

The lack of information on the roles of metabolites secreted by oil palm roots hindered the understanding on mechanism underlying plant's response to fluctuations in the abiotic and biotic environments. Investigation of root metabolites may provide information on the concentration levels of hundreds of known and not yet identified metabolites (Dubey *et al.*, 2010; Schmidt *et al.*, 2014). The use of metabolomics tools such as Liquid Chromatography Mass Spectrometry/Mass Spectrometry (LC-MS/MS) profiling technology holds great promises for investigating and understanding of metabolism associated with root metabolome. The LC-MS/MS method developed for profiling and identification of oil palm root metabolites can also be used as a model for developing a reliable tool towards distinguishing tolerant palms to oil palm disease such as basal stem rot (Nusaibah *et al.*, 2011; Chong *et al.*, 2012; Nurazah *et al.*, 2013). Possible sources of genetic resistance to *Ganoderma*

in oil palm have been reported (Idris *et al.*, 2004; Durand-Gasselin *et al.*, 2005; Breton *et al.*, 2006; Cooper *et al.*, 2011). Metabolites are final products of gene expression where changes in gene expression are reflected in metabolite profiles. Hence, identifying metabolites associated with resistance promises development of biomarker tool for screening oil palm resistant to *Ganoderma boninense*. In addition, understanding resistance mechanisms at the metabolome level helps breeders for better understanding of resistant gene function and pyramiding suitable resistant gene in elite variety. In parallel the high-throughput LC-MS/MS characterisation of metabolite can be applied to study metabolite changes in response to environmental stimuli, chemical or nutritional supplementation and genetic changes in oil palm.

METHODOLOGY

A metabolomics approach for screening metabolites from oil palm root has been developed (Figure 1).

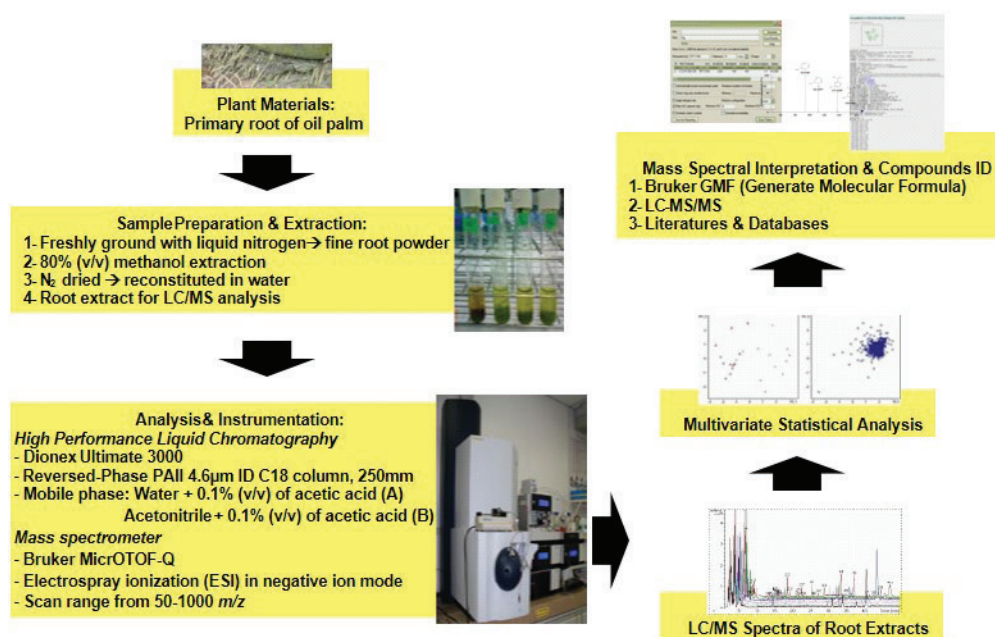


Figure 1. Established metabolomics pipeline for screening oil palm root metabolites (Nurazah *et al.*, 2013).

The oil palm genetic materials comprise six MPOB-Cameroon *pisifera* parental palms as a source of partial resistance genes from Trial 0.219 and six Deli *dura* parental palms as a source of susceptible genes from Trial 0.212 of MPOB Research Station, Kluang, Johor. All palms were selected based on previous selection of partial resistance in oil palm progenies to *Ganoderma* basal stem rot (Idris *et al.*, 2004) with no visible symptoms of *Ganoderma* infection as observed by physical inspection. The primary roots were cut, cleaned and immediately frozen in liquid nitrogen and ground into fine powder using mortar and pestle. Metabolites extraction was performed according to the method described by Ferracane *et al.* (2010) with minor modification. Five ml of 80% (v/v) aqueous methanol was added to 0.5 g of frozen powdered root tissue powder in a Falcon tube. Then the mixture was sonicated in an ultrasonic bath for 30 min and centrifuged at 3000 rpm for 15 min, 25°C. The resultant clear supernatant was collected and

dried under nitrogen stream before reconstituted in 3 ml of Milli-Q water. The extract was filtered through a 0.2 μm cellulose acetate syringe filter and an aliquot of 150 μl was subjected to LC-MS analysis.

Under the experimental conditions, 40 peaks were detected from a single LC-MS run (Figure 2). Metabolite identities were tentatively assigned by comparing measured mass values with the theoretical exact masses, reference compounds, literature searches and collision-induced dissociation (CID) experiment.

Figure 3 shows metabolome investigation of Deli *dura* and MPOB-Cameroon *pisifera* oil palm as a source of susceptible and partial resistance genes, respectively, coupled with Principal Component Analysis (PCA) generates two clusters linked to the susceptible and partial resistance palms to *G. boninense*.

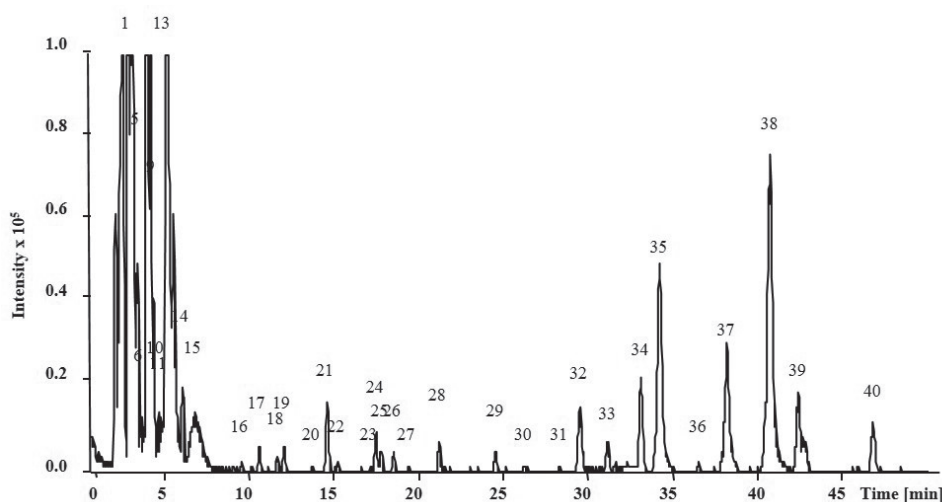


Figure 2. LC-MS Base Peak Chromatogram (BPC) of the oil palm root extracts.

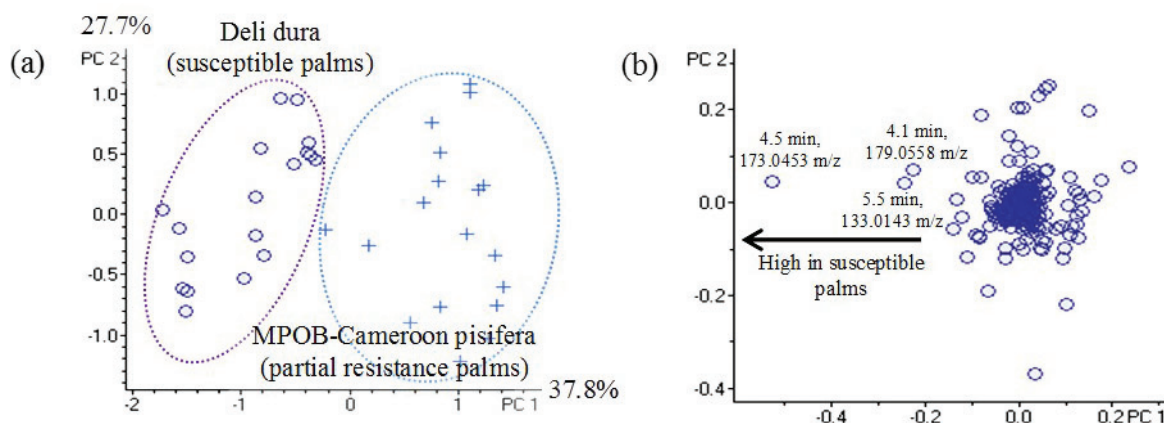


Figure 3. Scores and loadings plot of oil palm samples by PCA processing based on the MS data obtained in negative ion mode: (a) scores plot of Principal Component 1 (PC1) vs. Principal Component 2 (PC2), and (b) loadings plot of PC1 vs. PC2.

TABLE 1. TENTATIVE IDENTIFICATION OF POTENTIAL METABOLITES IN DISTINGUISHING PARTIAL RESISTANCE AND SUSCEPTIBLE PALMS TO *Ganoderma boninense*

No.	<i>m/z</i> (mass-to-charge ratio)	Molecular formula	Putative compound	MS/MS fragments (<i>m/z</i>)	Fold changes in concentration* (Deli <i>dura</i> /MPOB-Cameroon <i>pisifera</i>)
1.	173.0453	C ₇ H ₉ O ₅	Shikimic acid	155.0366, 137.0250, 111.0457, 93.0340	6.8781
2.	179.0558	C ₆ H ₁₁ O ₆	Glucose	161.0449, 149.0443, 143.0335, 131.0338, 119.0342, 113.0235, 101.0232, 89.0229	1.6034
3.	133.0143	C ₄ H ₅ O ₅	Malic acid	115.0025, 89.0242, 71.0139	1.9811

Note: *Significant at $p < 0.01$ using T-test.

Three potential metabolites (173.045 *m/z*, 179.0558 *m/z* and 133.0143 *m/z*) which were found high in susceptible palms, responsible for the segregation of clusters between Deli *dura* and MPOB-Cameroon *pisifera*. They were tentatively assigned as shikimic acid, glucose and malic acid based on collision-induced dissociation (CID) experiment which generated characteristic mass spectrometry/mass spectrometry (MS/MS) fragment ions that matched the metabolite identities. The fold changes in concentration of the three most influential metabolites (shikimic acid, glucose and malic acid) were significant at $p < 0.01$ (Table 1).

INTELLECTUAL PROPERTY

A patent has been filed - PI 2016700539 entitled *A Method for Identifying Oil Palm Plants that are at Least Partially Tolerant to Ganoderma based on the Roots of the Oil Palm Plants*.

BENEFITS

- Novel method for the analysis of oil palm root metabolites using LC-MS/MS.
- Prediction of oil palm phenotype tolerant and susceptible to basal stem rot (BSR) by evaluating significant metabolite determinants.
- Potential technique for understanding root metabolomics to formulate diagnostic kits for other important traits.

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