

METABOLITE PROFILING OF OIL PALM TOWARDS UNDERSTANDING BASAL STEM ROT (BSR) DISEASE

NURAZAH ZAIN*; IDRIS ABU SEMAN*; A KUSHAIRI* and UMI SALAMAH RAMLI*

ABSTRACT

Basal stem rot (BSR) which affects many oil palm plantations in Malaysia is a destructive disease caused by the wood decaying fungi *Ganoderma boninense*. Information on response of oil palm to BSR is scarce, particularly concerning changes in the level of plant metabolites as the ultimate consequence of biological systems to genetic or environmental changes. A previous study on root inoculation technique was conducted by MPOB to identify oil palm progenies partially tolerant and susceptible to *G. boninense* infection that causes BSR. Therefore, in this study, parental palms of these progenies were identified and root tissues of both partially tolerant and susceptible parental palms of different BSR susceptibility were used to analyse metabolites by using a metabolomic-based approach. In this report, we examine the application of liquid chromatography-mass spectrometry (LC-MS)-based metabolomics to study aqueous methanolic extracts of root tissues of selected parental palms that are partially tolerant and susceptible to *G. boninense*. Collectively, 39 peaks were observed from LC-MS analysis operated in negative mode and of these, nine distinctive peaks were further characterised using accurate mass, isotopic pattern, database match and MS/MS experiment for compound identification. Together with several other metabolites, MS/MS spectrum of procyanidin B was presented and all the major fragments were assigned with predicted sub-structures. The nine metabolites which were also successfully identified revealed a range of plant sugar and phenolics derivatives in parental palms root extracts. The novelty of the technique relies on the use of mass signals and the analytical strategy that are applicable to a wide range of analysis in oil palm root metabolomics. These findings provide information on the relationship between phenolic compounds as marker metabolites for disease detection and oil palm preventive mechanism against BSR.

Keywords: oil palm root, *Ganoderma boninense*, liquid chromatography-mass spectrometry, metabolite profiling.

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INTRODUCTION

Basal stem rot (BSR) of oil palm caused by the wood rotting fungus *Ganoderma boninense* is one of the most serious diseases that causes major losses in the oil palm industry. The BSR disease which usually affects matured palms is lethal and incurable (Idris *et al.*, 2000a, b). Moreover, the widespread losses have also been currently reported in young

plantings less than five years old (Mohd Zainuddin and Faridah, 2008). A potential of 30%-70% loss of oil palm which has been reported due to BSR after repeated planting cycles has substantial implications to planting resources and revenue (Idris *et al.*, 2000a, b). Thus, BSR disease caused by *Ganoderma* is a major concern in Malaysia, as palm oil is a major bulk export.

The available control measurements for BSR diseases, such as cultural practices for fungicides are unsatisfactory due to the fact that *Ganoderma* has various resting stages such as melanised mycelium, basiodio-spores and pseudosclerotia (Mohd Zainuddin and Faridah, 2008). It has also

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been established that biological control agents such as *Trichoderma harzianum* and *Gliocladium viride* (Susanto *et al.*, 2005) provide an alternative approach to decrease the strong dependence on chemical fungicides which cause environmental pollution. On the basis of the importance of early detection for controlling the BSR disease, research on molecular and biochemical aspects to enhance the understanding of oil palm-*Ganoderma* interactions is currently on-going. The changes in defense-related genes, protein and metabolites abundance from artificially *Ganoderma*-inoculated and healthy oil palm seedlings have been monitored at different weeks intervals (Mohamad Arif *et al.*, 2007).

Information on metabolites associated with plants resistance to diseases caused by fungi is scarce. Defense metabolites in plants against pathogens or termed as 'anti-microbial compounds' can be both pre-formed/constitutive (phytoanticipins) and inducible (phytoalexins). Phytoanticipins were reported to provide disease resistance and defensive properties against herbivores (Zagrobelny *et al.*, 2008; Benderoth *et al.*, 2006; Bidart-Bouzat and Kliebenstein, 2008) while several classes of phytoalexins which involve phenylpropanoids groups have important role in plant resistance against pathogen attack (Mansfield *et al.*, 2000; DiCenzo and Vanetten, 2006; Jeandet *et al.*, 2002). Over the years, studies have been carried out to analyse plant-pathogen interactions through metabolomic approaches. According to Jones *et al.* (2011) alanine exhibited correlation between time and *Magnaporthe grisea* fungal penetration into the leaf of *Oryza sativa* as evaluated by the use of nuclear magnetic resonance (NMR) spectroscopy and gas and liquid chromatography-tandem mass spectrometry (GC/LC-MS/MS). Another study conducted by Desbrosses *et al.* (2005) showed the application of gas chromatography-mass spectrometry (GC-MS)-based metabolite profiling of *Lotus japonicus* to study plant-microbe interactions where several marker metabolites have been identified which include octadecanoic acid, asparagine, glutamate, homoserine, cysteine, putrescine, mannitol, threonic acid, gluconic acid, glyceric acid-3-phosphate and glycerol-3-phosphate. Besides the use of mass spectrometry (MS) in metabolomics, the utilisation of NMR-based metabolomics also serves as a platform to study metabolic response of *Solanum lycopersicum* (tomato) leaves infected with *Citrus exocortis* viroid (CeVd) and *Pseudomonas syringae* bacteria where glycosylated gentisic acid is found as marker for viroid infection while phenylpropanoids and flavonoids (*e.g.* rutin) are for bacterial infection (Lopez-Gresa *et al.*, 2010).

Plant metabolomics include various methodological approaches and bioanalytical knowledge for the characterisation of many

chemically diverse classes of metabolites (Hegeman, 2010). Metabolite profiling is one of the techniques in metabolomics that involves rapid analysis, often not quantitative, of a large number of different metabolites with the objective to identify a specific metabolite profile that characterises a given sample or plant tissues (Villas-Boas *et al.*, 2005). Various combinations of analytical instruments consisting of a mass spectrometer and combined chromatography-MS are often employed in metabolite profiling. Liquid chromatography coupled with mass spectrometry (LC-MS) allows molecular identification of polar, less polar and neutral metabolites, even in relatively low concentrations level and complex matrix (Villas-Boas *et al.*, 2005). By the use of LC-MS, a wide range of chemical diversities of known and unknown compounds which are present in a biological matrix can be detected and identified without prior knowledge of the chemical structure (Villas-Boas *et al.*, 2005). Thus, LC-MS is an important tool in metabolite profiling that has been used to screen phytochemicals and bioactive compounds in metabolome studies (Roux *et al.*, 2011; Gomez-Romero *et al.*, 2010; Quirantes-Pine *et al.*, 2009; Dan *et al.*, 2008).

There has been a general consent within the oil palm industry that the primary route for BSR infection is through oil palm roots. *G. boninense* colonises the roots and produces enzymes that degrade tissues in basal stems (Paterson *et al.*, 2009). In plants ecosystem, the plants have to cope with potentially unfavourable conditions by developing their own chemical defence metabolites, which are involved both in resistance against pathogens and in tolerance towards abiotic stressors (Iriti and Faoro, 2009). Consequently, the induction of plant metabolites acting as defense compounds in oil palm root may involve in defense mechanisms against *G. boninense* in oil palm. There are certain classes of plant metabolites in oil palm root that may contribute to the oil palm resistance against disease, either pre-formed or inducible metabolites as reported by Diabate *et al.* (2009). According to Diabate *et al.* (2009), oil palm resistance against vascular wilt disease is due to the presence of phenolic compounds in *Fusarium oxysporum* infected oil palm roots which act as an indicator of defence reaction.

A comparison of metabolite profiles between partially tolerant and susceptible palms root extract is one of the techniques to uncover the biochemical pathways involved in BSR disease. As the field of metabolomics focuses on the unbiased and generally applicable strategies for metabolite extraction, fractionation and detection, significant challenges in fundamental activities such as compound identification still exist. For this reason, in this current research, a metabolite profiling technique

was developed and applied to oil palm root samples to identify compounds from two groups of partially tolerant and susceptible parental palms towards *G. boninense*. Information on the initial experiment on oil palm root metabolite profiling with the use of LC-MS, which allows separation and detection of a wide range of metabolites in a single run was developed. This metabolite profiling approach will have potent capacities in the areas of oil palm BSR disease detection and biomarker discovery. In the long run, this metabolomics approach will provide additional information on metabolic changes in oil palm for the improvement of understanding of BSR disease or chemical processes involving small molecules.

MATERIALS AND METHODS

Chemicals

Acetonitrile and acetic acid for HPLC and methanol for metabolites extraction were purchased from Merck, Germany. Procyanidin B1 standard ($\geq 90\%$) was purchased from Sigma-Aldrich, USA. All solvents used were HPLC grade. Water was purified by a Milli-Q system (Milipore, USA).

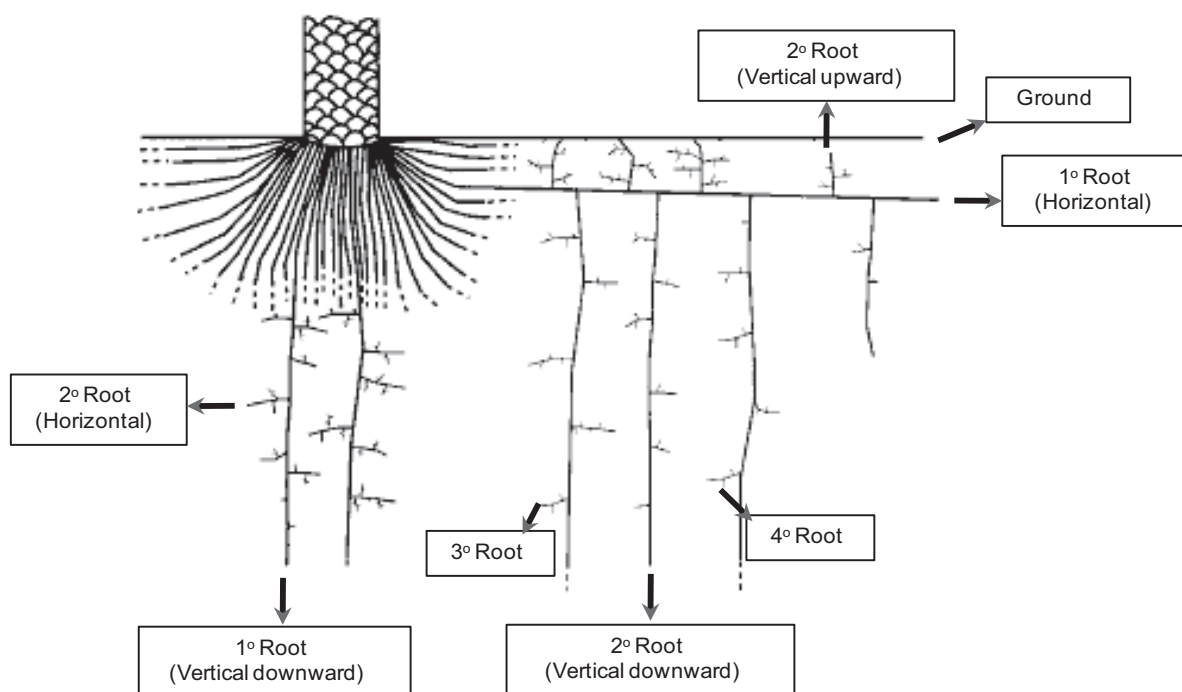
Plant Material

Root tissues from parental palms (0.221/1340, Zaire and 0.175/345, Dumpy selfs) were collected from MPOB Kluang Research Station, Kluang, Johor. In the present study, the palms were

identified based on the previous investigation on progenies screening for selection of partial resistance in oil palm to *Ganoderma* by Idris *et al.* (2004). Healthy parental palms without any visible symptoms of BSR were selected based on age and similar planting location. The root tissues were harvested in the morning from 9 to 11 am by cutting the primary root (Figure 1). The primary root tissues were rinsed with distilled water and immediately frozen in liquid nitrogen. The tissues were then ground to a fine powder using pestle and mortar in liquid nitrogen and stored at -80°C until further analyses.

Metabolite Extraction

Metabolites were extracted from a 500 mg frozen root tissue powder using a methanol-water extraction (Ferracane *et al.*, 2010). The choice of the extraction method is an important factor in any metabolomics study. Powdered tissue samples (500 mg) were first mixed with 5 ml 80% (v/v) aqueous methanol (HPLC grade, Merck Germany) in 10 ml Falcon tube (Greiner Bio-One, Germany). The extraction of root metabolites was performed by sonication in ultrasonic bath (Townson-Mercer, United Kingdom) for 30 min, centrifuged for 15 min at 4000 rpm, 25°C . The resultant clear supernatant was collected and dried under nitrogen stream before it was reconstituted in 3 ml of Milli-Q water. The extract was filtered through 0.2 μm pore-size filter (Sartorius, Germany) before analysis by LC-MS. All experiments were performed in three technical replicates.



Source: Jourdan *et al.* (2000).

Figure 1. Diagram of an adult oil palm root system showing the different types of roots observed.

LC-electrospray Ionisation (ESI)-MS Analysis

Analyses were carried out using an Ultimate 3000 HPLC system equipped with standard autosampler and photodiode array detector (Dionex, USA). The HPLC column used was a Reversed-Phase Acclaim PolarAdvantage II (C18 4.6 x 250 mm in length, 5 μ m particle size, Dionex, USA). The chromatographic separation was carried out at 37°C with a gradient elution program at a flow rate of 1.0 ml min⁻¹ and injection volume of 5 μ l. The mobile phase consisted of: (A) water containing 0.10% (v/v) acetic acid and (B) acetonitrile containing 0.10% (v/v) acetic acid and the gradient applied started at 5% of B and increased linearly to 25% B for 45.5 min. The column was washed for 5 min and equilibrated for 4 min before the next injection.

The HPLC system was coupled to a mass detector, Quadrupole/Time-of-Flight (Q/TOF) mass spectrometer (Bruker Daltonics, Germany) equipped with an ESI interface operating in negative ion mode and controlled by HyStar software (Bruker Daltonics, Germany). As ESI interface was used, the effluent from the HPLC column which was set at 1.0 ml min⁻¹ was reduced using a 'T' type splitter before being introduced into the mass spectrometer (split ratio 1:4). The flow which arrived to the detector was 250 μ l min⁻¹. Nitrogen was used as nebulising gas at pressure

of 4.1 bar with the flow rate of 9.0 litres min⁻¹. The temperature and the voltage of the capillary were set at 200°C and +3.5 kV, respectively. The full scan covered the mass range from 50-1000 m/z .

The accurate mass data of the molecular ions were processed through the Data Analysis 3.4 software (Bruker Daltonics, Germany), which provides a list of possible elemental formulas by using Generate-MolecularFormula (GMF) Editor. The GMF Editor uses a CHNO algorithm, which provides standard functionalities such as minimum/maximum elemental range, electron configuration and ring-plus double bonds equivalents, as well as a comparison of the theoretical with the measured isotope pattern (Sigma value) for increased confidence in the suggested molecular formula (Bruker Daltonics Technical Note No. 008, Molecular Formula Determination Under Automation).

External instrument calibration was performed using a syringe pump (kdScientific, USA) directly connected to the interface, with a sodium acetate cluster solution. The calibration solution was injected at the beginning of each run and all the spectra were calibrated prior to the compound identification.

All procedures in the metabolite analysis of parental palms root extract were done in triplicates. Figure 2 summarises the metabolite analysis work-flow of parental palms root extracts.

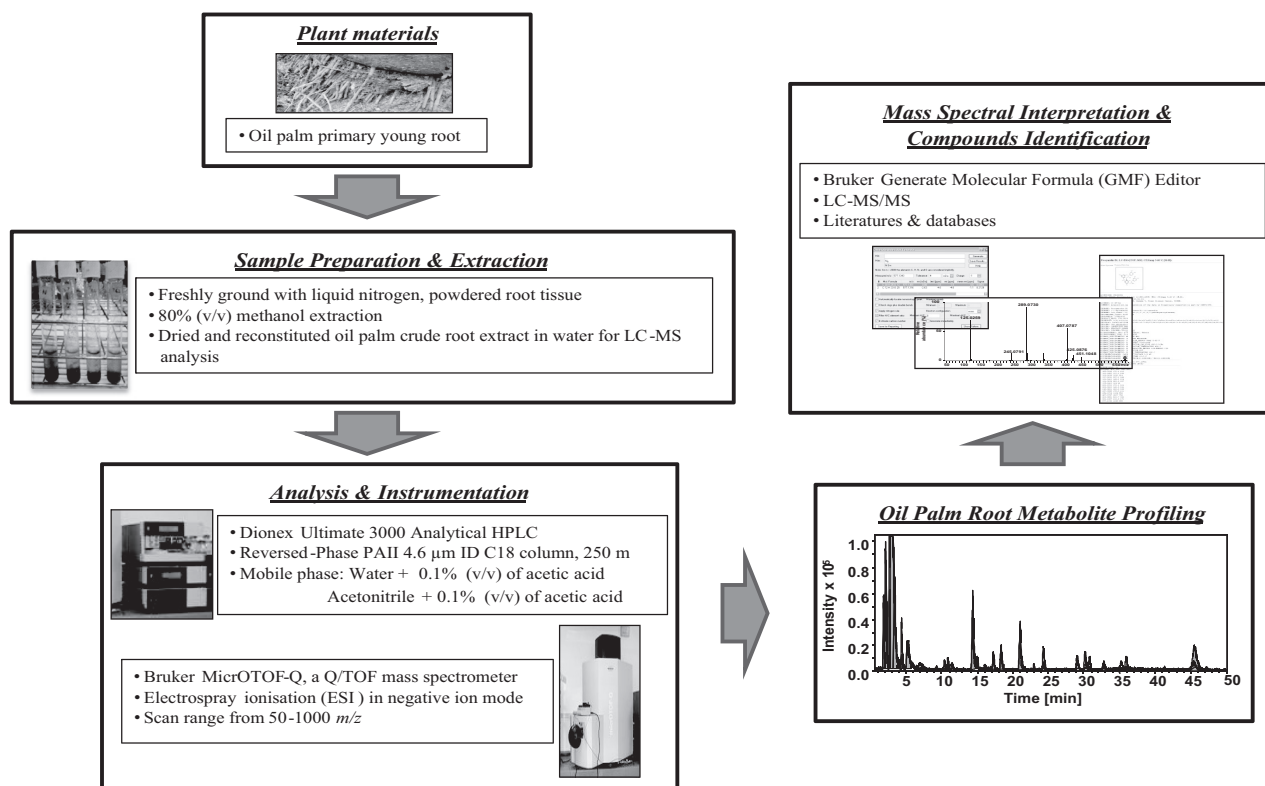


Figure 2. Parental palm root metabolite analysis work-flow.

RESULTS AND DISCUSSION

Identification of resistant breeding material is very important because they are the most common source of enhanced plant disease resistance. Ariffin *et al.* (1999) reported differences in susceptibility to *G. boninense* among 20 commercial DxP materials by using a root inoculation technique. In another study conducted by Idris *et al.* (2004), different oil palm progenies were selected for resistance to *G. boninense* infection. Data by Idris *et al.* (2004) were translated into a diagram as given in Figure 3. Partial resistance was expressed by low severity of foliar symptoms and slow progress of *G. boninense* infection in the roots and stem tissues. Among 23 oil palm progenies of different crosses, the most susceptible progeny was PK 2724 [DxD, Deli (Elmina) x Deli (Elmina)] whilst the partially resistant progeny was PK 2567 (DxP, Zaire x Cameroon). Obviously, the differences detected by the root inoculation technique on these progenies require further studies to predict field performance by the parental palms, where the progress of the disease may take several years to develop visible symptoms (Idris *et al.*, 2004).

However, it seems unlikely that correlation between metabolite status and differences in susceptibility between seedlings of these progenies to be examined since most of them were dead due to *G. boninense* infection. Therefore, the work

described here was undertaken to study the metabolite status of the parental palms for the partially tolerant and most susceptible progenies. The most susceptible and partially tolerant parental palms were reviewed and visually inspected to assure no visible symptoms of BSR. From our experiment, the oil palm root metabolites extracted in 80% aqueous methanol were amenable to analysis by LC coupled to photodiode-array (PDA) detector for screening of the different classes of metabolites, whereas MS and MS/MS fragmentation data were employed for compounds identification. An LC-MS Base Peak Chromatogram (BPC) of the oil

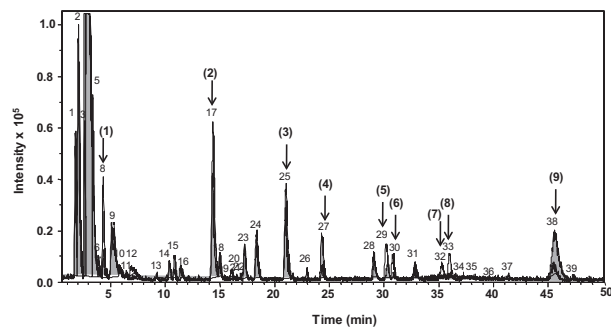
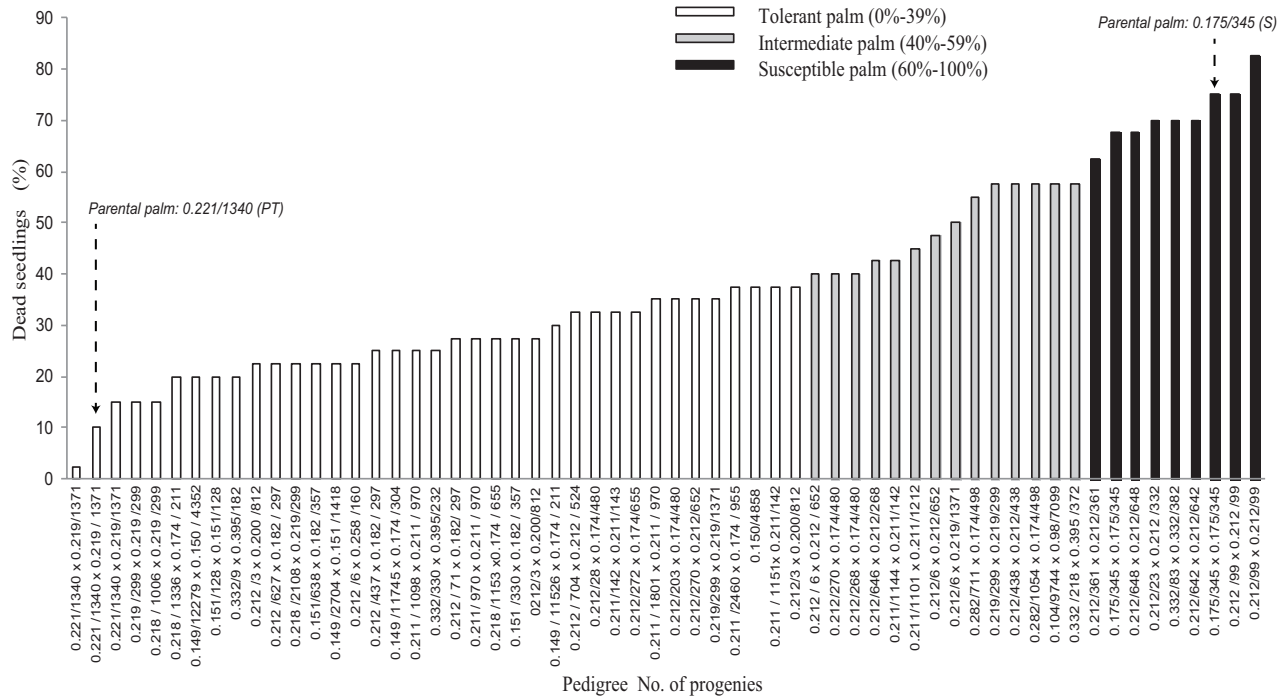


Figure 4. Liquid chromatography-mass spectrometry (LC-MS) base peak chromatogram (BPC) of partially tolerant and susceptible (shaded) parental palm root extracts. Nine peaks numbered in bold (1-9) show qualitative difference based on peak height.



Source: Idris *et al.* (2004).

Figure 3. Pedigree number of progenies according to the percentage of dead seedlings. The arrow indicates the partially tolerant and susceptible mother palm samples that were used in this study (PT: partially tolerant, S: susceptible).

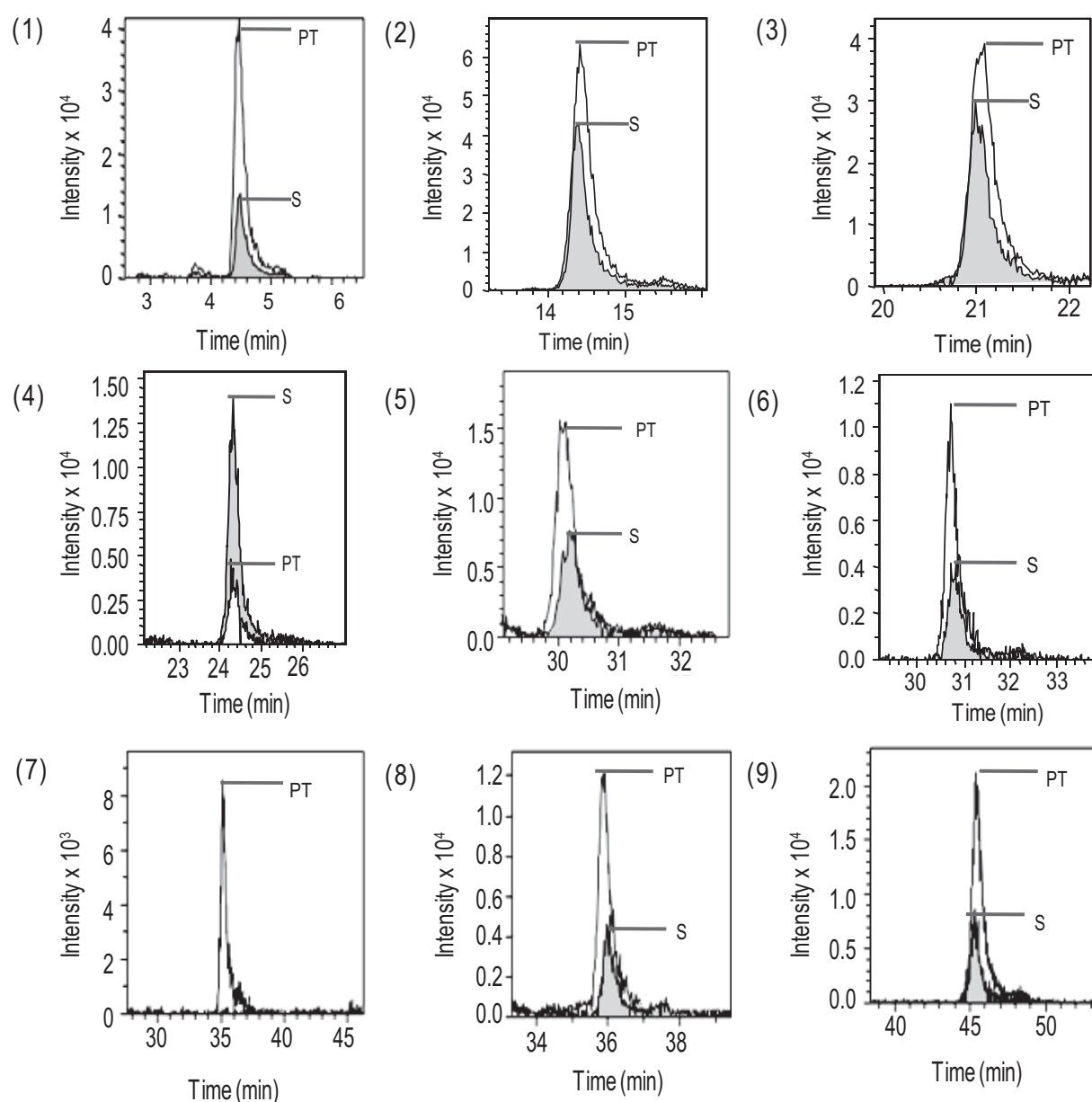


Figure 5. Extracted ion chromatogram (EIC) of liquid chromatography-mass spectrometry (LC-MS) analysis of the negatively charged molecular ion ($[M-H]$) of nine peaks from partially tolerant (PT) and susceptible (S) (shaded) parental palms that show qualitative difference in terms of peak height: 1 (209.0680 m/z), 2 (463.1446 m/z), 3 (477.1582 m/z), 4 (475.1818 m/z), 5 (577.1341 m/z), 6 (489.2080 m/z), 7 (503.1269 m/z), 8 (577.1359 m/z) and 9 (439.0843 m/z).

palm root extracts from the partially tolerant and susceptible parental palms is illustrated in Figure 4 where nine distinctive peaks based on peak height were identified with number (1-9).

Figure 5 shows the extracted ion chromatogram (EIC) of negatively charged molecular ion ($[M-H]$) in the partially tolerant and susceptible parental palm root extracts according to the elution order: (1) 209.0674 m/z , (2) 463.1446 m/z , (3) 477.1582 m/z , (4) 475.1818 m/z , (5) 577.1341 m/z , (6) 489.2080 m/z , (7) 503.1269 m/z , (8) 577.1359 m/z and (9) 439.0843 m/z .

The LC-MS detection in negative ionisation mode was used to obtain more information on the structural features based on the interpretation

of the elemental composition data, accurate mass and isotopic pattern information (DataAnalysis ver 3.4, Bruker Daltonics, Germany). Identification of the compounds within each classes, based on chromatographic behaviour and mass spectra and comparison with literature is summarised in Table 1 where compounds were numbered according to their retention times (t_R) in the chromatogram. Information on experimental and calculated m/z for the provided molecular formulas, error and the main fragments obtained by tandem MS, the proposed compounds for each peak and as well as the fold increment of ion intensity in the tolerant and susceptible parental palms are included.

Identification of Plant Sugars

The MS/MS analysis of sedoheptulose (Compound 1, t_R : 4.5 min, m/z 209.0674) gives characteristic fragments of sedoheptulose at m/z 159.0296, 129.0190, 99.0080 and 85.0292 as shown in Figure 6a. This plant sugar shows 2.0-fold increment in tolerant palm compared to susceptible palm. Sedoheptulose plays an important role in the primary and secondary metabolism of plant during synthesis of carbohydrates and biosynthesis of shikimic acid (Lee *et al.*, 1989). As shown in Table 1, the higher recovery of sedoheptulose (2.0-fold) in the partially tolerant palm suggests that pentose phosphate pathway metabolites might be activated against environmental effect. Sugar production is a fundamental activity in a plant system which also determines the ability to serve as a control mechanism and integration to external environmental conditions including light, other nutrients and abiotic and biotic stresses (Sheen *et al.*, 1999).

Identification of Plant Phenolics

Compounds 5 and 8 with m/z 577. Plants have evolved defense mechanisms, including production and accumulation of anti-microbial metabolites to protect themselves against microbial attack. Phenolics, powerful antioxidants that work in multiple ways to prevent diseases are plant metabolites that have been studied widely

(Strack, 1997). They are essential for the growth and reproduction of plants, and are produced as a response for defending injured plants against pathogens (Koskimaki *et al.*, 2009; Diabate *et al.*, 2009; Jones *et al.*, 2011). Phenolics occur naturally in plant tissue and can be classified into two groups, flavonoids and non-flavonoids. The flavonoids are sub-classified as flavonols, flavones, flavanols, anthocyanidins, flavanones and isoflavones. The tentatively identified procyanidin B1 and its isomer are classified under the sub-class of flavanols. The B-type procyanidins occur the most frequently in plant tissues which consist of procyanidins B1, B2, B3 and B4 (Xie and Dixon, 2005). Compound 5 was identified as procyanidin B1 based on the characteristic MS/MS fragments (Figure 7) and similar retention time (Figure 8) of the commercially available standard. The similar MS/MS fragments of compound 8 suggest that this compound is an isomer of procyanidin B. However, due to the limitation of the available B-type procyanidins standard, the identity of the procyanidin isomer could not be identified for compound 8. Procyanidin B1 and procyanidin B isomer (Compounds 5 and 8, t_R : 30.1 min and 35.9 min, m/z 577.1341 and 577.1359) increased over 3.3- and 2.5-fold in the partially tolerant parental palm, respectively, compared to susceptible parental palm. The characteristic fragments of procyanidins B at m/z 451.1048, 425.0876, 407.0787, 289.0730, 245.0791 and 125.0259 from MS/MS analysis is shown in Figure 6h.

TABLE 1. MASS SPECTROMETRY (MS) AND MS/MS DATA FOR EACH COMPOUND AND THEIR PROPOSED STRUCTURES

Peak	t_R (min)	m/z Experimental	m/z Calculated	Error (ppm)	Molecular formula	Tandem MS (MS/MS) fragments (m/z)	Proposed compound	Increase in abundance	Literature
1	4.5	209.0674	209.0667	-3.4	$C_7H_{13}O_7$	159.0296, 129.0190, 99.0080, 85.0292	Sedoheptulose	2.0-fold	-
2	14.4	463.1441	463.1457	3.5	$C_{19}H_{27}O_{13}$	293.0857, 233.0660, 169.0510, 125.0233	Dimethoxyphenyl-O-hexose-O-pentoside	1.4-fold	Kanchanapoom <i>et al.</i> (2002a)
3	21.1	477.1614	477.1614	-0.1	$C_{20}H_{29}O_{13}$	293.0838, 233.0643, 183.0668, 125.0222	Trimethoxyphenyl-O-hexose-O-pentoside	1.3-fold	Kanchanapoom <i>et al.</i> (2002b)
4	24.3	475.1805	475.1821	3.3	$C_{21}H_{31}O_{12}$	415.1601, 269.1022, 191.0533, 125.0185	Dimethoxybenzyl-O-hexose-O-rhamnoside	2.0-fold	Koike <i>et al.</i> (2010)
5	30.1	577.1341	577.1351	1.7	$C_{30}H_{25}O_{12}$	451.1047, 425.0859, 407.0761, 289.0699, 125.0235	Procyanidin B1	3.3-fold	Rodrigues <i>et al.</i> (2007); Falleh <i>et al.</i> (2011)
6	30.7	489.1969	489.1978	1.7	$C_{22}H_{33}O_{12}$	429.1749, 265.0935, 205.0708, 163.0602	Dimethoxyphenylethyl-O-hexose-O-rhamnoside	2.5-fold	Ono <i>et al.</i> (2009)
7	35.2	503.1178	503.1195	3.4	$C_{24}H_{25}O_{12}$	255.0728, 137.0257, 125.0246	Pinocembrin malonylhexoside	Only in tolerant palm	Aguilera <i>et al.</i> (2011)
8	35.9	577.1357	577.1351	-0.9	$C_{30}H_{25}O_{12}$	451.1048, 425.0876, 407.0787, 289.0730, 125.0259	Procyanidin B isomer	2.5-fold	Rodrigues <i>et al.</i> (2007); Falleh <i>et al.</i> (2011)
9	45.3	439.0532	439.0552	4.5	$C_{15}H_{19}O_{13}S$	241.0020, 197.0456, 152.9855, 125.0327	Hydroxy-dimethoxybenzoyl-sulfohexoside	2.0-fold	-

Note : The 'abundance of ion intensity column' in bold and italic indicates higher intensity of molecular ions in tolerant parental palms compared to susceptible parental palms. Analysis was performed in triplicates. m/z : mass to charge ratio; t_R : retention time.

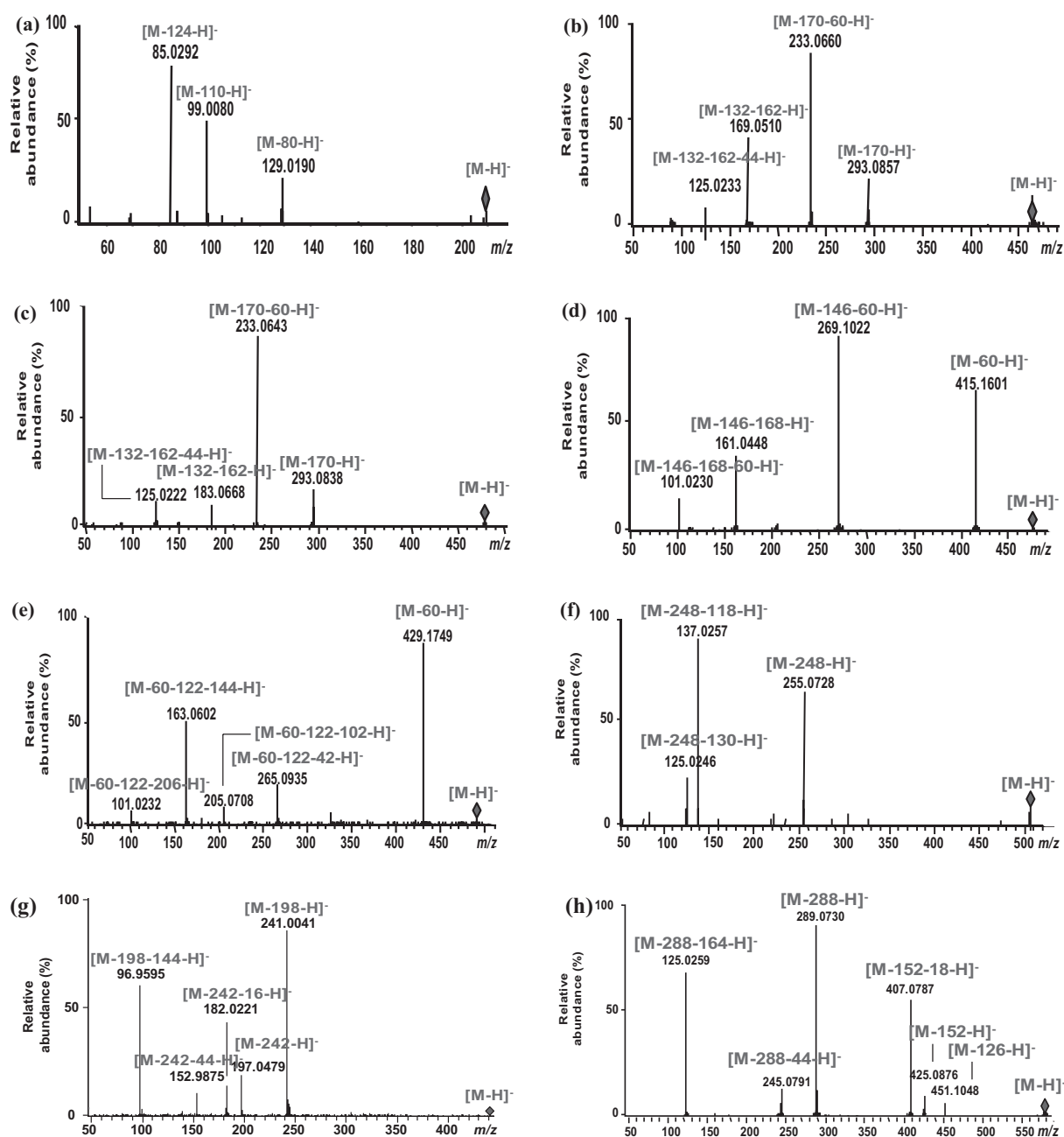


Figure 6. Tandem mass spectrometry (MS/MS) spectra of compounds in parental palms root extract. The spectra correspond to: (a) sedoheptulose, (b) dimethoxyphenyl-O-hexose-O-pentoside, (c) trimethoxyphenyl-O-hexose-O-pentoside, (d) dimethoxybenzyl-O-hexose-O-rhamnoside, (e) dimethoxyphenylethyl-O-hexose-O-rhamnoside, (f) pinocembrin malonylhexoside, (g) hydroxy-dimethoxybenzoyl-sulfo-hexoside and (h) procyanidin B.

Compounds 7 with m/z 503. Most of the flavonoids exist naturally as glycosides, in which the hydroxyl groups and sugars increase the water solubility of flavonoids (Crozier *et al.*, 2009). The proposed compound of pinocembrin malonylhexoside which was only present in the partially tolerant palm (Compound 7, t_R : 35.2 min, m/z 503.1269) represents flavonoids glycosides and shows characteristic fragments in MS/MS spectrum at m/z 255.0728, 137.0257 and 125.0246 as shown in Figure 6f.

Compounds 2, 3 and 6 with m/z 463, 477 and 489. The non-flavonoids groups were also detected in the LC/MS spectra, which were then identified as dimethoxyphenyl-O-hexose-O-pentoside by MS/MS analyses (Compound 2, t_R : 14.4 min, m/z 463.1446), trimethoxyphenyl-O-hexose-O-pentoside (Compound 3, t_R : 21.1, m/z 477.1582) and dimethoxyphenylethyl-O-hexose-O-rhamnoside (Compound 6, t_R : 30.7 min, m/z 489.2080). Figures 6b, 6c and 6e show characteristic fragments for

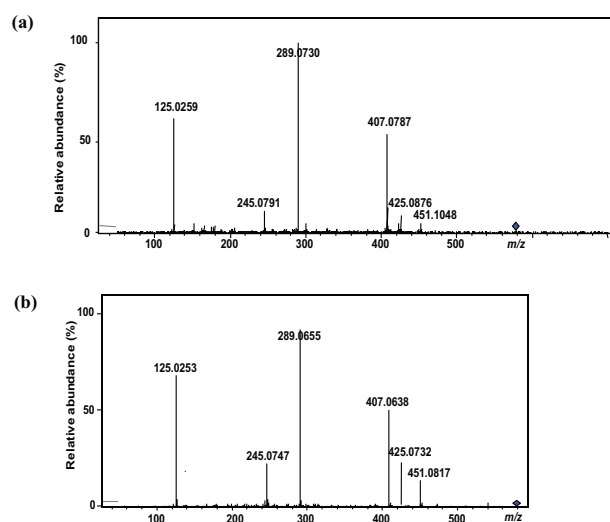


Figure 7. Similar mass spectrometry/mass spectrometry (MS/MS) fragments of compound 5 and procyanidin B1 standard. (a) Compound 5 and (b) procyanidin B1 standard.

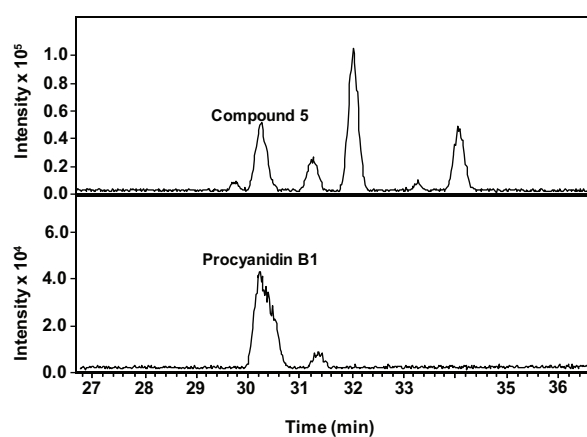


Figure 8. Similar retention time (t_R) of compound 5 and procyanidin B1 standard. (a) Compound 5 and (b) procyanidin B1 standard.

compounds **2** at m/z 293.0857, 233.0660, 169.0510 and 125.0233, **3** at m/z 293.0838, 233.0643, 183.0668 and 125.0222 and **6** at m/z 429.1749, 265.0935, 205.0708 and 163.0602. Each compounds shows 1.4-, 1.3- and 3.3-fold increment, respectively, in the partially tolerant palm.

Compounds 4 and 9 with m/z 475 and 439. The tentatively identified dimethoxybenzyl-*O*-hexose-*O*-rhamnoside (Compound **4**, t_R : 24.3 min, m/z 475.1818) and hydroxy-dimethoxybenzoyl-sulfo-hexoside (Compound **9**, t_R : 45.3, m/z 439.0843) show 2.0- and 2.5-fold increment in the susceptible parental palms. The MS/MS analyses that give characteristic fragments of compound **4** at m/z 415.1601, 269.1022, 191.0533 and 125.0185 and **9** at m/z 241.0041, 197.0479, 182.0221, 152.9875 and 96.9595 are shown in Figures 6d and 6g, respectively.

Figure 9 shows the proposed structures of the significant compounds in the partially tolerant and susceptible parental palms root extract.

LC-MS/MS Characterisation of Procyanidin B

The procyanidin B fragmentation behaviour as observed under negative ionisation mode is presented in Figure 10 for structure elucidation via MS/MS. The MS/MS spectra in the negative mode show six key fragments: m/z 451.1048, 425.0876, 407.0787, 289.0730, 245.0791 and 125.0259. Compounds **5** and **8** which were identified as procyanidin B1 and its isomer by comparison of their LC-MS/MS data with authentic standard and literature reports are among the well-studied phytochemical compounds (Falleh *et al.*, 2011; Sun and Miller, 2003; Gu *et al.*, 2003; Rodrigues *et al.*, 2007). Procyanidins or condensed tannins are commonly composed of flavan-3-ol units. The sensitivity and selectivity of procyanidin B under negative ionisation mode were due to the acidity of the phenolic hydrogens (Sun and Miller, 2003; Sannomiya *et al.*, 2005).

Three different pathways are involved in procyanidin B fragmentation in ESI source, which are retro-Diels-Alder (RDA), heterocyclic ring fission (HRF) and quinine methide (QM) fragmentation. Details on the fragmentation scheme of procyanidin B are shown in Figure 11. According to Gu *et al.* (2003) and Rodrigues *et al.* (2007), RDA reaction resulted in the ion at m/z 425. The RDA was found to be the most important fragmentation for structure elucidation of the dimers. The product of subsequent water elimination (m/z 407) was often detected in significant amounts, sometimes greater than those of the RDA product. Additionally, the MS/MS fragmentation gave a signal at m/z 451, corresponding to the loss of the A-ring by HRF fragmentation pathway, while m/z 287 and m/z 289 resulted from cleavage of the interflavan linkage through the QM mechanism (Falleh *et al.*, 2011; Rodrigues *et al.*, 2007). The fragmentation continued with the presence of the fragment at m/z 245 which was proposed to be the possible result of the loss of a $-\text{CH}_2-\text{CHOH}-$ group (Perez-Magarino *et al.*, 1999). The formation of molecular ion at m/z 125 is considered to be a diagnostic MS/MS fragment for the presence of two hydroxyl groups on the A-ring of flavan-3-ols (Miketova *et al.*, 2000).

This polyphenol-type compound with several $-\text{OH}$ groups shows anti-bacterial and anti-viral activities and its beneficial effects are probably due to its antioxidant activity protecting the cells against oxidative stress (Okuda, 2005). The elevated level of procyanidin B in healthy partially tolerant palms might suggest the involvement of this polyphenol-type compound to potentiate

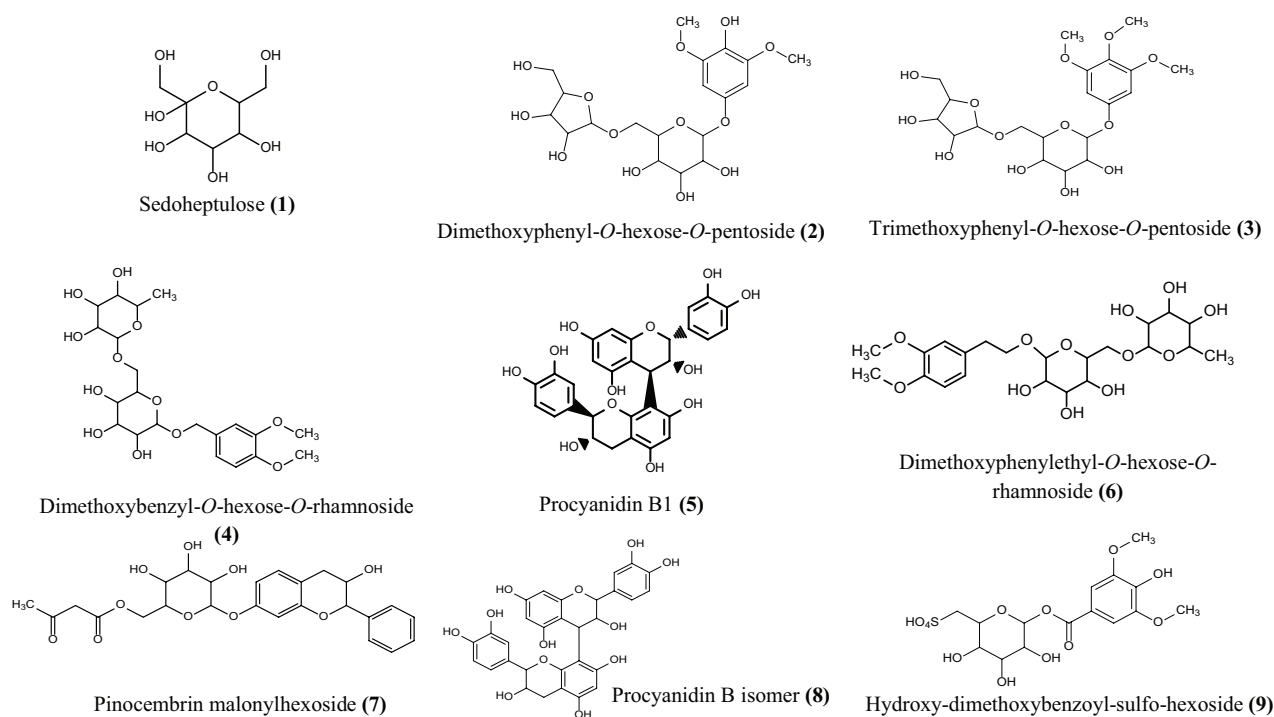


Figure 9. Proposed structures of compounds in the partially tolerant and susceptible parental palms.

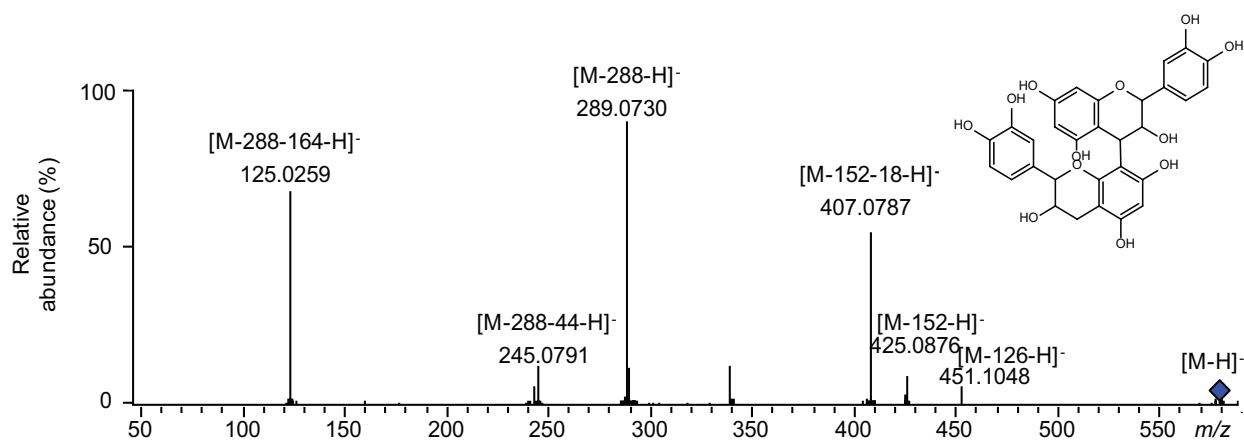


Figure 10. Product ion spectra of the [M-H]⁻ ion at *m/z* 577 identified in root extract of partially tolerant parental palm.

a preventive mechanism against BSR. Bell *et al.* (2010) reported that some components of resistance which are present in healthy plants are part of the 'constitutive defense'.

CONCLUSION

This article provides an overview of the basic metabolomics approach and its application to oil palm roots as well as future directions of metabolite profiling of oil palm towards understanding BSR disease. Oil palm metabolite profiling provides an improvement to the understanding of the

biosynthetic pathways response to environmental changes and serves as platform for further investigation and identification of marker metabolites for BSR disease detection. This research demonstrates that LC is successfully combined with MS in metabolite profiling analyses to have an insight into the composition of intercellular metabolite pools of specific tissues. This work represents the first comparative metabolite profiling analysis of oil palm roots metabolites from partially tolerant and susceptible parental palms to describe their progenies that showed differences in susceptibility to *G. boninense*, a causal agent of BSR disease in oil palm. The developed LC-MS

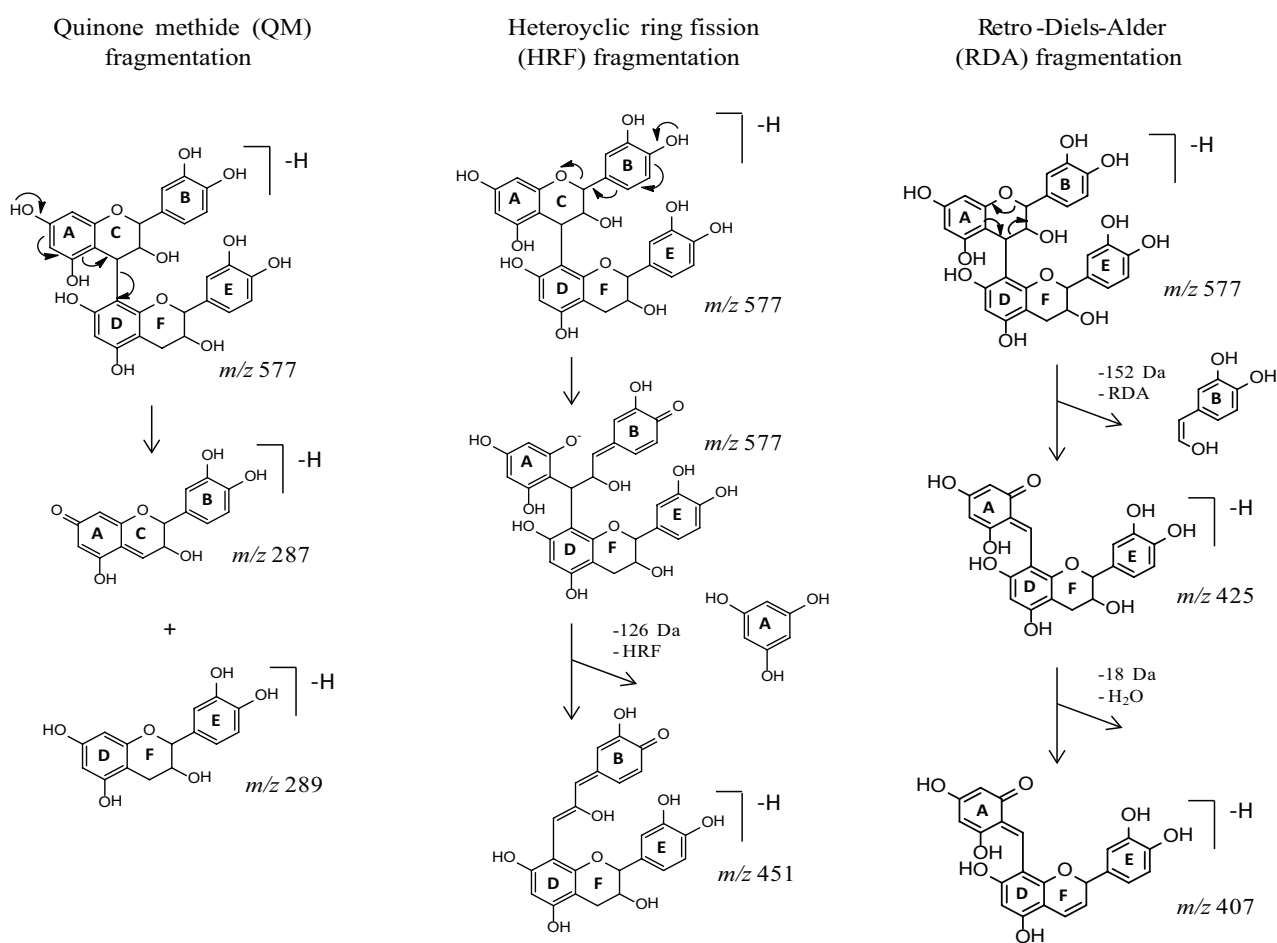


Figure 11. Main fragmentation pathways of procyanidin B (B-type proanthocyanidins) as described by Rodrigues et al. (2007).

and LC-MS/MS metabolite profiling can also be used to characterise the chemical composition of oil palm across cultivars and enables groups of palms to be differentiated. Thus, the next step will be towards the clearly laborious path of analysing larger group of tolerant and susceptible parental palms and to discriminate them using multivariate analysis for marker metabolites identification. In the long run, the metabolomics approach and technological development may lead to enhance the understanding of 'G. boninense-oil palm metabolites' interaction or cell-to-cell communication of oil palm physiology.

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