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METABOLOME ANALYSIS OF OIL PALM CLONE P325 OF DIFFERENT PLANTING TRIALS

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ABSTRACT

The production of oil palm clones via tissue culture technique will increase productivity and yield of the oil crop by providing elite and uniform material for the industry. To exploit their full potential, these clones need to be planted on prime acreage. However, limitation of arable land has pushed oil palm growers towards more challenging environment such as peat soil. In this study, oil palm clone P325 planted on peat and mineral soil trials were subjected to metabolome and multivariate analyses to capture their response to their planting sites. The investigation recorded spear leaf metabolome differentiation according to the field trials of different soil types, marked by the asparagine and dopamine abundance difference in principal component analysis (PCA) loadings plot. Liquid chromatography-mass spectrometry (LC-MS) demonstrates a sensitive and rapid method in acquiring information that to date has been scarce for this important crop. Multivariate statistical analysis of PCA further enables the visualisation of the chemical distinction of the tissue from the complex data.

Keywords: oil palm clone P325, metabolome, soil, principal component analysis (PCA), liquid chromatography-mass spectrometry (LC-MS).

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INTRODUCTION

As we are fast surpassing the seventh billion headcount of human population, the demand for vegetable oil products for food and non-food sectors is ever increasing. In line with the mission and vision of palm oil stakeholders and the Roundtable on Sustainable Palm Oil (RSPO), productivity on existing agrarian land is to be increased to avoid the opening of new terra firma for oil palm plantation (Corley, 2009). To achieve this goal, an effective approach to improve oil palm planting material must be identified to complement conventional breeding and seed production techniques such as crossing of selected parental palms, populations and germplasm collections (Kushairi *et al*., 2011a).

One of the methods to allow rapid multiplication of oil palm planting material is by tissue culture (Tarmizi *et al*., 2011; Rohani *et al*., 2000). Tissue culture improves the productivity of planting material through enhanced availability and uniformity of identified planting stock with desired traits. By means of tissue culture, selected oil palm that have superior traits such as high yield of bunches, high bunch weight and high oil yield per bunch can be cloned into new plantlets (Corley and Tinker, 2003). Propagation of these elite planting materials would then improve the national 10 years average fresh fruit bunch (FFB) yield of 19.08 t ha^{-1} yr^{-1} (MPOB, 2016a) and facilitate the achievement of 26.2 t FFB ha^{-1} yr^{-1} by the year 2020 (MPOB, 2011).

Oil palm cultivation has expanded to 5.64 million hectares in 2015 from 5.39 million hectares

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the previous year and now covers more than 17% of total land in Malaysia (MPOB, 2016b; Loh *et al*., 2015). Limited acreage especially in Peninsular Malaysia has pushed oil palm cultivation towards marginal land such as peat. About 2.4 to 2.6 million hectares of land in Malaysia is composed of peat (Schrier-Uijl *et al*., 2013; Harun *et al*., 2011). It is a soil of many faces; to land owners, it is desolate and problematic, to ecologists it is sensitive, and to oil palm stakeholders it is challenging. Nevertheless, farming activities on peat land has increased over the years (Tarmizi and Othman, 2011; Hassan *et al*., 1990). The physical and chemical properties of peat such as high water table, high acidity, low bulk density and low nutrient content provide a challenge for agricultural activity but yet oil palm establishment has proven to be economic and return good yields, greatly facilitated by best management practices and application of peat research and development efforts (Tarmizi and Othman, 2011). Productivity of oil palm on peat can be improved through better understanding of the properties of the soil and associated environmental factors. These dynamics can influence the biosysnthesis of metabolites in crucial plant organ such as the leaves which will ultimately affect the yield of crops. However, there is yet sufficient information on the chemical response and adaptations of oil palm to the soil type and its biotic and abiotic properties.

Oil palm spear leaves have been sampled as one of the foliar component or as individual descriptive organ for various study. An assessment of genetic diversity of oil palm genotypes was conducted using microsatellite markers from spear leaf samples (Okoye *et al*., 2016). Expression of homogentisate phytyltransferase (HPT) gene responsible for formation of tocopherols was found to be significantly upregulated in oil palm spear leaf sample presumably in preparation for protection from photooxidation (Kong *et al*., 2016). In an investigation on stress and its effect on oil palm yield, spear leaf extension rate was found to be highly correlated to drought and water supply and has provided the most sensitive and practical criterion for water status assessment (Carr, 2011; Henson *et al*., 2005). This is due to the fact that different locations and soil types contribute to variations in the physiology of the oil palms leaves (Latiff, 2000).

Environmental metabolomics is an emerging approach in investigating the response of organisms to a variety of environmental stress factors (Badri *et al*., 2013; Scherling *et al*., 2010). In this work, the spear leaf metabolome of oil palm clone P325 cultivated on peat and mineral soils were analysed using liquid chromatography (LC) combined with mass spectrometry (MS) in an attempt to investigate their characteristics under different planting conditions such as soil types. An advanced data mining tool such as multivariate principal component analysis (PCA) is then applied onto the metabolome data to find substantial differences or/and commonalities between the metabolome profiles of the samples.

MATERIALS AND METHODS

Reagents, Solvents and Standards

Methanol (MeOH), acetonitrile (CH_3CN) and acetic acid (CH_3COOH) of high performance liquid chromatography (HPLC) grade were purchased from Merck (Darmstadt, Germany) and purified water was prepared from Milli-Q system (EMD Millipore Corporation, Billenica, MA, USA) water purification unit. L-asparagine (≥98%) and (±)-octopamine hydrochloride (≥95%) were purchased from Sigma-Aldrich (St Louis, MO, USA) while 3-hydroxytyramine (dopamine) hydrochloride (99%) was from Acros Organics (Geel, Belgium).

Plant Samples

Spear leaves (unopened frond 0) from the clone P325 were sampled from the Malaysian Palm Oil Board (MPOB) Research Stations at the same period of time (10-11 am) to minimise diurnal effects. Details of the leaf samples are tabulated in *Table 1*.

The planting density for both trials were 160 palms ha^{-1} in a randomised complete block design (RCBD). The clone P325 was produced via tissue culture process (Kushairi *et al*., 2011b) from an ortet that was originated from commercial *tenera* (*dura* x *pisifera*, DxP) palm of 0.191/326. The clone P325 was considered high potential for oil yield and has exhibited zero mantling in commercial field

TABLE 1. DETAILS ON OIL PALM SPEAR LEAF SAMPLES FROM DIFFERENT TRIALS

Sampling sites	Clone	Age during harvest	Soil type
Keratong Research Station (Trial No. 0.461)	P325 (*n*=6 x 3 technical replicates)	4 years	Mineral (Serdang Series)
Teluk Intan Research Station (Trial No. 0.436)	P325 (*n*=6 x 3 technical replicates)	4 years	Peat (Penor Series)

Note: n-biological replicates.

trials. The ortet for this clone has shown superior performance with FFB production of 198.8 kg palm^{-1} yr^{-1} and oil-to-bunch (O/B) values of 35.2% (Zamzuri, 2011). The P325 palms in Trial No. 0.461 were grown under standard fertiliser regime of 'MPOB F1' (10% N, 5.4% P_2O_5, 16.2% K_2O added with boron and MgO) for inland soil while palms in Trial No. 0.436 were given 'MPOB F2 Super K' (7% N, 3% P_2O_5, 30% K_2O added with boron and zeolite) with application rate of 8.0 kg palm^{-1} yr^{-1}. MPOB F2 Super K was specially formulated to overcome and to compensate for nutrient deficiency due to leaching and debilitation through organic decomposition owing to its low pH, a phenomenon common for peat soil (Othman *et al*., 2014; 2005; Hassan *et al*., 1990) and to provide sufficient nutrient input for various peat soil types.

The oil palm spear leaf samples from six biological replicates from each planting sites were cut into small pieces of 2.54 cm length and shock-frozen in liquid nitrogen before pulverised under liquid nitrogen using mortar and pestle. The powdered samples were then lyophilised using a Labconco FreeZone® Freeze Drier System (MO, USA).

Extraction of Metabolites from Oil Palm Spear Leaf

The freeze dried leaf powder samples (0.1 g) were extracted with 5 ml 80% methanol by vortexing the mixture for 30 s prior to sonication for 30 min at room temperature for optimum metabolite recovery (Li *et al*., 2007). After centrifuging at 4000 rpm for 15 min at 25°C, the supernatant was collected and dried under a stream of nitrogen before reconstituted in 1 ml water. The extracts were then filtered through a 0.25 μm syringe filter (Sartorius AG, Goettingen, Germany) for HPLC injection. The extraction was carried out in triplicates for each biological replicates.

Liquid Chromatography-mass Spectrometry (LC-MS) Analysis

Oil palm spear leaf extract was separated using a C18 [octadecyl carbon chain (C18)-bonded silica] Reversed-Phase Acclaim® 120Å column of 150 mm length, 4.6 mm internal diameter (ID) and 5 μm particle size (Dionex, Sunnyvale, CA, USA) at 35°C (thermostated column compartment) on Dionex Ultimate 3000 HPLC with a diode-array PDA-3000 detector. Gradient elution was performed with water containing 0.1% acetic acid (solvent A) and acetonitrile containing 0.125% acetic acid (solvent B). LC gradient was programmed as follows: 0-2.5 min:5% B, 2.5-59.5 min:22% B and 59.5-60.0 min: 95% B. The injection volume was 1 μl with a constant flow rate of 1.00 ml min^{-1} and the flow was split to allow 200 μl min^{-1} of eluent into MS.

MS analysis was performed on MicrOTOF-Q™ quadrupole-time-of-flight (QTOF) mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The source conditions were: nebuliser gas (N_2) at 3.5 bar, dry gas (N_2) at 8.0 l min^{-1}, dry temperature at 200°C, capillary voltage at -3500 V and end plate offset at -500 V. The MS acquisitions were performed in negative electrospray ionisation (ESI) mode, in the mass range of *m/z* 50 to 1000. Data acquisition was performed by HyStar Application version 3.2 while data processing was carried out with DataAnalysis Version 3.4 by Bruker Daltonik GmbH. While both modes ionises efficiently, previous screening using oil palm spear leaf from commercial *tenera* (*dura* x *pisifera*, DxP) oil palm showed negative mode ESI-MS allowed higher recorded intensity of metabolite peaks for control sample (*Figure 1*).

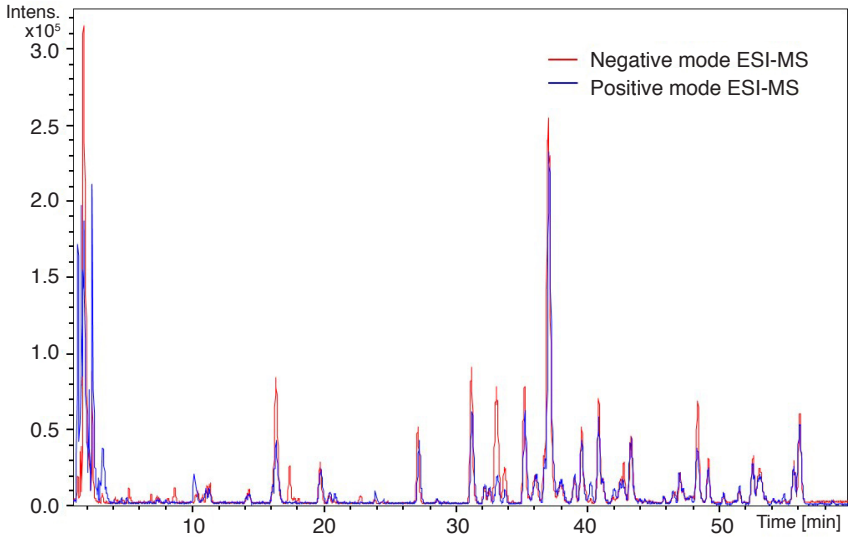

Figure 1. Negative and positive electrospray ionisation-mass spectrometry (ESI-MS) chromatograms of commercial oil palm spear leaf sample from previous work.

Principal Component Analysis (PCA) and *t*-test

PCA and *t*-test were performed on ProfileAnalysis™ (Version 2.0) from Bruker Daltonics. LC-MS profile data from 1.0 to 59.5 min run time pre-processed with Find Molecular Features (FMF) function in DataAnalysis software with 'bucketed' components from *m*/*z* 50 to 1000. Bucketing is a procedure of 'matrixing' LC-MS peaks in each data file into 'mass-to-charge (m/z) ratio: retention time' pairs containing peak intensities (ProfileAnalysis™ User Manual). A signal-to-noise (S/N) threshold of 5 was applied to the traces of the peak clusters in which a signal must exceed the set value to be used in peak detection. The buckets of 1 min and 1 *m*/*z* deltas were organised into tabular dataset format with retention times (rows) against peak intensities (columns). Normalisation was set to the 'sum of bucket values in analysis' by which it considers the total intensity in an analysis and is most appropriate for unknown sample and sample without internal standard. 'Pareto' scaling was performed on the data. PCA results were displayed as scores and loadings plots in a coordinate system of calculated variables called principle components (PC) while *t*-test results was reported for bucket(s) of *p*-value < 0.05. A confirmation of model fitness (R2) and predictive ability (Q2) with values closer to 1.0 was performed using similar buckets in SIMCA-P+ Version 12.0.1 by MKS Data Analytics Solutions (formerly MKS *Umetrics*), Malmö, Sweden.

Compound Identification using Tandem MS (MS/MS)

Collision-induced dissociation (CID) MS/MS of metabolites discovered as variable(s); bucket pairs that contribute most to the orientation of the PC as observed in PCA loadings plot were conducted in automatic scan mode using 20-30 eV CID energy in the MS collision cell with similar source and transfer settings in MS analysis.

RESULTS AND DISCUSSION

Figure 2 shows one of the clone P325 palms spear leaf extracts in negative mode ESI-LC-MS chromatograms. The data set for the PCA modelling was cross-validated (n=36) and the model was proven to be robust with all data points located in the distance 1 (D1) quadrant in the influence plot (*Figure 3*). D1 quadrant corresponds to analyses lying within the model space while D3 quadrant is the area of analyses that are calculated to be outside the model space but within the same plane. If samples are found on D2 and D4 quadrants, they are potentially different from the rest of the samples and should be studied for irregularities or errors that may be caused by sample preparation, instrument performance, sample composition or unique biological variation.

The results of PCA are illustrated as scores and loadings plots. The PCA model generated from the LC-MS data of oil palm spear leaf extracts of clone P325 palms planted on different soil trials exhibited a discriminative pattern between the two sample

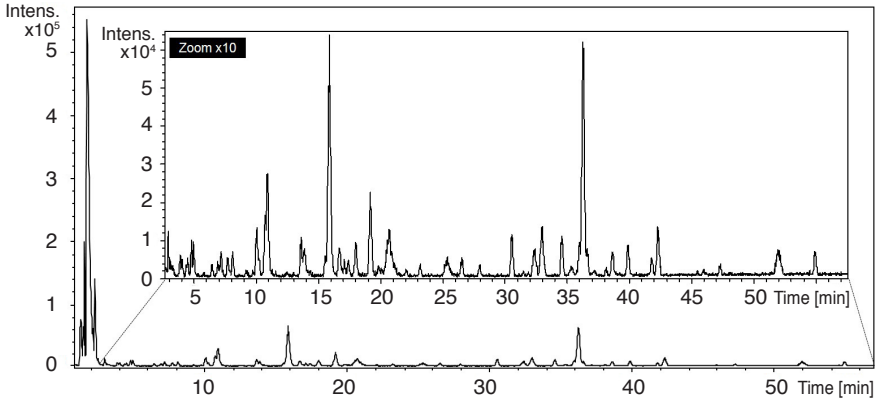

Figure 2. Representative liquid chromatography-mass sepectrometry (LC-MS) chromatograms of clone P325 palms.

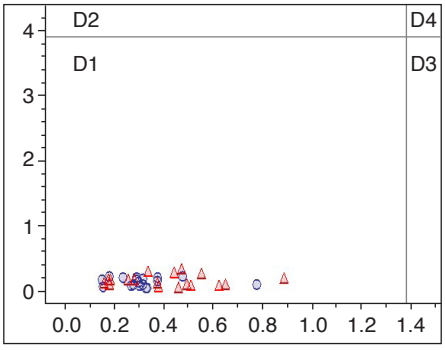

Figure 3. Principal component analysis (PCA) influence plot of clone P325 palms planted on mineral and peat soil trials with distance (D) quadrants.

groups (*Figure 4*). The scores plot contains individual palm numbers as labelled at the planting sites of Trials 0.461 (mineral soil) and 0.436 (peat soil).

Principal components 1 (PC1) and 2 (PC2) accounted for 28.7% and 37.5% of variances in the PCA model, respectively. Groupings or patterns in an investigated data set can be observed in the scores plot while metabolite(s) responsible for the separation in the data can be detected using loadings plot (Shuib *et al*., 2011). The furthest variable or bucket in a loadings plot has the most effect on the differentiation of the data set in the scores plot (Wagner *et al*., 2006). The variables in the loadings plot are also used to identify marker candidates that are responsible for group clustering (Meinicke *et al*., 2008). The goodness (R2) and predictive ability (Q2) of the model were found to be sound, with R2 of 0.888386 and Q2 of 0.860202 for component 1 and R2 of 0.94550 and Q2 of 0.920901 for component 2 (*Figure 5*).

PC2 at y-axis allowed segregation between the two groups of palms and the variable buckets found furthest from the centre cloud of the loadings plot were labelled as number 1 and 2 in *Figure 4b*. The compared to calculated *m/z* 131.0462 [M-H]$^-$ for the formula. This chemical formula corresponded to that of asparagine, a nitrogen carrier in plants (Neuberg *et al*., 2010; Snapp and Vance, 1986). The identity of metabolite bucket 1 was confirmed as asparagine by LC-MS retention time and MS/MS spectrum comparison with a commercial standard as shown in *Figure 6*.

Asparagine is one of the most profuse free amino acids in the xylem and phloem, allowing it to be used as marker for nitrogen (N) assimilation and utilisation study. It is synthesised from inorganic nitrogen, ammonium or nitrate in the soil and the process is highly influenced by environmental elicitors (Oliveira *et al*., 2001). Compared to plants fertilised with nitrate, those getting N from ammonium were observed to contain higher amino acids (Neuberg *et al*., 2010).

The difference in asparagine abundance detected in spear leaf of palms planted on peat and mineral soils could be due to their distinct ecosystem,

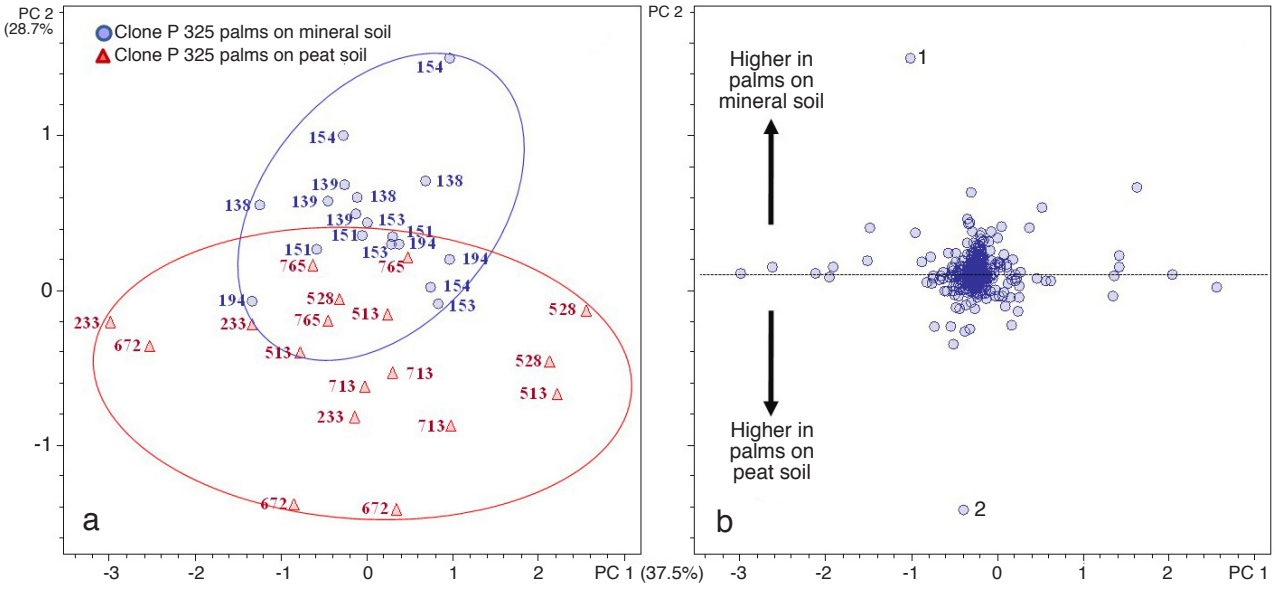

Figure 4. Principle component analysis (PCA) scores (a) and loadings (b) plots of spear leaf metabolome of clone P325 palms planted on mineral and peat soil trials.

results of *t*-test for a total of 27 significant variables including furthest buckets 1 and 2 from buckets generated from all samples are listed in *Table 2*. The values in the buckets are displayed in the gap of 0.5 for their retention time (t_R) and *m/z* due to equal raster width in the delta specification of the processing software.

Metabolite of bucket labelled as 1 (*m/z* 131, t_R 1.7 min) was found in lower abundance in data points representing the palms planted on peat soil trial. The molecular ion mass of metabolite of bucket 1 was recorded in accurate mass at *m/z* 131.0434 [M-H]$^-$ and calculated theoretically as deprotonated ion of $[C_4H_7N_2O_3]^-$ with errors of 2.82 mDa and 21.5 ppm

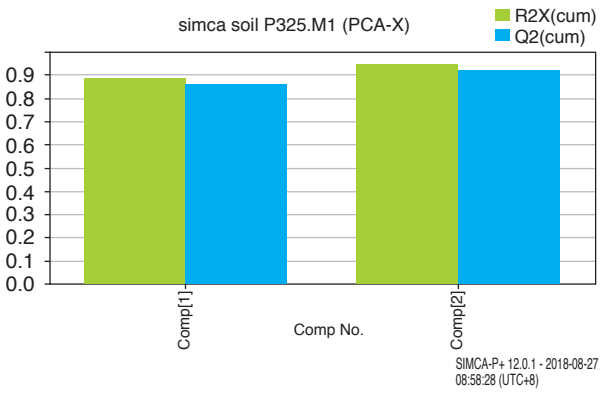

Figure 5. The R2 and Q2 for first two components of the principal component analysis (PCA) model.

TABLE 2. SIGNIFICANT VARIABLES FROM *T*-TEST

No.	t_R:*m/z* bucket	*p*-value	Fold change mineral/peat	Max signal intensity
1	**2.5 min:152.50 *m/z***	**0.00006**	**-1.42**	**273 720**
2	1.5 min:359.50 *m/z*	0.00071	1.49	10 596
3	1.5 min:358.50 *m/z*	0.00080	1.41	10 596
4	1.5 min:165.50 *m/z*	0.00142	-1.77	12 864
5	1.5 min:501.50 *m/z*	0.00175	1.34	6 140
6	2.5 min:165.50 *m/z*	0.00311	-2.03	12 864
7	1.5 min:485.50 *m/z*	0.00783	1.16	16 544
8	2.5 min:398.50 *m/z*	0.00845	2.05	1 756
9	2.5 min:399.50 *m/z*	0.00845	2.05	1 756
10	16.5 min:289.50 *m/z*	0.00878	-2.39	35 960
11	**1.5 min:131.50 *m/z***	**0.01161**	**1.31**	**232 752**
12	9.5 min:355.50 *m/z*	0.01170	2.80	14 796
13	1.5 min:215.50 *m/z*	0.01786	-1.90	9 624
14	3.5 min:358.50 *m/z*	0.01852	-2.49	7 560
15	36.5 min:446.50 *m/z*	0.01874	1.38	11 876
16	31.5 min:596.50*m/z*	0.02433	5.79	7 888
17	1.5 min:164.50 *m/z*	0.02460	-1.60	12 864
18	1.5 min:500.50 *m/z*	0.02511	1.29	6 140
19	1.5 min:201.50 *m/z*	0.02798	1.21	95 216
20	1.5 min:195.50 *m/z*	0.04070	-1.75	17 524
21	3.5 min:457.50 *m/z*	0.04234	1.43	19 444
22	3.5 min:456.50 *m/z*	0.04243	1.42	19 444
23	1.5 min:216.50 *m/z*	0.04294	1.70	10 996
24	1.5 min:217.50 *m/z*	0.04424	1.85	10 996
25	42.5 min:430.50 *m/z*	0.04665	5.14	22 308
26	16.5 min:486.50 *m/z*	0.04873	-1.53	11 828
27	1.5 min:179.50 *m/z*	0.04914	-1.50	24 112

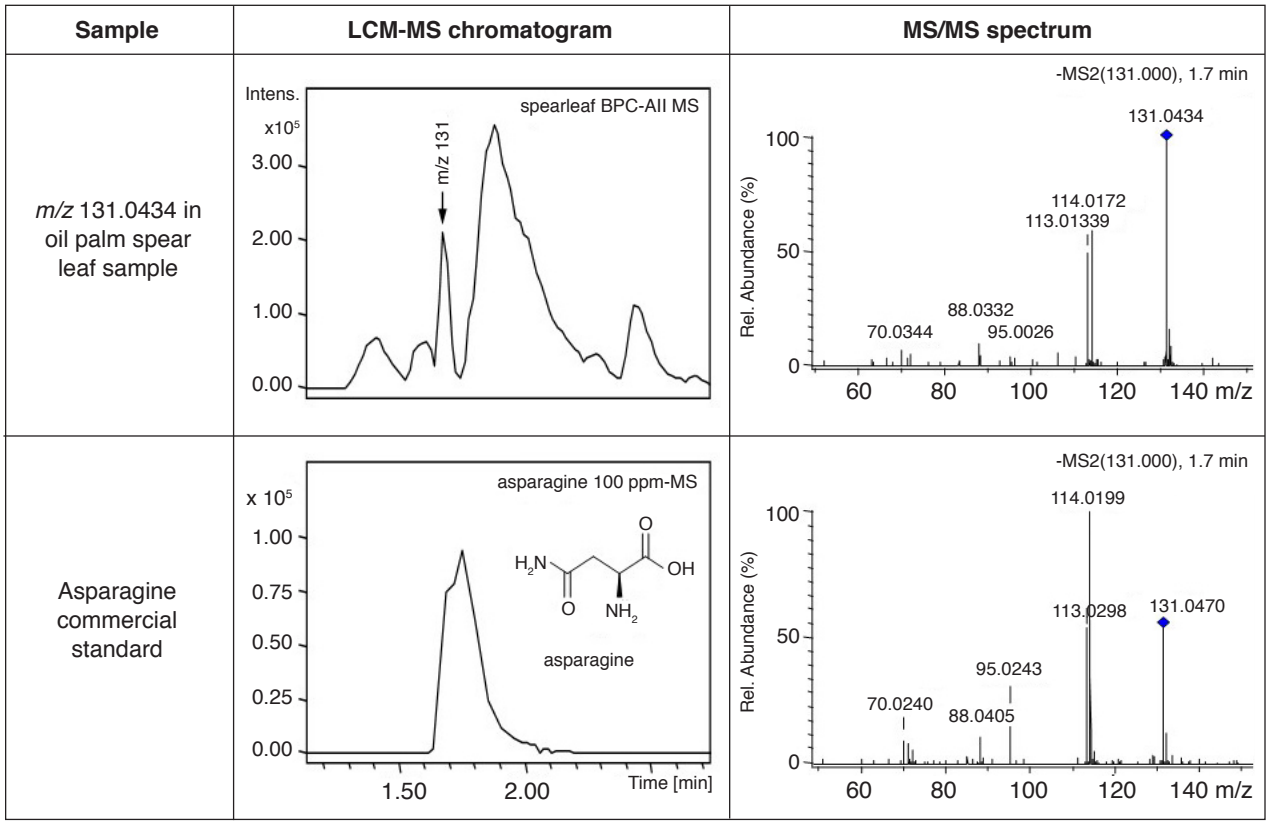

*Figure 6. Liquid chromatography-mass spectrometry (LC-MS) chromatograms and MS/MS spectra of metabolite 1 (*m/z *131.0434) in spear leaf sample and asparagine commercial standard.*

e.g. physical and chemical soil characteristics of the two planting locations and the organisms related to the sites instead of their fertiliser application. Trial 0.461 of mineral soil was applied with MPOB F1 with N in ammonium sulphate form while Trial 0.436 of peat soil was fertilised with MPOB F2 Super K with N in ammonium nitrate (Kushairi *et al*., 2011b). Both trials received N from similar cation ($NH4^+$) for N assimilation with regards to amino acid level.

Peat soil has high carbon-to-nitrogen ratio (C: N) of about 40:1 which result in slower composting rate and low mineralisation (Melling *et al*., 2002). The foremost notion of dealing with this fact is evidently to apply higher nitrogen fertiliser. However, peat soil trials carried out on several sites in Malaysia have shown that N treatment has no significant effect on leaf N level and fresh fruit bunch yield, even with zero application rate (Othman *et al*., 2014). Moreover, albeit the recorded disparity of asparagine abundance that generally presumed to be correlated to the nitrogen uptake by the palms, there is no evident symptom of nitrogen deficiency observable from the oil palm planted on the peat soil trial such as delayed growth or pinnae necrosis at the time of sampling. It is prudent however to take into account the scenario of minor peat subsidence that is causing the exposure of root systems and haphazard leaning of the palms in the peat soil trial.

Metabolite from bucket labelled as 2 (*m*/*z* 152, t_R 2.5 min) was recorded as accurate mass of *m*/*z* 152.0719 $[M-H]^-$ with errors of 0.20 mDa and 1.3 ppm to calculated *m*/*z* 152.0717 for $[C_8H_{10}NO_2]^-$. The MS/MS spectrum of metabolite from bucket 2 was found to be similar to dopamine [4-(2-aminoethyl) benzene-1,2-diol] as deposited in MassBank, record number KO000580 (Kakazu and Horai, 2007), with fragment ions at *m*/*z* 137, 123 and 122. However, the molecular formula and *m*/*z* value are also similar to that of another amine and its isomer, octopamine [4-(2-amino-1-hydroxyethyl)phenol] and norfenefrine [3-(2-amino-1-hydroxyethyl) phenol]. Thus, metabolite 2 could be any one of these three possibilities. A comparative LC-MS and MS/MS analysis with dopamine and octopamine commercial standards validated the identity of metabolite 2 as dopamine (*Figure 7*).

This metabolite was recorded in higher abundance in spear leaf metabolome sample of palms planted on peat soil trial. Unlike humans and animals, plants do not possess nervous systems of integrated neural networks. However, more findings reveal that they synthesise neurotransmitters such as dopamine, exerting specific activities probably as deterrent when interacting directly with other living organisms (Roshchina, 2010; Kulma and Szopa, 2007). In various plants such as tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), pepper (*Piper nigrum*), tobacco (*Nicotiana tabacum*) and onions (*Allium cepa*), derivatives of tyramines such as dopamine- and octopamine-conjugates of hydroxycinnamic acids are synthesised in response to infections from microorganisms, stress and wounding (Zacarés *et al*., 2007; Von Roepenack-Lahaye *et al*., 2003). Dopamine is also an alkaloid biosynthesis intermediate (Guidotti *et al*., 2013).

There have been reports of higher incidence of termite (*Coptotermes curvignathus*) incursions in oil palm of less than five years old in peat soil plantation (Othman *et al*., 2010). Peat soil also harbors bunch moths and *Ganoderma* fungus due to its high organic matter content and humidity. However, since no visible damage or infection symptoms were found on the oil palms when the sampling was carried out, it could not be ascertained as to whether the high presence of this metabolite has any correlation with the infestations.

The Penor soil series in Trial 0.436 is overlaid with sulfidic clay (Paramananthan, 2000) and is categorised as sulfihemists (*Table 3*), a histosol with sulphidic materials. Excess sulphur in the environment particularly with relevance to soil sphere causes stress and stimulate the endeavour for survival of the plant (Capaldi *et al*., 2015). This situation could lead to the biosynthesis of myriad range of metabolites such as dopamine as mediator and/or activator for stress response, adaptation and acclimatisation.

Asparagine and dopamine are organic bases containing nitrogen atoms in their molecules. As shown in *Figure 8*, both compounds are interrelated between amino acid biosynthesis and shikimate pathway (Kanehisa *et al*., 2014). Asparagine and other amino acids exist as weak alkaline in vascular bundles before being metabolised in plant tissue (Raven and Smith, 1976) and its substantial lower presence in spear leaves of palms planted on peat soil trial could be due to intracellular osmotic balance influenced by the external cellular condition. Dopamine on the other hand is synthesised for more restricted functions, either independent or in concert with other phytochemicals for stress responses such as adjusting stomatal behaviour, regulating mineral elements absorption and lowering oxidative damages (Li *et al*., 2015; Iriti, 2013). Its higher abundance detected in oil palm spear leaves of peat soil trial is befitting for a tracheophyte (higher plant) responding to challenging environment.

The different abundance of asparagine and dopamine of two different chemical routes in the oil palm spear leaves planted on these two different trials demonstrated the magnitude of the biotic and abiotic environment towards the palms. This finding instigate the necessity of a more in-depth study to fully understand and delineate the response and acclimatisation of the oil palm in a less favourable soil condition in expressing these metabolites. As this investigation is conducted in actual plantation environment, a lot of factors

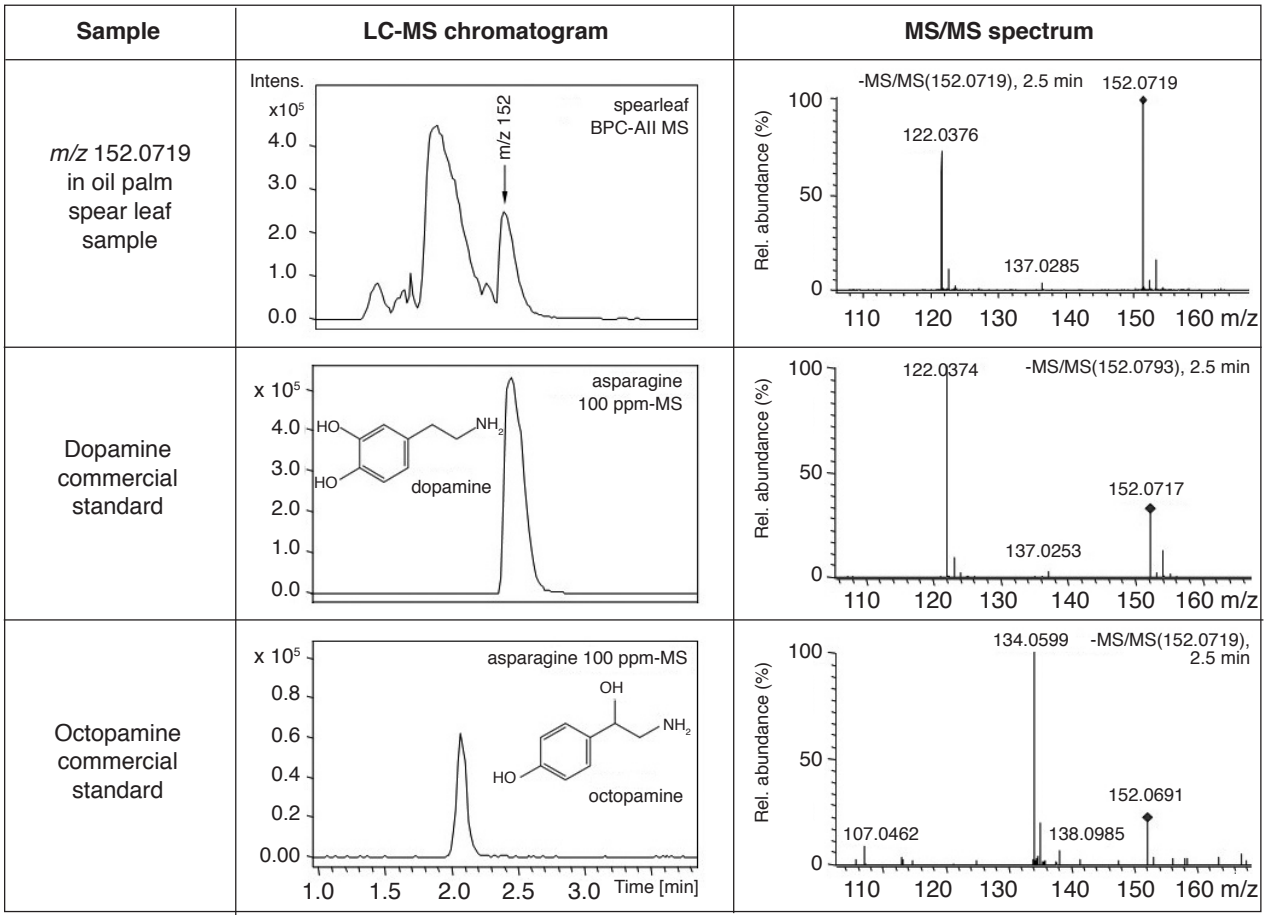

Figure 7. Liquid chromatography-mass spectrometry (LC-MS) chromatograms and MS/MS spectra of metabolite 2 (m/z 152.0719) in spear leaf sample, dopamine and octopamine commercial standards.

TABLE 3. SERDANG AND PENOR SOIL SERIES PHYSICO-CHEMICAL CHARACTERISTICS

	Serdang mineral	Penor peat
pH	4.8	3.7
Texture	Sandy loam	Organic sapric material
Soil taxonomy	Typic Kandiudults	Terric Sulfihemists
FAO classification	Haplic Nitisols	Thionic Histosols
C%	1.15	49.22
N%	0.12	1.22
C:N ratio	9.58:1	40.34:1

Note: *Adapted from Ariffin *et al*. (2015); Sabrina *et al*. (2009) and Sinnakkannu *et al*. (2005)

affect the species regardless of their equivalent clonal background. An experiment in a controlled environment will probably help to narrow down the variables for a specific interpretation of the metabolome status especially in understanding genetic versus environment (GxE) factors towards this important crop.

CONCLUSION

In general, this study recorded the influence of planting conditions and the environment onto the metabolome of oil palm spear leaves despite their similar origin (ortet). From the PCA of the LC-MS data, it was found that the abundance of asparagine and dopamine contributed to the deviation of the samples based on their planting site; peat and mineral soils.

PCA provides a powerful tool for descriptive statistical analysis of multivariate data. Similarities and differences between biological samples can be investigated and their details assessed with high level of confidence. The application of multivariate statistical analysis such as PCA on LC-MS data is one of the systems biology approach allowing interpretation of large scale data-mining of latent information.

This approach can be considered to be new to oil palm agronomists and physiologists, in which the

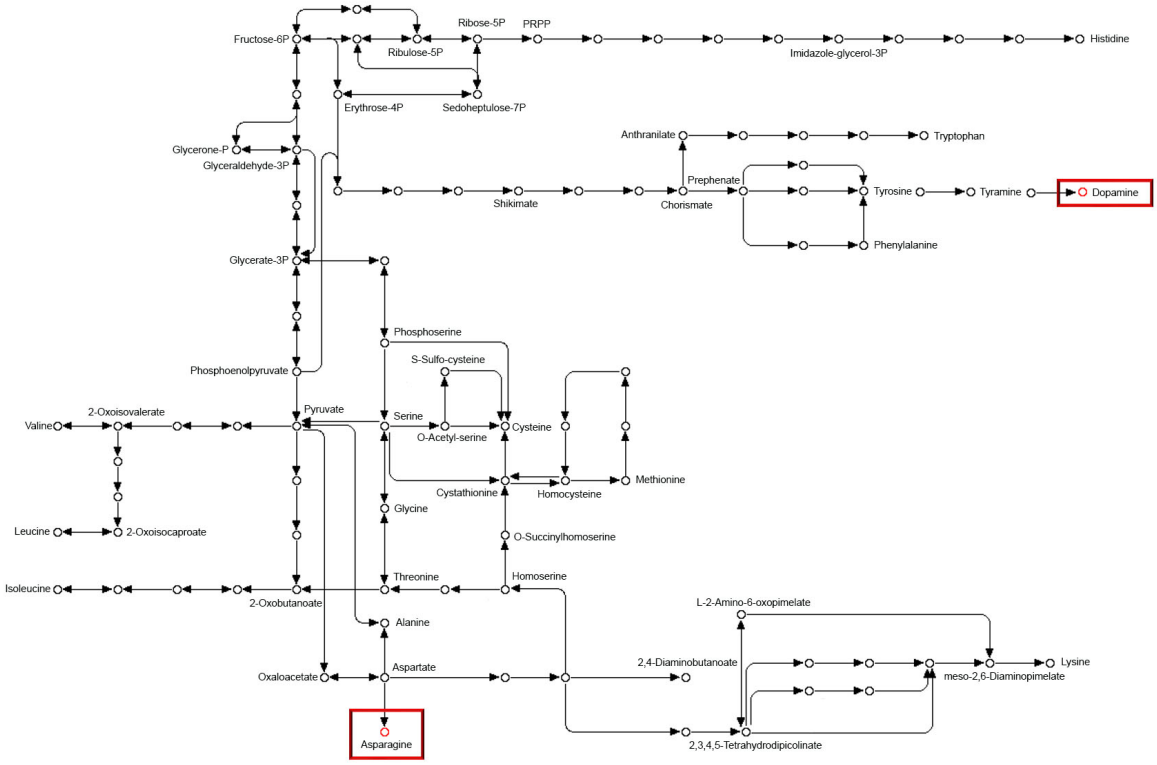

Figure 8. Asparagine and dopamine in interrelated metabolic pathways adapted from Kyoto Encyclopedia of Genes and Genomes (KEGG) Reference Pathways.

snapshot of metabolome status of the species can be acquired and can display variation in response to their environment or perturbations. The prospect of employing this technique on other investigations involving different oil palm tissues and various agricultural practices in oil palm cultivation is very appealing.

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