM H+ATPase activities have been manipulated by evolving pathogens during infection, and are targeted by pathogens to increase plant susceptibility (Elmore and Coaker, 2011).

Receptor kinases are important in plant immunity, growth, and development. They deploy many receptor kinases as pattern recognition receptors that detect microbe- and host-derived molecular patterns as the first layer of inducible defence (Tang et al., 2017). The serine/threonine protein phosphatase 2A controls pathogenesis responses in various plant species. It contributes to the regulation of receptor signalling, organellar signalling, gene expression, metabolic pathways, and cell death, all of which essentially impact plant immunity (Durian et al., 2016).

**CONCLUSION**

Research using a combination of omics technologies is useful for providing a universal view of understanding the molecular systems that underlie various functions in oil palm seedlings infected with BSR. The present work explored both the transcripts...
### TABLE 7. PROTEINS WITH DIFFERENT ABUNDANCE PRESENT IN THE ROOTS OF SYMPTOMATIC OIL PALM SEEDLINGS

| Accession | UniProt | Name                                                                 | Fold change | Unused ProtScore | Total ProtScore | % Coverage | Peptide (95%) |
|-----------|---------|----------------------------------------------------------------------|-------------|-----------------|-----------------|------------|--------------|
| gi|3106891355 | E3VH6 | 6-phosphogluconate dehydrogenase [Pinus sylvestris]                  | 87.90       | 2.00            | 4.42            | 17.4       | 7            |
| gi|514814998 | K4A649 | PREDICTED: 5-methyltetrahydropyroloretigulatum ate--homocysteine methyltransferase-like [Setaria italica] | 87.90       | 3.01            | 8.83            | 18.7       | 7            |
| gi|1192910882 | B3TLW8 | Beta-1,3-glucanase [Elaeis guineensis]                               | 6.14        | 12.64           | 12.64           | 38.1       | 28           |
| gi|7271955 | Q9MS11 | Cytosolic pyruvate kinase [Lilium longiflorum]                       | 6.08        | 3.16            | 3.17            | 8.4         | 2            |
| gi|192910890 | B3TLX2 | Early flowering protein 1 [E. guineensis]                            | 2.11        | 16.06           | 16.06           | 55.1       | 17           |
| gi|192911934 | B3TLZ4 | Temperature-induced lipocalin [E. guineensis]                        | 1.29        | 2.03            | 2.03            | 15.4       | 1            |
| gi|502136998 | A5BUU4 | PREDICTED: 40S ribosomal protein SA-like [Cicer aritinum]             | 1.19        | 8.00            | 8.00            | 20.9       | 7            |
| gi|148927382 | A6N8F8 | Cysteine proteinase [E. guineensis]                                  | 1.18        | 4.76            | 4.76            | 19.2       | 7            |
| gi|752295898 | Q6L544 | RecName: Full--Germin-like protein 5-1; Flage: Precursor             | 1.14        | 5.87            | 5.87            | 18.6       | 8            |
| gi|22534046 | Q6F2Y7 | Heat shock protein 101 [O. sativa]                                   | 1.06        | 2.34            | 2.34            | 4.6        | 1            |
| a) Proteins with higher abundance |
| gi|166452940 | B6RF01 | 14-3-td protein [Gossypium hirsutum]                                 | 90.09       | 2.00            | 2.00            | 6.5        | 1            |
| gi|192913044 | B3TM49 | Aci-reductone dioxygenase-like protein [E. guineensis]                | 90.09       | 2.44            | 2.44            | 1.3        | 1            |
| gi|448872690 | M1H922 | Alpha-1,4-glucan-protein synthase [E. guineensis]                    | 90.09       | 4.00            | 4.00            | 27.8       | 2            |
| gi|192910882 | B3TLW8 | Beta-1,3-glucanase [E. guineensis]                                   | 90.09       | 2.16            | 2.16            | 6.4        | 2            |
| gi|192910744 | B3TL9 | Cyclophilin [E. guineensis]                                          | 90.09       | 2.00            | 2.00            | 4.0        | 1            |
| gi|255539693 | B9RN6 | Enolase isoform X2, putative [Ricinus communis]                      | 89.29       | 1.57            | 1.59            | 10.7       | 2            |
| gi|255539693 | B9RN6 | Enolase isoform X2, putative [R. communis]                           | 77.52       | 3.48            | 9.68            | 26.5       | 7            |
| gi|511537412 | R9VW8 | FVE protein [Litchi chinensis]                                       | 42.02       | 2.22            | 4.30            | 23.8       | 2            |
| gi|508785294 | A0A0161GR98 | General regulatory factor 7, NU [Theobroma cacao]               | 40.16       | 2.00            | 5.13            | 17.1       | 3            |
| gi|192910866 | B3TLW0 | Glutathione S-transferase [E. guineensis]                            | 36.36       | 4.05            | 4.05            | 5.2        | 2            |
| gi|82400215 | Q2QF4 | Glyceraldehyde 3-phosphate dehydrogenase [E. guineensis]             | 33.11       | 15.00           | 15.00           | 62.4       | 12           |
| gi|302805526 | D8SM1 | Hypothetical protein SELMODRAFT_156635 [Selaginella moellendorffii]  | 25.84       | 2.42            | 2.42            | 10.8       | 1            |
| gi|192910730 | B3TL2 | Light-inducible protein ATLS1 [E. guineensis]                        | 22.08       | 2.85            | 7.17            | 9.3        | 5            |
| gi|351724907 | Q7TE8 | Methionine synthase [Glycine max]                                    | 18.18       | 4.01            | 10.91           | 59.3       | 9            |
| gi|192910736 | B3TLP5 | Mitochondrial F1-ATP synthase D chain [E. guineensis]                | 16.00       | 3.62            | 3.62            | 19.8       | 1            |
| gi|897618 | C0HH32 | Mitochondrial F-1-ATPase subunit 2 [Zea mays]                        | 15.70       | 5.89            | 5.89            | 21.9       | 3            |
| gi|192910872 | B3TLW3 | Pathogenesis-related protein [E. guineensis]                         | 12.24       | 11.21           | 11.21           | 30.3       | 9            |
| gi|211090450 | D2DZ8 | Phosphoglycerate kinase [Gossypium hirsutum]                         | 9.73        | 4.16            | 4.16            | 12.5       | 2            |
| gi|508712850 | A0A061JK37 | Phosphoglycerate mutase, 2,3-bisphosphoglycerate-independent isoform 2 [Theobroma cacao] | 9.63 | 2.00 | 2.00 | 7.2 | 1 |
| gi|359429244 | F6HYG1 | PREDICTED: 97 kDa heat shock protein-like [Vitis vinifera]            | 8.47        | 12.60           | 12.60           | 39.1       | 8            |
| gi|470102123 | Q8S9S4 | PREDICTED: IAA-amino acid hydrolase IRL1-like 4-like [Fragaria vesca] | 8.02        | 4.21            | 4.21            | 8.6        | 3            |
| gi|514779022 | K3XN7 | PREDICTED: malate dehydrogenase, mitochondrial-like [Setaria italica] | 7.45        | 5.33            | 5.33            | 30.7       | 3            |
| gi|514814448 | K4A6V8 | PREDICTED: phosphoglucomutase, cytoplasmic 2-like isoform X2 [S. italica] | 6.92 | 8.93 | 8.93 | 35.3 | 8 |
| gi|1225452974 | Q4220 | PREDICTED: probable mitochondrial-processing peptidase subunit beta [V. vinifera] | 5.34 | 15.75 | 15.75 | 36.2 | 12 |
| gi|514798087 | K3YL3 | PREDICTED: proteasome subunit beta type-1-like [S. italica]          | 5.01        | 4.05            | 23.44           | 49.2       | 28           |

Note: Unused ProtScore- A measure of the protein confidence for a detected protein, calculated from the peptide confidence for peptides from spectra that have not already been completely “used” by higher scoring winning proteins. For 95% confidence, the required Unused ProtScore is 1.3; Total ProtScore- A measure of the total amount of evidence for a detected protein; % Coverage- The percentage of matching amino acids from identified peptides divided by the total number of amino acids in the sequence.
### TABLE 8. PROTEINS WITH DIFFERENT ABUNDANCE PRESENT IN THE ROOTS OF ASYMPTOMATIC OIL PALM SEEDLINGS

| Accession | UniProt | Name | Fold change | Unused ProtScore | Total ProtScore | % Coverage | Peptide (95%) |
|-----------|---------|------|-------------|------------------|-----------------|------------|---------------|
| gi|502093390| P34788 | PREDICTED: 405 ribosomal protein S18-like | 99.1 | 1.48 | 1.48 | 17.8 1 |
| gi|115945541| P55195 | Phosphoribosylaminomimidazole carboxylase, eukaryotic-type | 87.9 | 1.35 | 1.35 | 3.2 1 |
| gi|146430485| Q7SN96 | Plasma membrane H+-ATPase [Daucus carota] | 87.9 | 2 | 5.24 | 7 3 |
| gi|151478521| K3XF69 | PREDICTED: probable inactivator kinase | 87.1 | 2 | 2.5 1 |
| gi|1514782147| Q8950 | PREDICTED: serine/threonine-protein phosphatase 2A | 86.3 | 2.7 | 2.7 | 9.1 1 |
| gi|131069155| E3VH16 | 6-phosphogluconate dehydrogenase [Pinus sylvestris] | 77.3 | 2 | 4.42 | 17.4 7 |
| gi|192591934| B3TLZ4 | Temperature-induced lipocalin [Elaeis guineensis] | 6.9 | 2.03 | 2.03 | 15.4 1 |
| gi|374256013| H6TNN9 | Putative methionine synthase protein, partial | 5.4 | 7.68 | 10.61 | 28.9 7 |
| gi|192591908 | B3TL1Y | Fructose-bisphosphate aldolase [E. guineensis] | 5.1 | 18.23 | 18.23 | 58.4 20 |
| gi|145827831 | Q5JI3K | Elongation factor 1-alpha [E. guineensis] | 5 | 12.62 | 12.62 | 27.3 14 |
| gi|12506645 | A9TW99 | Alpha tubulin [Physcomitrium patens] | 4.4 | 19.07 | 19.07 | 46.3 14 |
| gi|147026989 | Q67B8 | Nucleoside diphosphate kinase [Haploepithus orientalis] | 4 | 15.45 | 15.45 | 26.7 14 |
| gi|192910730 | B3TL2P | Light-inducible protein ATSL1 [E. guineensis] | 3.9 | 4.09 | 4.09 | 42.6 2 |
| gi|192910882 | B3TLW8 | Beta-1,3-glucanase [E. guineensis] | 3.8 | 16.88 | 16.88 | 36 28 |
| gi|14126976 | Q9ZV68 | Vacuolar H+-pyrophosphatase [Chara corallina] | 3.5 | 1.47 | 1.47 | 4.3 1 |
| gi|50251203 | Q9EOU9 | Putative vacuolar proton-ATPase [Oryza sativa] | 3.5 | 15.45 | 15.45 | 26.7 14 |
| gi|192910967 | B3TL4 | Triose phosphate isomerases cytosolic isoform | 2.8 | 4.49 | 4.49 | 26 5 |
| gi|181686725 | Q9D55 | NAD-dependent isocitrate dehydrogenase c2 [O. sativa] | 2.5 | 4.03 | 4.03 | 16.9 2 |
| gi|357167236 | P08926 | PREDICTED: nRisCO large subunit-binding protein subunit alpha, chloroplast-like [Brachypodium distachyon] | 2.3 | 9.85 | 9.85 | 19.9 8 |
| gi|192910890 | B3TLX2 | Early flowering protein 1 [E. guineensis] | 2 | 13.68 | 13.68 | 56.3 15 |
| gi|502082135 | P54774 | PREDICTED: cell division cycle protein 48 homolog [Cicer arrietum] | 1.7 | 6.77 | 6.77 | 13.1 4 |
| gi|125435632 | P82732 | PREDICTED: formate-tetrahydrofolic acid-ligase-like [Vitis vinifera] | 1.4 | 2.79 | 2.79 | 4.9 1 |
| gi|177535629 | Q9QRX7 | Pyruvate kinase family protein expressed [O. sativa] | 1.4 | 4 | 4 | 12.5 2 |
| gi|147010771 | Q8KNW6 | PREDICTED: succinyl-CoA ligase [ADP-forming] subunit beta, mitochondrial-like [Fragaria vesca subsp. vesca] | 1.2 | 4.13 | 4.13 | 22 4 |
| gi|353441078 | G8FGH1 | Putative enolase [E. guineensis] | 1.2 | 4.01 | 10.91 | 59.3 9 |
| gi|192910928 | B2CZK7 | Plasma membrane intrinsic protein [E. guineensis] | 1.2 | 5.07 | 5.07 | 22.3 5 |
| gi|508722839 | A0A061G0U1 | Sugar isomerase (SIG) family protein isoform 2 [Theobroma cacao] | 1.1 | 5.12 | 5.12 | 9.5 3 |
| gi|192910860 | B3TL7V | Adenine phosphoribosyltransferase 1 [E. guineensis] | 1.1 | 2.59 | 2.59 | 18.8 1 |
| gi|353441104 | G8FCJ2 | Putative cytosolic malate dehydrogenase [E. guineensis] | 1.1 | 1.51 | 32 4 |
| gi|122545274 | Q4Z290 | PREDICTED: probable mitochondrial-processing peptidase subunit beta [V. vinifera] | 1.1 | 4.53 | 4.53 | 9 2 |
| gi|192910964 | B3TM09 | Protein disulfide isomerase 2 precursor [E. guineensis] | 1.1 | 10.67 | 10.67 | 25.7 6 |
| gi|1897618 | C0HPF2 | ATPI synthase subunit beta [Zea mays] | 1 | 38.01 | 38.01 | 56.2 42 |

Note: Unused (ProtScore)- A measure of the protein confidence for a detected protein, calculated from the peptide confidence for peptides from spectra that have not already been completely “used” by higher scoring winning proteins. For 95% confidence, the required Unused ProtScore is 1.3, Total (ProtScore)- A measure of the total amount of evidence for a detected protein; % Coverage- The percentage of matching amino acids from identified peptides divided by the total number of amino acids in the sequence.
and proteins in artificially inoculated seedlings after a 12 month prolonged exposure to *Ganoderma*. The biological functions of asymptomatic seedlings resembled those of healthy, uninoculated ones where cellular processes for regular growth and development were observed. Stress-responsive proteins and transcripts related to carbon fixation, glycolysis and pyruvate metabolism were detected in symptomatic seedlings. The profiles of asymptomatic and symptomatic seedlings can be used to understand the behaviour of host plants towards the mechanisms of disease infection after prolonged exposure. These findings add value to previous publications where seedlings were usually destructed after a three to four month exposure to the disease, and comparisons were made only for control and treated seedlings. Further investigations into the mechanisms of disease tolerance in oil palm could be proposed, where asymptomatic seedlings are thoroughly scrutinised. Integration of transcriptomic and proteomic approaches will also provide a better understanding of host-pathogen interactions, leading to new insights which would not be easy to attain using either the protein or mRNA differential analysis alone.

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**REFERENCES**

Asemota, O and Shah, F (2004). Detection of mesocarp oleoyl-thioesterase gene of the South American oil palm *Elaeis oleifera* by reverse transcriptase polymerase chain reaction. *Afr. J. Biotechnol.*, 3(11): 595-598.

Bahari, M N A; Sakeh, N M; Abdullah, S N A; Ramli, R R and Kadhodaei, S (2018). Transcriptome profiling at early infection of *Elaeis guineensis* by *Ganoderma boninense* provides novel insights on fungal transition from biotrophic to necrotrophic phase. *BMC Plant Biol.*, 18: 377.

Bai, L; Sun, H B; Liang, R T and Cai, B Y (2019). iTRAQ proteomic analysis of continuously cropped soybean root inoculated with *Funnelliformis mossaeae*. *Front. Microbiol.*, 10: 61.

Balasubramanian, V; Vashisht, D; Cletus, J and Sakthivel, N (2012). Plant β-1,3-glucanases: Their biological functions and transgenic expression against phytopathogenic fungi. *Biotechnol. Lett.*, 34: 1983-1990.

Breton, F; Hasan, Y; Hariadi; Lubani, Z and de Franqueville, H (2006). Characterization of parameters for the development of an early screening test for basal stem rot tolerance in oil palm progenies. *J. Oil Palm Res.*, Special Issue: 24-36.

Chen, X R; Brurberg, M B; Elameen, A; Klemsdal, S S and Martinussen, I (2016). Expression of resistance gene analogs in woodland strawberry (*Fragaria vesca*) during infection with *Phytophthora cactorum*. *Mol. Genet. Genomics*, 291: 1967-1978.

Chen, Y T; Liu, H; Stone, S and Callis, J (2013). ABA and the ubiquitin E3 ligase KEEP ON GOING affect proteolysis of the *Arabidopsis thaliana* transcription factors ABF1 and ABF3. *Plant J.*, 75(6): 965-976.

Cho, M and Cho, H T (2013). The function of ABCB transporters in auxin transport. *Plant Signal. Behav.*, 8: e22990.

Crandall, S G; Gold, K M; Jiménez-Gasco, M D M; Filgueiras, C C and Willett, D S (2020). A multi-omics approach to solving problems in plant disease ecology. *PLoS ONE*, 15: e0237975.

Crone, M; McComb, J A; O’Brien, P A and Hardy, G E S J (2013). Assessment of Australian native annual/herbaceous perennial plant species as asymptomatic or symptomatic hosts of *Phytophthora cinnamomi* under controlled conditions. *Forest Pathol.*, 43: 245-251.

Dai, T; Chang, X; Hu, Z; Liang, L; Sun, M; Liu, P and Liu, X (2019). Untargeted metabolomics based on GC-MS and chemometrics: A new tool for the early diagnosis of strawberry anthracnose caused by *Colletotrichum theobromicola*. *Plant Dis.*, 103: 2541-2547.

Daim, L D J; Ooi, T E K; Yusof, H; Abdul Majid, N and Karsani, S (2015a). Optimization of protein extraction and two-dimensional electrophoresis protocols for oil palm leaf. *Protein J.*, 34: 304-312.

Daim, L D J; Ooi, T E K; Ithnin, N; Mohd Yusof, H; Kulaveerasingam, H; Abdul Majid, N and Karsani, S A (2015b). Comparative proteomic analysis of oil palm leaves infected with *Ganoderma boninense* revealed changes in proteins involved in photosynthesis, carbohydrate metabolism, and immunity and defense. *Electrophoresis*, 36: 1699-1710.

De Bruyne, L; Höfte, M and De Vleesschauwer, D (2014). Connecting growth and defense: The
emerging roles of brassinosteroids and gibberellins in plant innate immunity. *Mol. Plant*, 7: 943-959.

Dubrow, Z; Sunitha, S; Kim, J G; Aakre, C D; Giriya, A M; Sobol, G; Teper, D; Chen, Y C; Ozbaki-Yagan, N; Vance, H; Sessa, G and Mudgett, M B (2018). Tomato 14-3-3 proteins are required for Xv3 disease resistance and interact with a subset of *Xanthomonas euvesicatoria* effectors. *Mol. Plant Microbe Interact.*, 31: 1301-1311.

Durian, G; Rahikainen, M; Alegre, S; Brosché, M and Kanagasjärvi, S (2016). Protein phosphatase 2A in the regulatory network underlying biotic stress resistance in plants. *Front. Plant Sci.*, 7: 812-812.

Elmore, J M and Coaker, G (2011). The role of the plasma membrane H+-ATPase in plant-microbe interactions. *Mol. Plant*, 4: 416-427.

Eremina, M; Rozhon, W; Yang, S and Poppenberger, B (2015). ENO2 activity is required for the development and reproductive success of plants, and is feedback-repressed by AtMBP-1. *Plant J.*, 81: 895-906.

Friedman, E; Wang, H; Jiang, K; Perovic, I; Deshpande, A; Pochapsky, T; Temple, B; Hicks, S; Harden, T and Jones, A (2011). Acireductone dioxygenase 1 (ARD1) is an effector of the heterotrimeric G protein subunit in Arabidopsis. *J. Biol. Chem.*, 286: 30107-18.

Glass, M; Barkwill, S; Unda, F and Mansfield, S D (2015). Endo-β-1,4-glucanases impact plant cell wall development by influencing cellulose crystalization. *J. Integr. Plant Biol.*, 57: 396-410.

Ho, C L; Tan, Y C; Yeoh, K A; Lee, W K; Ghazali, A K; Yee, W Y and Hoh, C C (2019). Leaf transcriptome of oil palm (*Elaeis guineensis* Jacq.) infected by *Ganoderma boninense*. *Trees*, 33: 943-950.

Huang, X and Madan, A (1999). CAP3: A DNA sequence assembly program. *Genome Res.*, 9: 868-877.

Hushiarian, R; Yusof, N A and Dutse, S W (2013). Detection and control of *Ganoderma boninense*: Strategies and perspectives. *SpringerPlus*, 2: 555.

Hwang, J U; Song, W Y; Hong, D; Ko, D; Yamaoka, Y; Jang, S; Yim, S; Lee, E; Khare, D; Kim, K; Palmgren, M; Yoon, H S; Martinoia, E and Lee, Y (2016). Plant ABC transporters enable many unique aspects of a terrestrial plant’s lifestyle. *Mol. Plant.*, 9: 338-355.

Jones, J D G (1996). Plant disease resistance genes: Structure, function and evolution. *Curr. Opin. Biotechnol.*, 7: 155-160.
Moin, M; Bakshi, A; Saha, A; Dutta, M; Madhav, S M and Kirti, P B (2016). Rice ribosomal protein large subunit genes and their spatio-temporal and stress regulation. *Front. Plant Sci.*, 7: 1284.

Murase, K; Hirano, Y; Sun, T P and Hakoshima, T (2008). Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. *Nature*, 456: 459-463.

Neik, T X; Amas, J; Barbetti, M; Edwards, D and Batley, J (2020). Understanding host-pathogen interactions in *Brassica napus* in the omics era. *Plants*, 9: 1336.

Pilotti, C A (2005). Stem rots of oil palm caused by *Ganoderma boninense*: Pathogen biology and epidemiology. *Mycopathologia*, 159: 129-137.

Qin, X; Liu, J H; Zhao, W S; Chen, X J; Guo, Z J and Peng, Y L (2013). Gibberellin 20-oxidase gene *OsGA20ox3* regulates plant stature and disease development in rice. *Mol. Plant Microbe Interact.*, 26: 227-239.

Qistina Othman, N; Paravamsivam, P; Tan, J S; Lee, Y P; Kwan, Y Y and Syed Alwee, S S R (2018). Validation of differential gene expression of transcriptome assembly via Nanostring® Technologies analysis platform. *J. Oil Palm Res.*, 30(4): 36-46.

Rees, R W; Flood, J; Hasan, Y; Potter, U and Cooper, R M (2009). Basal stem rot of oil palm (*Elaeis guineensis*); Mode of root infection and lower stem invasion by *Ganoderma boninense*. *Plant Pathol.*, 58: 982-989.

Reinhold, B; Maes, T; Gemmer, S; Van Montagu, M and Hurek, T (2006). An endoglucanase is involved in infection of rice roots by the not-cellulose-metabolizing endophyte *Azoarcus* sp. strain BH72. *Mol. Plant Microbe Interact.*, 19: 181-188.

Saha, A; Das, S; Moin, M; Dutta, M; Bakshi, A; Madhav, M S and Kirti, P B (2017). Genome-wide identification and comprehensive expression profiling of ribosomal protein small subunit (RPS) genes and their comparative analysis with the large subunit (RPL) genes in rice. *Front. Plant Sci.*, 8: 1553.

Sariah, M and Zakaria, H (2000). The use of soil amends for the control of basal stem rot of oil palm seedlings. *Ganoderma Diseases of Perennial Crops* (Flood et al. eds.). CABI Publishing, United Kingdom. p. 89-100.

Singh, H; Kaur, K; Singh, M; Kaur, G and Singh, P (2020). Plant cyclophilins: Multifaceted proteins with versatile roles. *Front. Plant Sci.*, 11: 585121.

Su, Y; Wang, Z; Liu, F; Li, Z; Peng, Q; Guo, J; Xu, L and Que, Y (2016). Isolation and characterization of ScGluD2, a new sugarcane beta-1,3-glucanase D family gene induced by *Sporisorium scitamineum*, ABA, H₂O₂, NaCl, and CdCl₂ stresses. *Front. Plant Sci.*, 7: 1348.

Tan, B A; Daim, L D J; Ithinin, N; Ooi, T E K; Md-Noh, N; Mohamed, M; Mohd-Yusof, H; Appleton, D R and Kulaveerasingam, H (2016). Expression of phenylpropanoid and flavonoid pathway genes in oil palm roots during infection by *Ganoderma boninense*. *Plant Gene*, 7: 11-20.

Tang, D; Wang, G and Zhou, J M (2017). Receptor kinases in plant-pathogen interactions: More than pattern recognition. *Plant Cell*, 29: 618-637.

Tarazona, S; García-Alcalde, F; Dopazo, J; Ferrer, A and Conesa, A (2011). Differential expression in RNA-seq: A matter of depth. *Genome Res.*, 21: 2213-2223.

Wanke, D and Kolukisaoglu, H U (2010). An update on the ABCC transporter family in plants: Many genes, many proteins, but how many functions? *Plant Biol.*, 12: 15-25.

White, M D and Flashman, E (2016). Catalytic strategies of the non-heme iron dependent oxygenases and their roles in plant biology. *Curr. Opin. Chem. Biol.*, 31: 126-135.

Yang, D L; Li, Q; Deng, Y W; Lou, Y G; Wang, M Y; Zhou, G X; Zhang, Y Y and He, Z H (2008). Altered disease development in the eui mutants and Eui overexpressors indicates that gibberellins negatively regulate rice basal disease resistance. *Mol. Plant*, 1: 528-37.

Zhang, X; Kuivenhoven, J and Groen, A (2015). Forward individualized medicine from personal genomes to interactomes. *Front. Physiol.*, 6: 364.

Zhou, B and Zeng, L (2017). Conventional and unconventional ubiquitination in plant immunity. *Mol. Plant Pathol.*, 18: 1313-1330.