

OMICS PLATFORM TECHNOLOGIES FOR DISCOVERY AND UNDERSTANDING THE SYSTEMS BIOLOGY OF OIL PALM

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ABSTRACT

Palm oil is the leading vegetable oil in terms of volume in the world market. Indonesia and Malaysia are the top producers and exporters of the commodity. The global production of palm oil reached 73.5 million tonnes in the period 2018/2019, up from approximately 70.5 million tonnes in 2017/2018. Oil palm is the most productive crop in the world being 10 times more productive than soyabean which produces only about 0.45 t oil per hectare. Nonetheless, the industry is continuously under pressure to improve productivity and sustainability. This will require concerted innovations across the entire palm oil supply chain and fully committed research efforts including upstream technologies to expedite crop improvement. At the Malaysian Palm Oil Board (MPOB), research efforts at development of tools for improving oil palm traits by genetic modification have been augmented more recently with omics-based approaches and all of these innovations are synchronised towards improved product quality. Omics is a multi-disciplinary field encompassing genomics, epigenomics, transcriptomics, proteomics and metabolomics. Each of these fields provides an opportunity to understand and view oil palm biology from a global perspective, enabling accelerated discoveries for improved productivity and the development of new and improved varieties. An integrative omics approach promises great value in both phenotyping and diagnostic analyses. With the current technological capabilities, metabolomics is also being exploited for identifying unique chemical fingerprints to detect product contamination and adulteration in oil palm. This effort is actively being conducted in order to position the oil palm industry to meet and optimise the delivery of the highest quality oil with minimum environmental and social concerns. In this review, an overview is given on the current knowledge and progress made in oil palm research, focusing on the application of omics strategies and their integration with high-throughput technologies for oil palm crop improvement, development of geographical traceability system and ensuring that palm oil is free from residual oil contamination.

Keywords: omics research, proteomics, metabolomics, traceability, crop improvement.

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INTRODUCTION

Palm oil (from *Elaeis guineensis* Jacq.) is one of the most highly valued commodities for Malaysia and

contributes significantly to the economic growth of the country (Kushairi *et al.*, 2019). In terms of yield, the oil palm is characterised as the most productive oil crop with a potential yield capacity of more than 10 t of oil per hectare per year (Murphy, 2014). However, generally, yield gaps in oil palm plantation are large, with yields typically between 4-6 t of oil per hectare for the best commercial plantations and

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3-4 t oil per hectare for smallholders. Assessment of the underlying causes of yield gaps in oil palm production systems worldwide is still lacking (Woittiez *et al.*, 2017). Palm oil is used worldwide and contributes significantly to the world's oils and fats market (Nambiappan *et al.*, 2018; Parveez *et al.*, 2020). There are many factors that contribute to the global demand for palm oil. These include increasing awareness of its health attributes, competitive price and versatility that allows it to be used in both food and non-food applications (May and Nesaretnam, 2014; Khosla, 2011). Malaysia is the second largest producer of palm oil in the world behind Indonesia. The overall production in Malaysia in 2019 was over 19.86 million tonnes of crude palm oil from 5.9 million hectares of planted area. India and China are the two largest palm oil export destination, with the European Union (EU) also being among the main markets for Malaysia's key commodity. In 2019, Malaysia exported a total of 16.88 million tonnes of palm oil to the world indicating a strong demand from importing countries, especially India, China and the EU as compared to 15.36 million tonnes in the previous year (Parveez *et al.*, 2020).

Unstable commodity prices, labour shortage, ageing oil palm populations, and pests and diseases that seriously affect overall yield are among the key challenges faced by the oil palm industry (Alam *et al.*, 2015; Murphy, 2014). A viable solution for overcoming these challenges is through the development of varieties that can increase yield per unit land, are more resilient even to climate change and produce diversified and more high value products (Murphy, 2014). Oil palm breeding programmes focussed on specific traits such as high yield, water uptake efficiency, nutrient use efficiency, disease resistance and plant architecture will be essential in the future for improving commercial yields. While conventional breeding has its limitations, application of biotechnological tools will be a viable strategy for overcoming the above challenges. Fortunately, tools are available to assist crop improvement such as genetic modification for producing high value products and developing oil palm with pest and disease tolerance. This is to keep pace with global population growth and meet the increasing demand for palm oil for both food and non-food applications (Parveez *et al.*, 2015).

Tremendous progress has been made in recent years in the area of modern genetics through integration with other omics technologies (Teh *et al.*, 2017). Biochemical and genetic studies have revealed that in many crops including oil palm, the majority of important agronomic traits such as yield, plant height and nutrient content are complex quantitative traits that are controlled by multiple interacting genes (Seng *et al.*, 2016; Long *et al.*, 2007; Ramli *et al.*, 2002a, b). To understand the inheritance of complex traits, the establishment of reliable

screening tools and platforms that can precisely measure expression of physiological traits in realistic field environments is important. The emergence of the novel omics innovations, for example, genomics, proteomics and metabolomics has allowed scientists to identify the molecular mechanisms underpinning crop improvement (Tester and Langridge, 2010; Syrenne *et al.*, 2012; Appleton *et al.*, 2014; Chaudhary *et al.*, 2015). In this respect, genomics and epigenomics-based technologies have successfully provided the necessary tools that will support the sustainable development of oil palm (Low *et al.*, 2017; Ong-Abdullah *et al.*, 2015; Singh *et al.*, 2013a, b; 2020). Concomitantly, remarkable progress in omics research, combining innovative and accurate technologies with the requirement for intersection with bioinformatics has resulted in the discovery of key regulators of various traits for crop improvement (Hajduch *et al.*, 2011; Chaudhary *et al.*, 2015; Fernie and Schauer, 2009).

The use of proteomics and metabolomics in oil palm research was initiated about 10 years ago, aimed at developing tools to accurately monitor the proteome and metabolome profiles of clonal/transgenic palms and for a variety of important biological traits, *e.g.* yield, oil quality and disease resistance (Ramli *et al.*, 2016). Several technical and review reports are available on appropriate strategies for the application of molecular characterisation using proteomics and metabolomics in oil palm to improve our understanding of these complex traits, which are likely to underpin future gains in crop productivity (Teh *et al.*, 2017; Dzulkafli *et al.*, 2019; Hassan *et al.*, 2019; Lau *et al.*, 2018; Rozali *et al.*, 2017; Nurazah *et al.*, 2017; Vargas *et al.*, 2014; Neoh *et al.*, 2013; Tahir *et al.*, 2016; 2012). Recent developments in high-throughput omics technologies have also accelerated the accumulation of huge amounts of omics data from multiple sources – genome, epigenome, transcriptome, proteome and metabolome, posing new challenges and opportunities for developing novel computational approaches customised for integrative analysis (Mirza *et al.*, 2019). Consequently, simultaneous analysis of data obtained from different omics platforms, for the same biological specimen is expected to provide a holistic view of the complex biological interactions. The use of state-of-the-art machine learning (ML)-based approaches, which leverage prior knowledge of biological networks to integrate omics-datasets would be vital for robust biomarker modelling (Mirza *et al.*, 2019; Tafti *et al.*, 2017; Glaab, 2016; Price *et al.*, 2017). Thus, the various omics information is not just only assisting in the establishment of deeper understanding of the complex interactions of oil palm metabolic networks and their responses to environmental and genetic changes, but also providing screening and analysis platforms to support oil palm breeding programmes.

Such information will have major implications in enhancing the future production and sustainability of the oil palm industry.

TRANSLATING GENOMICS RESEARCH TO IMPROVE OIL PALM PRODUCTIVITY

Early application of genomics-based tools in oil palm breeding was initiated in the mid-1990s, where restriction fragment length polymorphism (RFLP) markers were employed for DNA fingerprinting (Cheah *et al.*, 1993; Mayes *et al.*, 1996). This was followed by the use of RFLP markers for the construction of a framework genetic map (Mayes *et al.*, 1997). Subsequent development of polymerase chain reaction (PCR)-based simple sequence repeat (SSR) markers led to the construction of a denser oil palm genetic map (Billotte *et al.*, 2005) and made available a more robust tool for fingerprinting (Singh *et al.*, 2008; Ting *et al.*, 2010). Systematic study of genes in oil palm via generation of expressed sequence tags (EST) (Jouannic *et al.*, 2005; Ho *et al.*, 2007; Low *et al.*, 2008; Chan *et al.*, 2010) was an important first step in building the sequence database for oil palm, which was followed by sequencing of the hypomethylated regions of the genome (Low *et al.*, 2014).

A high quality genetic blueprint of both species of oil palm, namely *E. guineensis* and *E. oleifera*, was eventually obtained using a combination of 454/Roche technology and bacterial artificial chromosome (BAC) end sequencing in 2013 (Singh *et al.*, 2013a). Scaffolds from the build were successfully aligned to the 16 linkage groups of the oil palm genetic map, and sequencing of 34 transcriptomes from various tissues enhanced the annotation of the assembly. The importance of the availability of this resource was quickly realised as it proved pivotal in identifying the *Shell* and *Virescens* genes responsible for the three different fruit forms and the exocarp colour of oil palm fruits (two important monogenic traits), respectively (Singh *et al.*, 2014; 2013b). The identification of these genes paved the way for the realisation of genomics guided breeding in oil palm, which was previously restricted to using molecular markers for deoxyribonucleic acid (DNA) fingerprinting and paternity testing of selected oil palm breeding lines and tissue culture clones (Thongthawee *et al.*, 2010; Singh *et al.*, 2007). Moving forward, and building on the experience of other crops, further refinements to the first oil palm genome assembly are necessary, especially to facilitate the identification of genes regulating complex (quantitative) traits.

Nevertheless, the availability of the *pisifera* *E. guineensis* genome sequence has facilitated the assembly of the *dura* *E. guineensis* genome apart from the resequencing of 17 other mostly advanced breeding lines (Jin *et al.*, 2016). The *dura*

genome as expected appeared to be similar to the *pisifera* genome in terms of the percentage of repeat sequences present (~40%), and the number of genes identified, 36 105, which was close to the number reported by Singh *et al.* (2013a). More importantly, the re-sequencing of the 17 palms revealed that the genetic diversity of palms in South-east Asia is rather low, consistent with earlier studies using molecular markers (Maizura *et al.*, 2006), which also suggested that the genetic variability of palms used for commercial cultivation in this region is low. The availability of the genome sequence had facilitated the resequencing of another 132 palms from both species and development of a high density single nucleotide polymorphism (SNP) array, consisting of 200 000 probes (Kwong *et al.*, 2016). The 200 000 single nucleotide polymorphisms were selected across the genome and thus, proved useful in a Genome Wide Association Study (GWAS) that led to the identification of loci influencing oil content in selected *E. guineensis* backgrounds (Teh *et al.*, 2016). A clear advantage of a fixed high density SNP array platform is that it can provide a rapid scan of the genome, with high SNP call rates and as the number of samples being genotyped increases, cost per sample gets lower (Thomson, 2014). In addition, use of a fixed array allows for a more robust comparison of fingerprints across multiple populations or accessions. However, one of the main disadvantages of the fixed array platform is that the single nucleotide polymorphisms are restricted to those found in the samples or accession used to design the array and in the case of oil palm, existing SNP platforms may not be incorporating rare single nucleotide polymorphisms found in wild germplasm samples. This can be a critical issue for oil palm breeding in South-east Asia, where the limited diversity requires the industry to introgress desirable alleles from wild oil palm collections into advanced breeding lines (Ong-Abdullah *et al.*, 2015; Zulkifli *et al.*, 2012; Singh *et al.*, 2008).

Developments in next generation sequencing (NGS) technologies paved the way for an alternative genotyping technique known as genotype by sequencing (GBS) (Nielsen *et al.*, 2011). GBS which entails low coverage skim sequencing of samples, reduces cost substantially, but nevertheless, requires a fairly good existing genome build to anchor the sequences and accurately call the SNP. The availability of the high quality oil palm genome build facilitated GBS studies in oil palm, where Pootakham *et al.* (2015), after further reducing the complexity by digesting the DNA samples with restriction enzymes, skim sequenced 108 palms from a mapping family. More than 20 000 high quality SNP were detected by mapping the GBS reads to the oil palm reference genome, which led to the construction of a genetic map, and more importantly, identification of genomic loci associated with key agronomic traits,

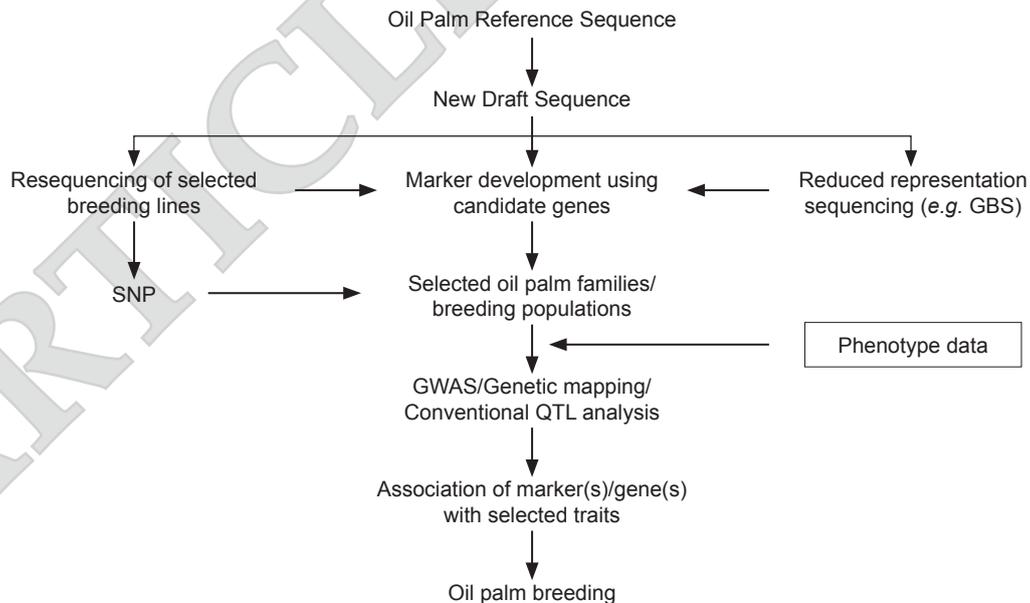
namely height increment and bunch weight. Using a similar GBS approach, again with the published oil palm genome sequence as reference, Bai *et al.* (2017) skim sequenced 155 palms (inclusive of both parental palms) of a F₁ family, and identified single nucleotide polymorphisms linked to oil content. The studies above clearly indicate how the availability of the genome build since 2013 has facilitated the application of the most current genomics-based technologies for marker-trait association in oil palm. Interestingly, the growing repertoire of sequences available for oil palm and research advancements in model crop systems, further facilitates the use of the candidate gene approach to uncover the molecular mechanisms influencing particular traits of interest. Exploiting this, Ong *et al.* (2018) designed SNP markers from selected candidate genes and in combination with the association mapping approach, identified a SNP marker linked to stem height in the MPOB-Angola germplasm collection - revealing an alternative but more focussed route to establish marker-trait association.

Undeniably, the genomic resources established thus far are an important foundation, that will help improve oil palm breeding efficiency (Figure 1). However, to capture the full complexity of a biological system like oil palm, a combined 'omics' approach is required to uncover complex traits such as disease resistance and adaption to abiotic stresses (e.g. drought tolerance). Genomics efforts are in full gear currently for the oil palm and integrating with recent developments in proteomics (Lau *et*

al., 2018) and metabolomics (Ramli *et al.*, 2016; Rodrigues-Neto *et al.*, 2018) will prove important to fully understand the regulatory systems that govern complex traits. However, rapid developments in each of the 'omics' platforms is resulting in vast amounts of complex data that require high end computational analysis by competent researchers to make incisive interpretations from such multi-omics datasets (Misra *et al.*, 2019).

PROTEOMICS AND METABOLOMICS – CURRENT AND EMERGING METHODOLOGIES IN CROP IMPROVEMENT

The essential functions of living cells, e.g. during cell differentiation and regulation or during disease development, are accomplished by gene products, mainly proteins. Thus, it is important to study the proteins involved in order to gain clues about their functional activity. In fact, proteomics is a tool for functional genomics and holds special promise for genetics and physiological studies towards crop improvement (Eldakak *et al.*, 2013; Fiehn *et al.*, 2001). Therefore, technologies aimed at studying proteins in a cell are a welcome complement to uncover the role of genes in cellular activities. Since proteins are involved in cellular enzymatic functions, regulatory switches and structural components, characterising the proteins expressed by a cell can give important clues to the function, organisation and responsiveness of a cell.



Note: GWAS - Genome Wide Association Study.
QTL - Quantitative trait loci.

Figure 1. An overview of the use of the oil palm reference genome for improving oil palm breeding efficiency. The reference genome proved useful as an anchor to establish new draft sequence, resequencing of selected lines which resulted in development of high-density single nucleotide polymorphism (SNP) array. Genotyping by sequencing (GBS) was also made possible as an alternative genotyping tool, which together with SNP arrays, assisted in establishing marker trait association. Interestingly, the availability of large repertoire of sequences also allows development of markers from candidate genes associated with traits in other crop system.

In proteomics research, all the proteins of a cell, the proteome are studied simultaneously in different physiological situations. It provides a dynamic reflection of genes and environment and offers a novel approach for gene (DNA) identification. Damerval *et al.* (1994) identified protein quantity loci (PQL) that explained some of the proteins analysed using proteomics for the purpose of implementing marker-assisted selection (MAS) to improve the traits under study. This type of research is particularly useful for breeding programmes as they provide molecular-based clues about nutritional value, yield and observed stress tolerance phenotypes and how these factors are affected by other adverse conditions (Salekdeh and Komatsu, 2007; Chen and Harmon, 2006). In addition, knowledge of key proteins that play a crucial role in the development of a plant has the potential to complement metabolomics to elucidate the biochemistry of the phenotype being expressed (Deshmukh *et al.*, 2014). There are many examples of proteomics-based studies that include the investigation of biotic and abiotic stresses, which negatively affect crop growth and productivity worldwide (Hashiguchi *et al.*, 2010; Bartolini *et al.*, 1999). Proteomics approaches are also useful to explore the molecular mechanisms underpinning plant-microbe interactions (Lodha *et al.*, 2013; Quirino *et al.*, 2010; Mehta *et al.*, 2008). Contrasting samples in respect to temporal protein accumulation during fruit development (*e.g.*, proteins expressed during early versus fruit ripening) or presence in a specific tissue (*e.g.*, proteins exclusively present in leaf, root and stem) have also been studied using proteomics (Bianco *et al.*, 2013; Nozu *et al.*, 2006).

The well-established International Rice Sequencing Protocols have delivered a substantial amount of information, where proteome studies have facilitated the detection of novel traits useful for breeding (Yu *et al.*, 2002). Accordingly, following proteome analysis, a number of previously unknown novel genes coding for enzymes in metabolic pathways were identified in a number of crops (Canovas *et al.*, 2004). Interestingly, proteome analysis of genetically modified crops has also been used to decipher substantial equivalence in comparative safety analysis to help increase the acceptance of transgenic crops and products (Zhao *et al.*, 2013; Barros *et al.*, 2010).

A complete understanding of plant systems requires filling the gap between proteome and phenotype. We can study genes being expressed, and what proteins are present, but the more important question is for instance what makes certain oil palms to be higher yielding than others or why specific oil palms are more tolerant to disease. Metabolomics is perhaps the ultimate level of post-genomic analysis as it can expose changes in metabolite fluxes that are caused by

only minor changes in gene expression (Allwood *et al.*, 2008). Metabolomics attempts to measure the complete set of metabolites as the end products of cellular regulatory processes, and their levels can be viewed as the response of biological systems to environment or genetic manipulations (Maloney, 2004). Metabolomics distinguishes what is really occurring in a system, whereas genomics and proteomics recognise what could occur. This fact demands a different perspective and requires the measurement of transcriptional, proteomic and metabolomic data in order to obtain a complete picture of the system's response to genetic or environmental stress stimuli.

There are increasing number of examples indicating that proteome and metabolome profiling approaches are becoming more and more important to explore significant sources of variation in crops caused by genetic background, breeding methods, growing environment, genotype-environment interactions and crop cultural practices (Davies *et al.*, 2010; Dixon *et al.*, 2006). The combination of proteomics and metabolomics is thought of as an advanced profiling technology that broadens the spectrum of detectable compounds, which improves the efficacy of assessment strategies (Barros *et al.*, 2010; Zolla *et al.*, 2008; Metzdorff *et al.*, 2006). Generally, the potential of applying proteomics and metabolomics in research is significant and a brief account of the various approaches in plants is given in *Table 1*. Nevertheless, proteomics and metabolomics are yet to be utilised directly on a large scale for crop improvement, and the most basic application currently is focussed on gaining an understanding of proteins and metabolites associated with complex traits such as, yield and disease resistance, both of which are major targets of crop breeding (Kumar *et al.*, 2017; Canovas *et al.*, 2004).

TECHNOLOGICAL PERSPECTIVES FOR PROTEOMICS AND METABOLOMICS

The advent of proteomics technologies for global detection and quantitation of proteins has generated new opportunities and challenges for scientists seeking a deeper insight into the systems biology of an organism. As the proteome more accurately reflects the dynamic state of a cell, tissue or organism, it has huge potential for yielding better markers for crop improvement and monitoring of disease. However, as proteins are much more diverse physically and chemically than nucleic acids, there are several difficulties in the study of proteins that are not inherent in the study of nucleic acids. Furthermore, proteins cannot be amplified like DNA; therefore, less abundant species are more difficult to detect (Van Wijk, 2001). More

importantly, this technique demands advanced technologies focusing on improved separation, resolution and automation for the identification and characterisation of proteins including the chemical and physical properties of the target proteins.

High throughput proteomics technologies coupled with advanced bioinformatics are increasingly leveraged to identify molecular signatures of economic importance based on protein pathways and signalling cascades.

TABLE 1. EXAMPLE OF INITIATIVES IN PROTEOMICS AND METABOLOMICS AS BIOTECHNOLOGICAL TOOLS FOR CROP IMPROVEMENT

Crop	Trait studied		Tools / techniques	References
Olive	Tolerance to cold <i>vs.</i> non-tolerant after cold treatments	Proteomics	Isoelectric focusing (IEF), 2-DE	Bartolini <i>et al.</i> (1999)
	Olive pulp proteins		Nano-LC/MS/MS, MALDI-TOF-MS	Capriotti <i>et al.</i> (2013) Esteve <i>et al.</i> (2011)
	Geographical characterisation	Metabolomics	NMR	Rongai <i>et al.</i> , (2017)
	Olive classification		GC-QTOF/MS	Sales <i>et al.</i> (2017)
	Determination of phenolic compounds in virgin olive oil		LC coupled to fluorescence detector	Monasterio <i>et al.</i> (2016)
Maize	Unintended effects of genetic modification (GM)	Proteomics	2-DE	Barros <i>et al.</i> (2010) Zolla <i>et al.</i> (2008)
	C4 leaf development		NanoLC-LTQ-Orbitrap	Majeran <i>et al.</i> , (2010)
	Ear rot infection		iTRAQ	Mohammadi <i>et al.</i> (2012)
	Seedlings		LC-MS/MS	Ning <i>et al.</i> (2016)
	Greening of leaves		LC-MS	Shen <i>et al.</i> (2009)
	Genetically modified organism (GMO)	Metabolomics	CE-TOF-MS, FT-ICR-MS	Leon <i>et al.</i> (2009)
	Salt stress tolerance		NMR	Gavaghan <i>et al.</i> (2011)
	Effect of environment and genotype		LC-MS	Baniasadi <i>et al.</i> (2014)
Rice	Etiolated shoot	Proteomics	2-DE	Komatsu <i>et al.</i> (1999)
	Unintended changes in GM rice		2-DE	Zhao <i>et al.</i> (2013)
	Rice proteome database		Shotgun proteogenomics	Helmy <i>et al.</i> (2011)
	Stress tolerance		iTRAQ	Wang <i>et al.</i> (2014)
	Chemical diversity	Metabolomics	GC-MS, LC-MS	Kusano <i>et al.</i> (2015)
	Rice quality and traceability		FT-IR, NMR	Uawisetwathana and Karoonuthaisiri (2019)
	GM-rice (insect resistance)		LC-ESI-Q/TOF MS	Chang <i>et al.</i> (2012)
	Nutritionally enhanced herbicide tolerance		GC-EI-TOF MS	Kim <i>et al.</i> (2013)
Soyabean	Salt tolerance	Proteomics	MALDI-TOF/TOF	Ma <i>et al.</i> (2012)
	Wild and cultivated soyabean genotypes		2-DE, MALDI, LC-MS/MS	Natarajan <i>et al.</i> (2006)
	Water and nutrient uptake		iTRAQ	Nguyen <i>et al.</i> (2012)
	Tolerance to <i>Phytophthora</i>		2-DE	Zhang <i>et al.</i> (2011)
	Drought and heat stress	Metabolomics	GC-MS/LC-MS	Das <i>et al.</i> (2017)
	Response to <i>Rhizoctonia</i> to foliar blight disease		NMR	Copley <i>et al.</i> (2017)
	Transgenic soyabean		CE-TOF-MS	García-Villalba <i>et al.</i> (2008)
Oil palm	Somatic embryogenesis	Proteomics	2-DE & MALDI, TOF-MS/MS	Tan <i>et al.</i> (2016)
	Fruit development proteomics		2-DE & MALDI, TOF-MS/MS	Hassan <i>et al.</i> (2019)
	Pathogen-studies (<i>Ganoderma</i>)		LC-MS Orbitrap	Syahanim <i>et al.</i> (2019)
	Pathogen-studies (<i>Ganoderma</i>)	Metabolomics	GC-GC-TOF-MS	Rozali <i>et al.</i> (2017)
	Phytochemicals		LC-ESI-TOF-MS	Tahir <i>et al.</i> (2012)

In the area of metabolomics research, every technique employed has specific advantages and limitations. Thus, clearly there is no single method that is ideal for all research purposes. Analytical techniques that are most often used for metabolite profiling include nuclear magnetic resonance (NMR) (Nicholson *et al.*, 1999), gas chromatography-mass spectrometry (GC-MS) (Roessner *et al.*, 2000), liquid chromatography-mass spectrometry (LC-MS) (Tolstikov *et al.*, 2003; Huhman and Sumner, 2002; Fraser *et al.*, 2000) and capillary electrophoresis-mass spectrometry (CE-MS) (Sato *et al.*, 2004). Generally, NMR instrument is arguably the most powerful technique for an organic chemist to determine structure in metabolomics research. MS excels at selective identification of a molecular entity, while NMR excels at identification of all proton containing species in a sample. Specifically, interfacing liquid chromatography with parallel MS or NMR (LC-NMR-MS) has been shown to give comprehensive structural data on metabolites of novel drugs in development. Although MS and NMR are the most common tools used for large scale analysis of metabolites whether targeted or non-targeted, metabolomics is not limited to these techniques. Other techniques such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC) with ultraviolet (UV)/visible absorbance, photodiode array (PDA) or electrochemical detectors, Fourier transform-infrared (FT-IR) and variety of other enzymatic assays have also been widely used to improve metabolite coverage and increase quantification limits (Shulaev, 2006; Gamache *et al.*, 2004; Kristal and Matson, 2002). Generally, specific platforms are not a prerequisite for metabolomics investigations, so in theory, any technique capable of generating comprehensive metabolite measurements can be used for metabolomics.

As seen today, metabolomics employs multiple complementary analytical devices starting from sample preparation to determining the measurement details of all analytical platforms, and finally to corresponding specific steps of data analysis (Table 1). The choice of technique primarily depends on the research strategies and the nature of the samples. Three basic strategies can be used in metabolomics: (i) targeted analysis, (ii) metabolite profiling, and (iii) metabolic fingerprinting (Halket *et al.*, 2004; Fiehn, 2002). These techniques are commonly used to reveal the phenotype of silent mutations (Raamsdonk *et al.*, 2001), studying responses to environmental stress (Huntingford *et al.*, 2005; Rosenblum *et al.*, 2005), assessing global effects of genetic manipulation (Catchpole *et al.*, 2005), comparing different growth/fruit development (Hurtado-Fernández *et al.*, 2015), nutrition (German *et al.*, 2002) and natural product discovery (Fiehn *et al.*, 2001). Specifically, the major analytical technologies driving metabolomics such

as LC-MS have enormous potential for metabolite profiling and for analysing selected metabolites as a complement to GC-MS. In addition, NMR spectroscopy has high discriminatory power at the level of metabolic fingerprints. However, NMR fingerprints provide limited information on individual metabolites and therefore purification of the metabolites is required for accurate identification (Jorge *et al.*, 2016; Weckwerth, 2003).

Like other functional genomics research, the future of proteomics and metabolomics lies in the combination of different techniques for identification and quantification of proteins and metabolites to achieve a complete view; no single technique alone can cope with the diversity and structural complexity of the proteome and metabolome. We believe that protein and metabolite analyses are much more powerful when used in combination than individually. Furthermore, the combination of the data generated from proteomics and metabolomics requires specific bioinformatics support in the form of databases, visualisation and data integration to provide a meaningful assessment of the research. A review by Weckwerth (2008) provides the strategy for data integration, which combines high throughput profiling methods with exploratory multivariate data mining. In fact, a combination of computer-aided metabolic modelling and multivariate data mining will enable a systematic comparison for identifying key steps involved in metabolism using extensive and multi-dimensional datasets from proteomics, transcriptomics and metabolomics studies (Mehrotra and Mendes, 2006; Morgenthal *et al.*, 2006).

Along with the establishment of a database platform, the streamlined workflow is now available to facilitate research in oil palm proteomics and metabolomics (Figure 2). This includes a combination of approaches, either gel-based or liquid chromatography (gel-free), with tandem mass spectrometry to optimally extract proteins by modifying existing methods described in literature. At present, proteomics technologies are being applied to identify potential biomarkers associated with oil production, fruit ripening, embryogenic lines and disease resistance (Hassan *et al.*, 2019; Lau *et al.*, 2018; Tan *et al.*, 2016; Syahanim *et al.*, 2013; Al-Obaidi *et al.*, 2017). Looking beyond the proteome, advanced metabolomics technologies also include key elements, starting from sample extraction to profiling of targeted and non-targeted metabolites using multiple technologies, mainly LC and GC coupled to tandem mass spectrometry (Nurazah *et al.*, 2017; Rozali *et al.*, 2017; Tahir *et al.*, 2016; 2012). We foresee that proteomics and metabolomics technologies will play a more significant role in the near future to address diverse issues related to the oil palm in the effort to assist on-going breeding activities to further improve the oil crop.

An important point to consider is that the large multi-dimensional datasets that result from proteomics and metabolomics require complex and high throughput statistical analysis to render the data meaningful and make rational conclusions in the study. Thus, bioinformatics tools are essential for the efficient processing of huge datasets. Proteomics data also have to be linked to the existing gene, transcripts and ribonucleic acid sequencing (RNAseq) datasets to develop tissue specific expression atlases and proteome maps. Integration of transcriptomics, proteomics as well as metabolomics with genotyping and phenotyping data and establishment of appropriate databases, with the corresponding bioinformatic infrastructure will lead to a functional understanding and improved prediction accuracy of yield and quality parameters for developing sophisticated strategies such as genome-wide MAS to improve breeding efficiency. Although the interpretation of the different 'omics' datasets is not straightforward, the combined information is an important step towards uncovering correlation(s) between the expressed genetic information and the phenotypic response of the oil palm crop.

Charting Proteome-based Knowledge for Improving Oil Palm Yield and Quality

Increasing oil production of a key crop such as oil palm is essential for a more sustainable and

greener future. A better understanding of how plants regulate oil biosynthesis will assist in efforts towards breeding palms that produce more oil, with specific fatty acid compositions which relate to quality (Lau *et al.*, 2018; Ramli *et al.*, 2002a, b). This provides a more sustainable way to produce more of the desired natural oils from existing hectareage instead of simply increasing the area of land used for agriculture. In this respect, several key enzymes and genes involved in the regulation of fatty acid biosynthesis of the oil palm have been characterised and purified, mainly for utilisation in the genetic modification programme (Masura *et al.*, 2017; Ramli *et al.*, 2012; Parveez *et al.*, 2010; Sambanthamurthi *et al.*, 2000). The information is also useful for genomics-based improvement of the crop. In fact, combining transcriptome and proteome analyses is essential for developing a comprehensive understanding of the molecular mechanisms regulating fruit development and fatty acid metabolism. Several studies which employed gel-based comparative proteomics, provided an overview of proteins that are abundant during oil palm fruit development, as well as insights into proteins that play a key role during fruit maturation. There has been much progress in technologies and chemometrics application that have revealed changes in proteins that accumulate during oil palm mesocarp development (Hassan *et al.*, 2019).

In its early stages, the oil palm fruit consists of storage and structural carbohydrates and

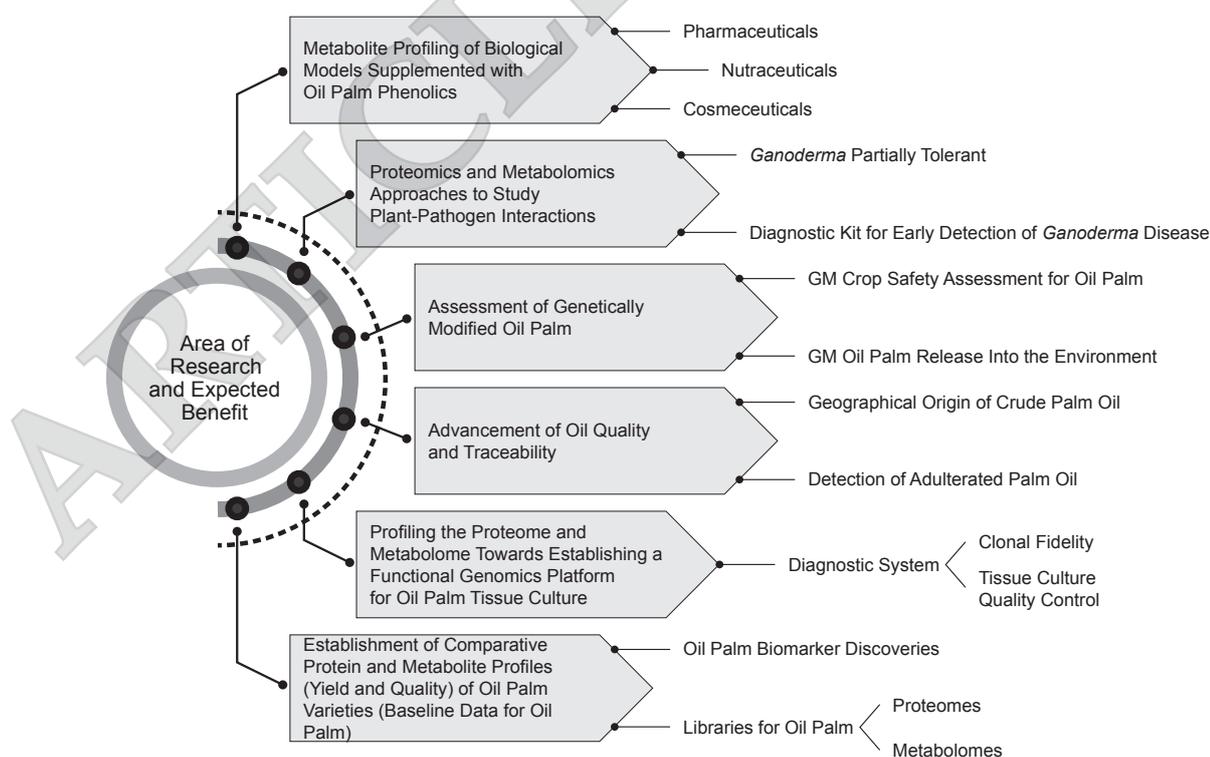


Figure 2. Priority research areas in oil palm proteomics and metabolomics.

membrane lipids but very little oil. As it approaches maturity, the formation of oil increases rapidly in the mesocarp tissue and the ripeness of the fruit bunch is marked by huge changes in the exocarp tissues. Several targeted and untargeted proteomics and metabolomics studies have been carried out to gain a holistic understanding of molecular mechanisms regulating fruit maturation to complement available genomic information. The metabolomics platform of MS in particular, which results in huge amounts of data has proven useful in the analysis of molecular fractions at specific time points during oil palm fruit development (Teh *et al.*, 2013). Hassan *et al.* (2019) developed proteomics protocol to study major metabolism including those involved in lipid production, energy, secondary metabolites and amino acid, which were found to be significantly altered during fruit development (Figure 3). Independent research which focused on the analysis of proteins and metabolites from high yielding oil palm and storage oil production (Ooi *et al.*, 2015; Loei *et al.*, 2013; Neoh *et al.*, 2013) has mostly complemented the earlier findings on the different proteins accumulated during fruit development. To further build capacity, more than 4000 proteins have been identified from oil palm fruit mesocarp (Hassan *et al.*, 2014; Lau *et al.*, 2018; 2020) can be catalogued in a database to facilitate future research. In another interesting research, Lau *et al.* (2015) studying high-oleate palms, described advanced proteomic techniques

to isolate, detect and identify chromoplast-based proteins associated with the fatty acid biosynthesis pathway. It was revealed that control of oleic acid production is not restricted to enzymes associated with fatty acid biosynthesis, supporting previous biochemical studies that the flux of fatty acids into most plant oils is controlled in large part by the relative activity of more than one pathway (Ramli *et al.*, 2002b). Lau *et al.* (2016) also reported the plausibility of phosphorylation involvement in the regulation mechanism.

Using the integrated omics approach, Appleton *et al.* (2014) discovered that subtle changes in carbon flux can lead to increased oil synthesis in oil palm. They reported that although genetic markers are important for oil palm crop improvement, marker accuracy varies across population backgrounds depending on genetic distance of markers from controlling genes and population diversity. Furthermore, development of accurate marker selection tools is especially challenging for highly polygenic traits like yields that are largely influenced by the environment. There is a recognition that biochemical omics techniques could act as a link between controlling genes and associated genetic markers by determining genes and biochemical pathways that are more directly associated with the final trait. Along this line, the team investigated the differences in biochemical regulation of high yielding individuals compared to average performers within a group of siblings planted in

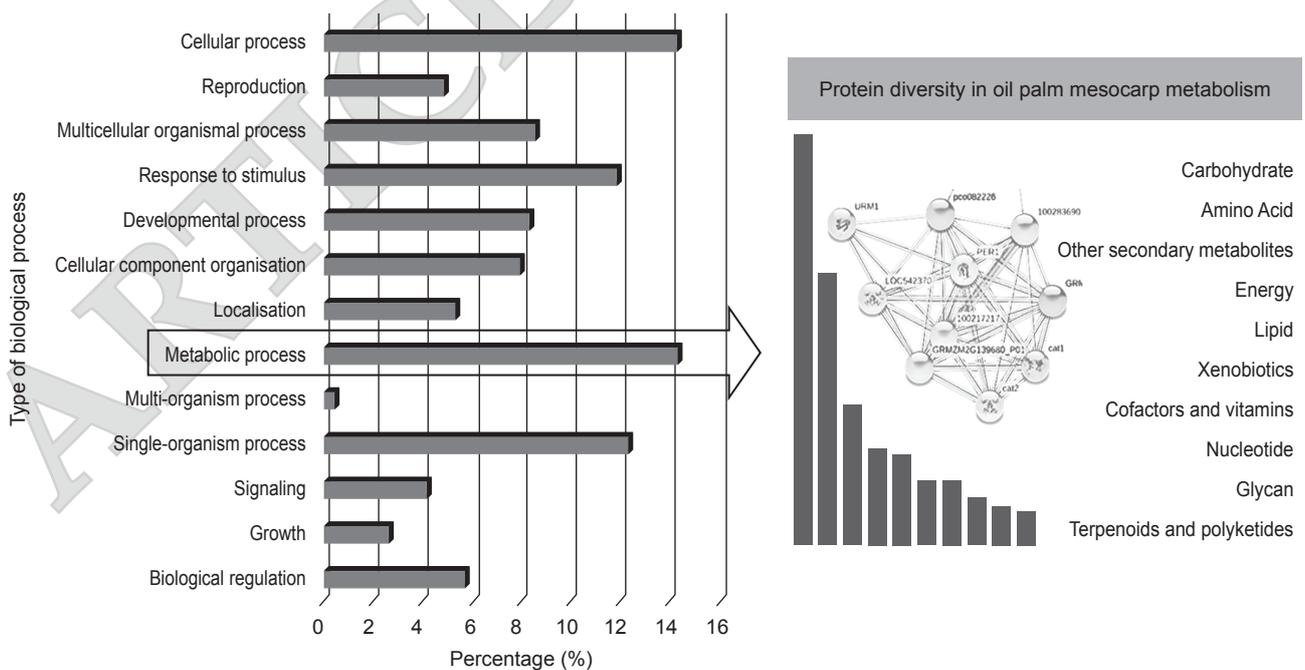


Figure 3. Functional classification of identified proteins from oil palm mesocarp tissue using gel-based proteomics approach (adapted from Hassan *et al.*, 2019).

the same location. Transcriptomics, proteomics and metabolomics were used to compare biochemical changes before, during and after oil biosynthesis in the mesocarp to obtain further understanding of regulation of oil biosynthesis in the oil palm. Genome polymorphisms associated with the genes identified were subsequently prioritised when conducting genome-wide association studies on larger sets of more diverse populations.

Teh *et al.* (2014) investigated changes in expression of polyamines, hormones and cell-wall-related genes, and metabolites in the oil palm mesocarp at different stages of fruit development and described the relationship between the structural and biochemical metabolism during ripening. A distinct reduction in auxin-responsive gene expression was observed from 18 to 22 weeks after anthesis. Fruit enlargement during lipid accumulation and later stage of fruit maturation coincided with high polyamine concentrations. Actin, expansin and polygalacturonase gene expressions were also observed to increase during fruit maturation. The combined omics approach provided a more detailed understanding of the coordinated process involved in fruit ripening and oil accumulation and could potentially lead to the identification of the master regulators of these important processes.

Oil palm and date palm exhibit an extreme difference in carbon partitioning, with oil palm accumulating high amount of oil in its mesocarp, and the closely related date palm accumulating almost exclusively sugars. Bourgis *et al.* (2011) compared the transcriptome and metabolite content of oil palm and date palm and discovered that despite more than a 100-fold difference in flux to lipids, most enzymes of triacylglycerol assembly were expressed at similar levels in oil palm and date palm. Oil palm had much higher transcript levels for all fatty acid synthesis enzymes, and transcripts of an orthologue of the WRI1 transcription factor were 57-fold higher in oil palm compared to date palm. The combination of transcriptomics and metabolomics confirmed earlier flux studies by Ramli *et al.* (2002a, b; 2009) that fatty acid synthesis plays a more predominant role than triacylglycerol assembly in the regulation of lipid synthesis in oil palm.

Deciphering the Molecular Interactions in Oil Palm Disease Using Proteomics and Metabolomics

The most important oil palm diseases are basal stem rot (BSR) (*Ganoderma* spp.), *Fusarium* vascular wilt (*Fusarium oxysporum* f.sp. *elaeidis*), red ring (*Rhadinaphelenchus cocophilus*), *Phytoplasma* sudden wilt (*Phytoplasma staheli*), *Phytohthora* bud rot (*Phytohthora palmivora*), Marchitez/lethal wilt (*Phytoplasma* sp), *Cercospora* leaf spot (*Cercospora*

elaeidis) and chlorotic ring (*Potyvirus*) (Ariffin *et al.*, 2000; Flood, 2006; Chinchilla, 1988; Torres *et al.*, 2010; Di Lucca *et al.*, 2013; Weir, 1968; Morales *et al.*, 2002). In Malaysia and other oil palm producing countries in South-east Asia, *Ganoderma boninense* is the dominant fungal pathogen responsible for BSR disease in *Elaeis guineensis* (Idris *et al.*, 2011; Susanto *et al.*, 2005; Turner and Gillbanks, 2003). Statistical data revealed that in Malaysia, losses due to *Ganoderma* disease can reach up to RM 1.5 million a year (Arif *et al.*, 2011) and it was estimated that the total area affected by *Ganoderma* disease in 2020 would be around 443 430 ha or 65.6 million of oil palms (Roslan and Idris, 2012). As yet no effective remedial method to prevent spread of the disease to healthy stands has been reported (Idris *et al.*, 2016).

As palm oil represents the largest share of the world's vegetable oil market, 80% of which is used by the food industry, BSR may have a serious effect on global food security. Continuous efforts have been made to select tolerant oil palm progenies (Idris *et al.*, 2004; Durand-Gasselien *et al.*, 2005) or to develop best management practices to control the spread of the disease (Susanto *et al.*, 2005). Metabolomics tools have been used to explore metabolite profiles in highly tolerant and susceptible genetic backgrounds to improve our current understanding of its biology. Although it is still preliminary, the developed method provides a basic tool as a standard protocol for future efforts aimed at selective breeding of oil palm via metabolomics-assisted breeding techniques (Rozali *et al.*, 2017). The Deli palms (in Malaysia and Indonesia) are more susceptible to *Ganoderma* than those from the African germplasm (Cameroon) (Idris *et al.*, 2004). The elucidation of potential metabolite markers, *i.e.* shikimic acid, glucose and malic acid that were responsible for separately clustering Deli and MPOB-Cameroon *duras* as highlighted by Nurazah *et al.* (2017) provides an initial clue into what may distinguish susceptible and tolerant planting materials. The results suggest that the employed methodology is a powerful approach to profile and portray leaf metabolome with different susceptibility to *G. boninense*. In the future, we envision additional studies that incorporate metabolomics as well as other different omics platforms for screening larger populations of truly resistant and susceptible palms, to obtain a holistic view of oil palm's response to *Ganoderma*, with a view to conduct detailed pathway analysis.

The use of omics technologies to better understand incidences of BSR in oil palm has been reported recently (Othman *et al.*, 2019a). The assignment of fatty acid composition in oil palm leaves in relation to severity of *Ganoderma* infection, and protein analysis of *G. boninense* using gel- and LC-based approaches are examples of how

proteomics and metabolomics studies are contributing to improve our current understanding of BSR (Othman *et al.*, 2017; Dzulkafli *et al.*, 2019; Nurazah *et al.*, 2013). Due to its great potential, proteomics specifically has been used to better understand the pathogen, *Ganoderma* spp. itself (Syahanim *et al.*, 2019; Dzulkafli *et al.*, 2016). The identification of differential proteins in two *Ganoderma* spp., namely *G. boninense* (pathogenic) and *G. tornatum* (non-pathogenic) will prove invaluable in understanding virulence and pathogenicity (Al-Obaidi *et al.*, 2016). This will also help in long-term efforts at elucidating the mechanisms of how the fungus infects oil palm. Protein identification depends not only on analytical technologies but also on the existence of complete genome sequence databases for comparison. The genome sequence information for this phytopathogen was recently published by a joint group of researchers from France and Malaysia who assembled a total of 63 Mb using Illumina and 454 platforms to identify microsatellite markers for genetic diversity studies (Mercière *et al.*, 2015). More recently, additional information on the sequence and number of reads generated for *G. boninense* and *G. tornatum* samples were also reported (Nagappan *et al.*, 2018).

Omics – A Tool to Assist in the Generation and Assessment of Transgenic Oil Palm

The identification of genes regulating specific traits will play a key role towards the improvement of the oil palm, with potential application in modifying oil content and fatty acid composition by genetic engineering (Parveez *et al.*, 2015; Ramli *et al.*, 2012; Othman *et al.*, 2000) and managing the *Ganoderma* disease (Mohamad Arif *et al.*, 2017; Safiza *et al.*, 2015; Lim *et al.*, 2017; Rasid *et al.*, 2014). Despite its economic importance, authorisation and commercialisation of genetically modified organism (GMO) has always been controversial within the scientific and public communities. The advancements in omics technologies are tremendously useful both to facilitate the generation and safety assessment of transgenic crops. First and foremost, omics platforms, especially the transcriptome data, are highly valuable for establishing the basic genetic resources required in the genetic engineering efforts. Identification and isolation of key genes, proteins or promoters are very much simplified. This is illustrated by several recent publications. On promoter isolation, Siti Suriawati *et al.* (2019) reported the identification of a gene that was highly expressed in mesocarp tissue through *in silico* analysis of oil palm transcriptome datasets. The expression specificity of the gene in the tissue was confirmed by real-time quantitative polymerase chain reaction (qPCR) analysis. Using a

similar approach, potential root specific genes were also identified (Masura *et al.*, 2019a). Identification of these tissue-specific sequences is crucial for the isolation of their promoter sequences and ultimately for directing the expression of transgenes into the desired tissues.

The application of transcriptome data has also led to the identification and isolation of a number of constitutive promoters from oil palm (Masura *et al.*, 2019b; 2011). In addition, omics data have also led to the identification of several indicative sequences and metabolites that are associated with several traits of interest (Masura *et al.*, 2017). All of these findings could potentially be used in the genetic engineering of oil palm. Oil palm is known to be recalcitrant to genetic transformation, and as such, the development of genotype-independent transformation technology is crucial for this crop. This could help improve oil palm transformation efficiency, which currently is quite low. One approach is through the use of *Baby boom* (*Bbm*) and *Wuschel2* (*Wus2*) genes, which can help establish a biological context to improve transformation efficiency.

The availability of oil palm omics data, especially transcriptome analysis of various tissues and development stages has facilitated the identification and isolation of key genes, which are being selectively applied in research related to genetic modification of oil palm (Masani *et al.*, 2018). The existing omics information also has huge potential for utilisation in the exciting area of genome editing. Genome editing offers several advantages over the conventional genetic engineering protocols. First, the technique results in a more predictable and precise gene modification. Since the technique does not add any external or foreign sequence to the recipient cells, it is also more readily acceptable to consumers as well as regulatory authorities. Research on the application of this in oil palm has already been initiated (Bahariah *et al.*, 2019), and will likely open up opportunities for genome editing-based improvement. The availability of omics data would facilitate the efforts in two primary ways. First, the omics data would allow more accurate prediction of the target locations. This would minimise the possibilities of off targets in the resultant modified plants. Besides, the data are also useful for the identification of the target or key genes. Increasing oleic acid content has been the main objective of oil palm genetic engineering research at MPOB. Extensive studies to identify the target genes and important regulatory controls over the biosynthesis pathway have been carried out and reviewed (Parveez *et al.*, 2015). This has resulted in the formulation of a comprehensive strategy to produce high oleic oil palms through conventional genetic engineering. However, the immense omics

data will allow even more comprehensive analysis of the fatty acid biosynthesis or other pathways.

The analysis will not only allow for the identification of target sequences of the pathway and discrimination of different isoforms but could also determine the crucial elements that are not directly involved in the pathway, such as transcription factors. For example, Dussert *et al.* (2013) identified paralogues of the *WRINKLED1* transcription factors that were highly expressed during oil deposition in the oil palm mesocarp and endosperm. Manipulation of these regulatory elements has proven to be effective in a number of recent studies. For example, overexpression of transcription factors *GmDof4* and *GmDof11* was shown to increase the oleic acid content of *Brassica napus* seeds (Sun *et al.*, 2018). Similarly, oil content of peanut seeds was significantly increased through overexpression of the transcription factor *AtLEC1* (Tang *et al.*, 2018). In tomato, lycopene content was increased up to 5.1-fold through CRISPR/Cas9 multiplex genome editing. It was further illustrated that the regulator protein *SGR1* has a greater control over the lycopene accumulation compared to a number of enzymes in the carotenoid biosynthesis pathway (Li *et al.*, 2018).

Gene editing is also relevant in the field of pests and diseases. A number of recent reports have demonstrated the feasibility of manipulating regulatory sequences to create disease or pest resistant or tolerant plants. For example, genetic manipulation of the disease susceptibility gene *CsLOB1* through CRISPR/Cas9 technique produced plants that are resistant to citrus canker. The *CsLOB1* is a member of the Lateral Organ Boundaries Domain (LBD) gene family of plant transcription factors that can be recognised and induced by the pathogen transcription activator-like (TAL) effectors (Jia *et al.*, 2017). Another example is demonstrated by the increased tolerance to rice blast using transcription factor *OsSRF922*. Mutation of the gene through CRISPR/Cas9 has resulted in significant decrease in pathogen infection in all mutant lines tested (Wang *et al.*, 2016). Initial development in this area has also started to take place in oil palm. Studies to unravel the interaction between oil palm and its most serious pathogen *G. boninense* have been carried out and reported using transcriptomic, proteomic and metabolomic analyses (Ho *et al.*, 2016; Nusaibah *et al.*, 2016; Al-Obaidi *et al.*, 2016; Syahanim *et al.*, 2013). The findings from these studies will eventually contribute towards creating *Ganoderma* tolerant oil palm.

As the research for developing genetically modified (GM) oil palm pushes forward, omics platforms will be essential to confirm that only the intended genomic alterations have occurred. Analysis of the genome, proteome and metabolome will increase the chances of detecting unintended

effects, if any (Gong and Wang, 2013). As an example, the proteomics-based analysis of the GM round-up ready soyabean revealed no significant alteration in the expression of allergens following genetic modification. Similarly, Gong *et al.* (2012) showed that genetic modification did not alter the protein profile of rice compared to those produced via conventional breeding. However, other studies have shown differences between GM products and non-GM controls (Luo *et al.*, 2009). Metabolomics analysis showed significant difference in selected metabolites between the conventional and GM insect resistant maize (Levandi *et al.*, 2008). The differences observed are likely due to exogenous gene(s) being inserted and the site(s) where the insertion occurs among other factors (Gong and Wang, 2013). This demonstrates the important role of omics-based platforms in evaluating GM oil palm in the near future.

Integrative Omics for Understanding Oil Palm Seed Germination

Despite the critical importance of germination of oil palm seeds, our knowledge on the physiology of the process is limited. Most metabolic studies were carried out in the 1980s and there has been little advancement in our knowledge of molecular players and metabolic controls involved in germination. Oil palm seeds have a low germination rate because of a long dormancy attributed to restrictions of embryo growth because of the hard endocarp which results in mechanical constraints and resistance to oxygen absorption. The germination of oil palm seeds can take years, under natural conditions. Elevated temperature and appropriate levels of seed moisture are necessary for rapid and optimal germination. For commercial seed production, oil palm seeds are heat treated under controlled conditions to break dormancy. However, the effect of heat treatment on phytohormone-related genes/proteins/metabolites and possible relationship with dormancy release is not well understood. Wang *et al.* (2019) carried out integrative omics analysis on the phytohormones involved in oil palm seed germination under conditions used for commercial production to discover the mechanisms involved in oil palm seed germination. Differentially expressed genes and proteins (DEG and DEP) related to seed germination were identified using RNA-seq and isobaric tags for relative and absolute quantitation (iTRAQ) and the results validated with qPCR and western blot. LC-MS/MS was used to determine endogenous phytohormones while exogenous phytohormones were also applied to validate their effects on seed germination. The authors confirmed the important role of phytohormones in oil palm seed germination with abscisic acid (ABA) acting as an inhibitor. Heat treatment breaks dormancy at

least partly by eliminating endogenous ABA. The results emphasised how the omics platforms of transcriptomics, proteomics and metabolomics in combination led to an improved understanding of oil palm seed germination.

Although key metabolic pathways such as β -oxidation are common in germination of all oleaginous seeds including oil palm, palm seeds have unique attributes such as the involvement of the haustorium that digests the endosperm and eventually invades the seed (Cui *et al.*, 2020). This involves important metabolic processes such as fatty acid transport and coenzyme A cycling. Despite the importance of these aspects, advances in germination metabolism have been scarce. It is increasingly obvious that integrated omics approaches will be key in resolving the complex processes underpinning germination. Functional genomics will identify gene functions and interactions relevant to seed germination. Proteomics analyses will be of importance to find key transporters, confirm the localisation of enzymes involved in mobilisation of reserves including but not limited only to lipids, and identify proteins associated with kernel oil bodies. Metabolomics may help unravel the spatial and temporal profile of metabolites in the various tissues of the germinating seed. This was recently achieved for maize (Feenstra *et al.*, 2017). Such information will be useful for identifying molecular markers associated with superior germination performance which will be advantageous for oil palm breeding.

Geographical and Authentication Tracing of Palm Oil Using Omics-based Technologies

Ensuring food safety requires the monitoring of its source of production and distribution throughout the supply chain. This will enable a sound certification system and also prevent the introduction of adulterants or contaminants during the production and processing stage (Goggin and Murphy, 2018). The ability to distinguish palm oil from different regions or sources requires the development of a geographical traceability system by using robust and precise methods that allow the palm oil consignment to be traced back to its origin at every phase of production. The attempt to differentiate palm oil according to its geographical source has been explored using a wide range of omics tools. These tools include advanced analytical techniques such as GC-flame ionisation detector (GC-FID), proton transfer reaction-MS (PTR-MS), UV-visible spectroscopy, elemental analyser coupled to an isotope ratio mass spectrometer (EA-IRMS), HPLC and GC-ion mobility spectrometry (GC-IMS) combined to cheminformatics of principle component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), orthogonal partial least squares-discriminant analysis (OPLS-

DA), hierarchical cluster analysis (HCA) and soft independent modelling of class analogy (SIMCA) (Ramli *et al.*, 2020). Promising markers that can facilitate geographical differentiation of palm oil include fatty acid profiles, triacylglycerols, volatile organic compounds, isotopic composition, phytosterols and carotenoid content, in combination with chemometrics (Goggin and Murphy, 2018; Tres *et al.*, 2013; Muhammad *et al.*, 2018). The possibility of utilising genomics-based DNA fingerprints of palm oil is also being explored due to its success in differentiating other vegetable oils (Costa *et al.*, 2012). However, the ability of DNA markers to track palm oil samples across the supply chain to their geographical origin is going to be challenging, as genotypes with different genetic backgrounds are usually planted, even in a single block of a large plantation (Ramli *et al.*, 2020; Ooi *et al.*, 2006). Nevertheless, the work is important to detect and nail down fraudulent and unsafe milling and processing activities, if any. With the current technological capabilities, metabolomics is also being exploited for identifying unique chemical fingerprints to detect product contamination and adulteration. This effort is actively being conducted in order to position the oil palm industry to meet and optimise the delivery of the highest quality oil with minimum environmental and social concerns. Efforts are being directed to specifically look into the potential of technologies to ensure that palm oil, especially crude palm oil (CPO) and refined palm oil meet international standards including those related to 3-monochloropropane-1,2-diol (3-MCPD) (Tarmizi *et al.*, 2019). Adherence to the practice of not adding sludge palm oil (SPO) or other residual oils, such as palm fibre oil (PFO) back to the CPO, will help maintain the Malaysian palm oil industry at the forefront of the vegetable oils and fats industry. Omics-based technologies can be applied to ensure that CPO is free from such residual oils. This was demonstrated in a recent study, where GC-IMS was used for rapid and cost-effective screening of CPO for detection of PFO and SPO based on their characteristic volatile organic compound (VOC) fingerprints. The study demonstrated that the identification of contaminants, adulterations and/or off flavours at very low concentrations is possible in CPO samples (Othman *et al.*, 2019b). This paves the way for this technology to be used for quality assurance and eventually for product traceability.

CONCLUSION

Genomic platforms represent a key resource towards achieving the target of producing superior oil palm. However, understanding the cellular biology and biochemistry underlying the specific traits is no substitute for studying proteins and

metabolites, since these are directly responsible for cellular activity. Therefore, to complete the view offered by genomics and transcriptomic-based analysis, proteomics and metabolomics offer a new comprehensive perspective to better understand regulatory mechanisms that govern complex phenotypes, especially those under environmental influence. As such, an integrated omics approach is essential to uncover the complexities and diverse physiological processes that govern complex traits in the oil palm (e.g. yield, quality and disease resistance), which can lead to biomarkers that can more accurately predict these traits. Undeniably, proteomics and metabolomics research on oil palm is still at its infancy and there is a need for further development of relevant methods and techniques for comprehensive analysis of proteins and metabolites towards understanding complex traits in oil palm. Although challenges are aplenty, on-going technical improvements will facilitate application of integrated omics technologies in developing new and improved oil palm varieties. The pressure of upholding sustainability in a situation where resources are limited warrants a shift in paradigm for the oil palm industry. At this juncture, strict regulations including risk assessment of transgenic oil palm, and making sure CPO is free of adulterants require established analytical tools that can be adopted by the industry. Thus, new technological advances which can provide fast and robust detection for quality and eventually traceability purposes, cannot be over-emphasised. The application of these techniques to agricultural systems is a step forward towards the implementation of translational proteomics and metabolomics in crop breeding. The integration of multi-layered omics data will allow for reconstruction of regulatory networks and provide a stereoscopic view of dynamic molecular processes and interacting networks of genes in the oil palm. This will lead to deeper insights into target traits and facilitate precision breeding of the oil palm.

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