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Proteomic Approaches to Unravel Complex Interactions Between *Ganoderma boninense* and Oil Palm in Basal Stem Rot Disease: A Scoping Review

Noor Azlina Mohd Nayan[1], Nur Maisarah Sarizan[1*], Umi Marshida Abd Hamid[2,3], Mohd Hefni Rusli[4], Muhammad Mukram Mohamed Mackeen[5,6] and Mohd Faiz Foong Abdullah[2,3]

[1]*Faculty of Applied Sciences, Universiti Teknologi MARA, Perlis Branch, Arau Campus, 02600 Arau, Perlis, Malaysia.*
[2]*Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.*
[3]*Molecular Microbial Pathogenicity Research Group, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.*
[4]*Malaysian Palm Oil Board, 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.*
[5]*Department of Chemical Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia.*
[6]*Institute of Systems Biology (INBIOSIS), Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia.*

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ABSTRACT

Ganoderma boninense poses the biggest threat to the oil palm industry by inducing basal stem rot (BSR) in Malaysia, a leading palm oil-producing nation. Protein analysis is one of the potential methods in early detection of infected plants by understanding the changes in protein molecules which are useful as biomarkers. However, proteomic methods are not widely used due to their complex protein analysis in nature and the diversity of protein characteristics, including variations in abundance, structure, and post-translational modifications. Thus, a scoping review was analysed based on related literatures by identifying gaps and limitations in protein analysis for managing *G. boninense*. This scoping review was conducted following PRISMA guidelines. The results were searched using different databases: Scopus, Asean Citation Index (ACI), ScienceDirect, PubMed, and Web of Science (WoS). The keywords used were "*Ganoderma boninense*" OR "basal stem rot" AND "proteomics" OR "protein analysis" OR "proteomic profiling" OR "mass spectrometry" OR "protein expression." 116 articles were collected from the various databases, and only 13 significant articles were included

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[1*] Corresponding author. *E-mail address*: maisarahsarizan@uitm.edu.my

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after reviewing the abstracts and removing the duplicates. The relevant articles showed that proteomic methods, particularly LC-MS, 2-DE, and MALDI TOF/TOF were effectively used to study the molecular reactions of oil palms to *G. boninense* infection. There was still a lack of proteomic data with other research on certain protein classes, and other post-translationally modified proteins which may contribute to an incomplete understanding of *G. boninense* pathogenicity. It is recommended that these gaps be addressed by employing advanced proteomic approaches in future studies to facilitate a deeper comprehension of complex protein interactions. The analysis of proteomic methods employed for studying *G. boninense* is essential for the oil palm sector, as this strategy can contribute to valuable insights that can advance strategies for early detection of the pathogen infection.

INTRODUCTION

Malaysia is a well-known palm oil-producing country, the second largest in the world [1]. However, the presence of fungi threatens the oil palm plantations. *Ganoderma boninense* (*G. boninense*) is a pathogenic fungus that causes two severe diseases: basal stem rot (BSR) and upper stem rot (USR) [2,3]. The problem related to BSR has been documented in almost all countries that involved in oil palm plantations. The prevalence of this severe disease has risen over time, particularly in Malaysia and Indonesia, since the massive replantation of oil palms [2]. BSR is transmitted to healthy oil palms from contaminated sources such as stumps, trunks, and diseased oil palms. *G. boninense* colonizes oil palms by entering through their root systems. Animals, insects, and wind can spread the basidiospores produced by *G. boninense* fruiting bodies [4]. The basidiocarp, a large, woody fruiting body of *G. boninense*, frequently manifests as leathery brackets with stems. Typically, these fruit bodies develop on tree trunks in a fan or hoof-like form. Additionally, they possess truncated, double-walled spores with inner layers ornamented in a yellowish-to-brownish colour. These features determine the morphological characteristics of *G. boninense* [5].

A broad spectrum of techniques has been developed to mitigate the growing severity of *G. boninense*, a fungus that substantially impacts Malaysia's crucial oil palm economy. Chemical and biological agents are becoming the frequent procedure to control *G. boninense* disease in oil palm plantations. Nonetheless, these techniques may possess drawbacks. Chemical procedures may cause environmental damage, whilst biological agents generally fail to provide enduring solutions [4,6]. In recent years, protein analysis has emerged as a promising method for detecting biomarkers in infected plants. This can enhance disease management by facilitating early detection and more focused interventions, such as targeted treatments, precision breeding for resistance, and real-time monitoring of pathogen progression. However, proteomic methods are not widely used due to the complexity of protein analysis, particularly because of the diversity of protein characteristics [7].

Technological advancements have improved the accessibility and efficiency of protein analysis, hence overcoming the past complexities that posed challenges in this sector. In the past, protein analysis relied on conventional methods like enzymatic hydrolysis and chromatography techniques (e.g., size exclusion chromatography, ion exchange chromatography, and immobilized metal affinity chromatography), which often involved manual work and were less efficient compared to modern automated approaches [8]. As a result, the protein analysis becoming the unfavourable solution to be used within the industry players. Although there have been significant technological breakthroughs, proteomics still faces a lack of mainstream awareness and acceptability among the research community. This is further compounded by technical challenges; for instance, Pauper et al. [9] highlight that while mass spectrometry-based proteomics offers tremendous potential, the lack of user-friendly, standardized platforms limits its

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accessibility to non-specialist laboratories. Despite proteomic methods have made significant advances in sensitivity and throughput, their adoption remains inconsistent across research fields. Therefore, a scoping study was carried out to identify relevant research areas of proteomic approaches that need further investigation. This review seeks to offer a thorough summary and direct future research initiatives in the oil palm industry.

Generally, a scoping review is a systematic process that aims to identify and collect existing literature on a subject, resulting in a comprehensive understanding of the topic. The primary purposes of a scoping review are to outline the existing literature's scope, breadth, and characteristics and to identify gaps [10]. Additionally, this scoping review is crucial to developing the focus and determining high-quality topics. A bibliometric analysis has been conducted on oil palm pests and their potential threat, but lack of synthesis and standardization. This highlights the significance of scoping review to consolidate the proteomic method and its relevance to oil palm application and disease management [11].

The main issue for this scoping review is that despite its massive impact on Malaysia's oil palm sector, there is still a poor understanding of protein analysis when studying the relationship of *G. boninense*. Previous studies mainly focused on developing products directly used to mitigate *G. boninense*. Therefore, the study of protein analysis is often overlooked because of their methods, commonly known as time-consuming and complicated procedures. The protein analysis may contribute to significant results on alterations of proteins related to the infection of *G. boninense.* The severity effect of *G. boninense* in oil palm plantations must be acknowledged, as this detrimental disease can lead to infection prior to identification, obstructing efficient management measures.

The purpose of this scoping review is to determine and underline the gaps or limitations of the proteomic methods used to lessen the burden of *G. boninense* infection in palm oil plantations, whilst highlighting its notable identification of proteins and the expression of proteins from the infection of *G. boninense* that could be useful for future improvement in disease management strategies.

EXPERIMENTAL

The 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses' (PRISMA) flowchart was used to ensure transparency and completeness in reporting the scoping review. A similar method in bibliometric analysis of oil palm disease literature was adopted by Corrêa et al. [12] which highlighted the importance of searching the database with certain keywords and selection criteria. Figure 1 illustrates an overview of the search process for this review. This process began with a comprehensive database search to identify relevant articles, followed by removing any duplicate records. Studies were then screened based on titles and abstracts to exclude unrelated ones, and full-text articles were reviewed for eligibility. Studies that met the inclusion criteria were included and analysed in the review. The PRISMA flowchart illustrates the included and excluded articles at each of these stages.

Study design

The scoping review employed multiple databases to gather extensive data to uncover the full range of research on this subject. The review was carried out using the framework suggested by Arksey and O'Malley [13] and improved by Levac et al. [14], which included several important stages. The research question is determined by outlining the scope and focus of the review. Relevant studies are identified by a comprehensive literature search across many databases. Studies are selected according to specified inclusion and exclusion criteria. The data from the chosen studies is subsequently extracted and systematically organized. In the final stage, the results are presented by synthesizing the data to emphasize significant findings, highlight the gaps, and offer recommendations for further studies.

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As an introduction to this scoping study, the research question must be defined clearly to guide the scope of the study which are population, intervention, comparison, and control (PICO). This PICO method is well known for developing the review question and focusing on the specific topic. In this study, the population refers to oil palms that have been infected by *G. boninense,* and intervention refers to protein analysis on the population. Meanwhile, the comparison refers to the instrument used to analyse the proteins either by conventional method or high-throughput technology; and outcome refers to the results of protein expression or profiles in the population. Therefore, the research question in this study is: "What are the conventional and high-throughput protein analysis methods used in detecting protein profiling in *G. boninense*-infected oil palms?"

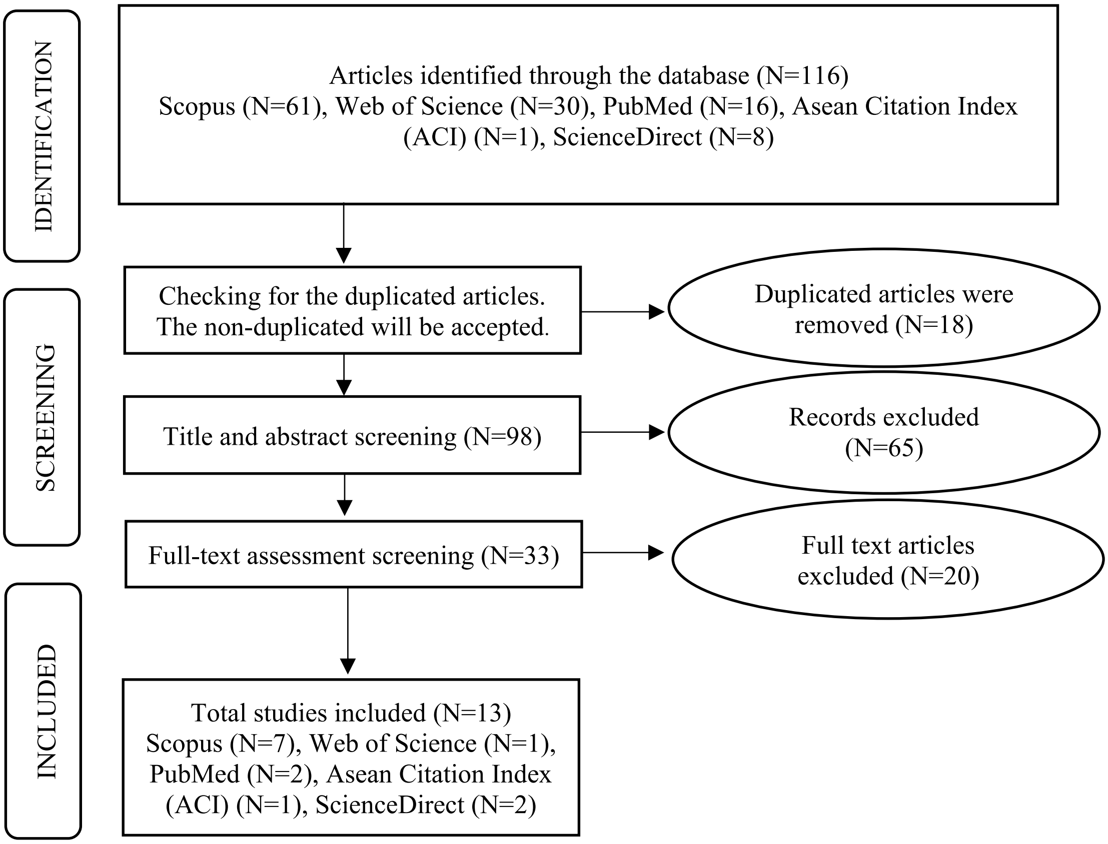

Figure 1. The search process of a scoping review.

Determine relevant studies

Proteome analysis represents a promising yet underexplored domain within BSR research. This is mainly because of its complex workflows, which have often been overlooked. Proteomic studies have the ability to reveal crucial insights, such as disease biomarkers, that can suggest the development of products that mitigate BSR efficiently. The inclusion criteria for this scoping review have two main requirements. Firstly, the studies must clearly explain the use of proteomic methods such as 2DE gel electrophoresis to detect

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proteins expressed in *G. boninense* that found in oil palms. Secondly, the studies must present the results related to *G. boninense* and oil palms, like the expression of proteins or the potential as biomarkers.

Search strategy

The proteomic interaction between *G. boninense* and oil palms was done by querying different databases, notably Scopus, ASEAN Citation Index (ACI), ScienceDirect, PubMed, and Web of Science (WoS). The search was performed to collect precious information on the proteome hallmarks of this disease. The selected keywords were thoughtfully chosen by using Boolean operators (e.g., OR, AND) and exact phrase (e.g., "basal stem rot"). Indeed, the use of Boolean query formulation improved retrieval accuracy along with rapid optimization [15]. The keywords are "*Ganoderma boninense*" OR "basal stem rot" AND "proteomics" OR protein analysis" OR "proteomic profiling" OR "mass spectrometry" OR "protein expression". In the results, the articles identified through the database (N=116); Scopus (N=61), Web of Science (N=30), PubMed (N=16), Asean Citation Index (ACI) (N=1), ScienceDirect (N=8).

Study selection

This study mainly focused on particular subjects, which are oil palms and the pathogenic fungus *G. boninense.* Another primary focus is the studies that utilized experimental designs using protein analysis tools to study interactions and alterations of proteins linked to BSR disease. Then, the papers were published from the year 2000 onwards and written in English or Malay language. Afterward, the papers were chosen and underwent the process of removing duplicate topics, screening the abstracts, and finalized with fully text assessment screening for each selected article.

The study selection was performed by using the PRISMA flowchart. The process showed abstract screening (N=98), which excluded 65 irrelevant abstracts to the topic. This was because their abstract mostly misaligned protein methodologies and did not directly discuss the protein results. The next step was a full-text assessment (N=33), which was critical to exclude 20 articles due to the studies on microbiological approaches that were mainly not applied to protein analysis. In the following stage, each chosen article was thoroughly examined to determine whether it should be included in the study's final selection. This stage ensures that every study area was comprehensively assessed and resolves inconsistencies through discussion.

Data reporting

The final results were 13 remarkable articles after thoroughly evaluating the scoping process. All of these data were updated and reviewed as these articles were conducted with meticulous attention, based on their particular relevance to the subject matter of oil palms and BSR, and confirmed that each study satisfied the required criteria for inclusion. Two independent reviewers have thoroughly examined the accuracy and suitability of data to ensure its inclusion in the study. This meticulous evaluation process ensures the reliability of the data and enables its trustworthy presentation. The data were classified according to some crucial criteria, including the publication year, the precise research methodology employed, and the result of the issue. The procedures applied in each study were documented, identifying the utilization of proteomic methods, and the corresponding annual findings were identified in a table. The data was gathered according to these two overarching categories, enabling a thorough comparison and analysis of the findings. Thus, this technique makes evaluating each methodology's comparative advantages and disadvantages feasible.

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RESULTS AND DISCUSSION

This scoping review has identified 13 notable articles, as listed in Table 1, that directly explained and performed protein analysis on *G. boninense* and its relationship with oil palms. Throughout this section, the findings of these 13 remarkable papers can provide valuable information on the current research and its consequences for managing BSR. This section is divided into three different discussions: comparative of proteomic analysis, proteomic analysis of oil palm interaction with *G. boninense,* and secretome analysis.

Comparative of proteomic analysis

The results showed and identified two-dimensional gel electrophoresis (2-DE), liquid chromatography-mass spectrometry (LC-MS), and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/TOF) as the proteomic method used in these papers. To begin with, 2-DE is the most common method used in the study of proteins as this is a basic method to spot and identify the proteins found in the sample tissues of oil palms to BSR. Generally, 2-DE can be defined as a powerful analytical method to separate complex protein mixtures according to their isoelectric point (pI) and molecular mass. This approach consists of two consecutive stages: isoelectric focusing (IEF) and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) [16].

After that, LC-MS is an analytical method that combines the separation skills of liquid chromatography with the detection and quantification abilities of mass spectrometry [17]. Commonly, LC-MS is used in the last stage of protein analysis as its function is to directly identify and characterize proteins. This analysis is crucial for establishing the structure and function of proteins involved in the sample tissues (roots, leaves, and mycelium) [18].

The most highlighted in these results is MALDI-TOF/TOF, method for identifying and characterizing proteins and peptides. The instrument functions by ionizing samples and measuring the duration it takes for ions to traverse a vacuum, enabling accurate mass calculation. This technique is greatly beneficial in the study of proteomics because it can look into complex biological samples with minimal pretreatment and achieve high sensitivity ranging from picomole to femtomole levels [19]. MALDI-TOF/TOF is highly proficient at discovering the structural details of proteins, such as post-translational modifications and protein interactions, which makes this method costly and requires the skills to perform this instrument compared to other proteomic methods [19]. In addition, MALDI-TOF/TOF is preferable to LC-MS because of their simple sample preparation step rather than the complex processes required with LC-MS [20]. Their findings indicate that the sample loss and contamination can be minimized with this high-throughput MALDI-TOF/TOF. Continuing with this instrument is also highly effective in identifying and examining post-translational modifications (PTMs). However, this instrument may not be available in many laboratories due to the need for substantial investment in infrastructure and training. Therefore, its utilization is less extensive than other techniques like LC-MS and 2-DE despite its higher capabilities in proteomic studies.

Table 1. 13 notable papers of scoping review after removing the duplicates and screening the abstract and full-text assessment.

Authors	Proteomic method	Sample type	Findings
[21]	LC-MS	Oil palm leaves	Altered proteins are associated with photosynthesis, carbohydrate metabolism, and immune defence.
[22]	2D Gel Electrophoresis, Mass Spectrometry	Oil palm leaves	24 differentially expressed proteins (photosynthesis, signalling, stress/defence, metabolism).

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[23]	MALDI-TOF/TOF Mass Spectrometry	Oil palm roots	36 proteins were discovered to be expressed differently in response to *Ganoderma* spp. inoculation (signalling, stress/defence response, and energy/lignin biosynthesis).
[24]	LC-MS	Oil palm roots and leaves	Identified unsaturated fatty acid and secondary metabolite pathways in partial resistant oil palm (TUP 1281) and biochemical differences between resistant and susceptible progenies (TUP 1309).
[25]	2-DE, MALDI TOF/TOF	*Ganoderma* spp. mycelium	The highest protein concentration and reproducibility were achieved through phenol/ammonium acetate extraction.
[26]	2-DE gel analysis, phenol/ammonium acetate extraction	Oil palms roots	12 potential proteins, including glucanase, were identified, emphasizing their involvement in stress response and defence.
[27]	Shotgun Proteomic Analysis under carbon and nitrogen starvation	Mycelial proteins of *G. boninense* and *G. tornatum*	A bioinformatics analysis identified 1645 proteins altered with catalytic activity, binding, and metabolic processes. Pathogenicity was associated with distinct protein groups.
[28]	2-DE & Mass Spectrometry	Oil palm roots	Identified 21 proteins that underwent substantial changes in abundance, including those associated with oil palm defence mechanisms.
[29]	Shotgun Proteomics for Secretome	Secreted proteins (secretome) of *G. boninense*	250 non-redundant proteins were identified and categorized as CAZymes, emphasizing the distinctive presence of oxidoreductases in *G. boninense.*
[30]	LC-MS/MS	*G. boninense* T10 secretome	Differential expression analysis identifies and analyses potential effector proteins (CEPs).
[31]	Protein Activity Assay	Oil palm roots	Exhibited antifungal activity of a defensin protein expressed in E. coli against *G. boninense.*
[32]	Nano-LC-MS/MS Analysis	Culture filtrate from *G. boninense*	Nine cerato-platanin homologs are present in the *G. boninense* related to the pathogenesis of fungal infections.
[33]	SDS-PAGE	*G. boninense* mycelium	Necrosis and ethylene-inducing protein (NEP) from *G. boninense* suggested the pathogenesis of fungal infections.

Proteomic analysis of oil palm interaction with G. boninense

Results in Table 1 showed that oil palms are analysed for protein content in various tissues, including leaves, roots, or a combination [21,22,23,24,26,28,31]. As an example, a study by Othman et al. [24] has shown that proteins disulfide isomerase (PDI) and NDR1/HIN1-like (NHL) contributed to defence response in oil palm root tissues infected with *G. boninense*.

Oil palm root tissues

Root tissues absorb nutrients, respond to stress, and interact with soil pathogens, *G. boninense*. The water status of root tissues can influence plant-pathogen interactions, as drought stress can increase susceptibility to pathogens by modifying plant water relations and facilitating pathogen establishment [34]. The presented information highlighted in Table 1, the studies that analysed oil palm roots utilized sophisticated proteomic methods are MALDI-TOF/TOF, phenol/ammonium acetate extraction, 2-DE, and LC-MS. Their studies successfully extracted and identified the proteins involved in the *G. boninense* pathogenicity. As mentioned earlier, the comparison of MALDI-TOF/TOF, 2-DE, and LC-MS has been discussed as the analytical method for uncovering and identifying the proteins in complex biological oil palm tissues. Meanwhile, phenol/ammonium extraction is well known for extracting proteins that are later used for subsequent analysis of proteins (MALDI-TOF/TOF, 2-DE, and LC-MS).

An excellent study by Al-Obaidi et al. [23] conducted protein analysis using MALDI-TOF/TOF to study proteins in oil palm roots with high sensitivity and specificity. The results showed 36 proteins expressed differently in response to *Ganoderma* spp. These proteins are crucial in activities of signalling, stress and defense response, and energy production and lignin. MALDI-TOF/TOF, in the study, was more advantageous due to its ability to precisely identify a diverse array of proteins. In addition, the study focused on *Ganoderma* spp. mycelium by employing the MALDI-TOF/TOF and phenol/ammonium extraction, which indicates the satisfied results of the proteins present in *Ganoderma* spp. mycelium. Therefore, the proteomic methods in his study clearly can identify protein functions in the initial phases of the interaction between oil palm and *Ganoderma* spp. and offer a better understanding of the plant's defence mechanisms.

Tissues of oil palm leaves

This scoping review has shown that studies have analysed proteins in tissues of oil palm leaves, suggesting the leaves are essential in photosynthesis, nutrient assimilation, and environmental stress responses. The proteomic methods that were used were 2DE and LC-MS, which are common methods in plant proteomics. These approaches are ideal for leaf analysis since extracting proteins from leaf tissues is typically easier than extracting proteins from roots. The simplicity is attributed to the distinct protein compositions in leaves, which frequently possess a higher concentration of proteins and a lower presence of complex substances such as lignin that are commonly found in roots. These methods can spot and determine the various proteins, including those that play crucial roles in photosynthesis, defence mechanisms, and metabolic regulation. 2DE elucidates alterations in photosynthetic proteins, defensive enzymes such as chitinases and peroxidases, and metabolic enzymes by their separation according to molecular weight and charge [26,35]. LC-MS enhances this by identifying and quantifying proteins, detecting post-translational changes, and assessing related metabolites [36]. Together, these approaches offer a comprehensive insight into the impact of *G. boninense* infection on photosynthesis, the activation of defence responses, and metabolic adaptations.

Mycelium tissues

Mycelium is the vegetative structure of basidiomycete fungi responsible for causing BSR [37]. One of the studies focuses on proteins in *Ganoderma* mycelium which is essential for knowing its ability to cause disease and its interactions with host plants, particularly with oil palm diseases [25]. This study mostly utilizes 2-DE and MALDI-TOF/TOF to examine protein profiles accurately. This study also performed the various extraction of protein methods, including the phenol/ammonium acetate in the methanol which produced the highest protein concentration and the most consistent results among the other protein extraction methods. The 2-DE method was used to detect protein spots. Later, MALDI-TOF/TOF analysis was used to determine the protein's identity in the *Ganoderma* mycelium. These approaches offer unique insights into the proteome of *Ganoderma*, contributing to the comprehension of its function in oil palm disease, BSR.

Another study by Dzulkafli et al. [27] used shotgun proteome analysis to analyse the protein expression of two *Ganoderma* species, *G. boninense* and *G. tornatum*, when subjected to carbon and nitrogen deprivation. Focused of the study is to have a deeper understanding of their role in oil palm disease,

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particularly BSR. *G. boninense* is widely known for its highly aggressive pathogenic meanwhile *G. tornatum* is considered non-pathogenic. Shotgun proteomics is a method that involves breaking down proteins into peptides and then using mass spectrometry to analyse them [38]. This study indicated the significant differences in protein expression between the two species, with proteins associated with virulence, stress responses, and metabolic adaptation exhibiting differential expression.

Combined sample tissues (roots and leaves)

A study by Othman et al. [24] presented a captivating case study of applying LC-MS in analysing root and leaf tissues in oil palms. This approach thoroughly explains the biochemical pathways associated with resistance to BSR. The study compared the protein and metabolite profiles, revealing significant differences between resistant and susceptible progenies in unsaturated fatty acid and secondary metabolite pathways.

The LC-MS method is highlighted as it allowed for a comprehensive analysis of the proteins and metabolites involved in the plant's defensive mechanisms by studying the root and spear leaf tissues. The approach facilitated the identification of crucial pathways, such as metabolites, chelidonic acid that could significantly impact the plant's reaction to infection *G. boninense* [24]. Thus, the utilization of LC-MS in this study demonstrated its efficacy in elucidating the molecular pathways underlying disease resistance in oil palms, offering it a crucial instrument for forthcoming research and breeding endeavors [39].

Secretome analysis

The secretome of *Ganoderma* spp. refers to the accumulation of proteins secreted during the infection process [29]. The secretome includes enzymes and effectors that play critical roles in plant pathogenesis such as CAZymes and hydrolases [29,40]. Based on Table 1, the results showed significant studies on secreted proteins from *G. boninense* by employing LC-MS and shotgun proteomics. First is a study by Dzulkafli et al. [29] applied shotgun proteomics of the secretome in *G. boninense* and *G. tornatum*, and identified proteins: oxidoreductases, transferases, hydrolases, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). These unique proteins have their functions in this *Ganoderma* pathogenicity. The abundance of present oxidoreductases suggested that these proteins respond to *Ganoderma* infection, specifically, *G. boninense* [29]. Meanwhile, transferase is an enzyme that facilitates transferring the functional group (e.g., methyl, glycosyl) [41], and hydrolase breaks down the oil palm cell wall to aid the infection of *Ganoderma* [42]. On the other hand, glyceraldehyde-3-phosphate dehydrogenase (GADPH) is present for contributing to the growth of *Ganoderma* especially *G. lucidum* [43].

Another remarkable study by Khairi et al. [30] revealed the results of candidate effector proteins (CEPs) involved in pathogenicity, including carbohydrate-active enzymes (CAZymes), hydrolases, and non-catalytic proteins. CAZymes are proteins involved in the breakdown of carbohydrates produced by *G. boninense* [42]. Then, hydrolases, and non-catalytic proteins are involved in functional roles in the pathogenicity of *G. boninense* [30].

CONCLUSION

In conclusion, high-throughput proteomic methods for protein analysis are significant, especially in identifying post-translational modifications. Although 2-DE, LC-MS, and MALDI TOF/TOF approaches present different difficulties in cost, demanding instrument management, and requirement for specialist skills, they still allow for effective and promising protein studies. The available study has presented the molecular mechanisms by which *G. boninense* induces the BSR in oil palms. It is recommended that future research studies advanced proteomic approaches to enhance the identification of proteins by offering insights into spatial protein distribution and accurate quantification. In addition, using advancements in

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computational tools could improve data processing, facilitating a deeper comprehension of complex protein interactions. Therefore, this recommendation may ultimately enable the discovery of crucial biomarkers and the development of more efficient ways to intervene in diseases. This would provide enhanced strategies for managing and eliminating the BSR disease.

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AUTHOR'S CONTRIBUTION

Noor Azlina Mohd Nayan conducted the study, gathered the data, authored the manuscript, and performed the revisions. Nur Maisarah Sarizan originated the study proposal, supervised the project as the corresponding author, and offered essential evaluations throughout the writing and revision phases. Umi Marshida Abd Hamid, Mohd Hefni Rusli, Muhammad Mukram Mohamed Mackeen, and Mohd Faiz Foong Abdullah participated in the evaluation and revision of the text and supplied insights to enhance the finalized version.

CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted without any self-benefits or commercial or financial conflicts and declare the absence of conflicting interests with the funders.

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