IDENTIFICATION OF LEAF METABOLOME CONTENT FOR DEVELOPMENT OF NEW VARIETIES

NURUL LIYANA ROZALI; NOOR IDAYU TAHIR; OTHMAN ABRIZAH; IDRIS ABU SEMAN; AHMAD KUSHAIRI AND UMI SALAMAH RAMLI

767

MPOB INFORMATION SERIES • ISSN 1511-7871 • JULY 2017 MPOB TS No. 167

In contrast to genetic markers, metabolite based biomarkers are relatively new in plant breeding. This is very different from the situation in the medicinal field, where clinical biochemistry has long been the basis for developing metabolite based biomarkers. Application in plant sciences has lagged behind, due to the slow application of related technologies that can help identify and characterise metabolites in the laboratory. Identification of metabolites not only assists in harnessing biomarkers to predict for phenotypes before the features are apparent, but also leads to the development of novel compounds with potential health benefits and in disease detection (Tahir *et al*., 2012; Syahanim *et al*., 2013; Nurazah *et al*., 2016; Ramli *et al*., 2016; Rozali *et al*., 2017).

Commonly, phenotyping of plants requires growing a set of plants and assaying the organs of interest in a time- and cost- intensive process. Current technology links these phenotypes to genetic markers to allow marker-assisted selection. However, recently, metabolomics has emerged as a highly promising approach for prediction of a variety of agronomically important phenotypes of crop plants grown in different environments, and particularly for discovering signature metabolites or biomarkers for traits of interest (Sumner *et al*., 2003).

DESCRIPTION OF THE SERVICE

The workflow for metabolite profiling and identification using Gas Chromatography Quadrupole Time-of-Flight Mass Spectrometer (GC/Q-TOF) is illustrated in *Figure 1*. Generally, metabolite identification involves the following steps:

- Leaf tissues are ground into a fine powder and methanol extraction is performed with ribitol as an internal standard.
- The samples are centrifuged at 4000 rpm for 30 min. Eight hundred microlitres chloroform and 1 mL water are added to the liquid phase. The extracts are centrifuged and the resulting upper layer collected.
- The lower phase is re-extracted in 1 ml methanol followed by centrifugation. Next, the collected supernatant is dried under nitrogen stream.
- For the derivatisation technique, the resulting pellet is dissolved in 20 µl methoxyamine hydrochloride in dried pyridine solvents (20 mg ml^{-1}) and incubated at 37°C for 90 min with vigorous shaking. About 180 µl N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) is added and the samples incubated at 37°C for another 30 min followed by vigorous shaking prior to analysis by GC/Q-TOF. A metabolomics approach for oil palm spear leaf using GC/Q-TOF is shown in *Figure 1*.

ADVANTAGES

- Potential technique for characterising leaf metabolites towards formulating metabolite biomarkers for important traits in plant breeding.
- Such metabolite biomarkers can assist in developing fast, targeted and low-cost diagnostic assays that will facilitate crop breeding programs and quality control by increasing prediction power.

NOVELTY

A comprehensive method for extraction and identification of key metabolites using gas chromatography-mass spectrometry (GC-MS). The technology holds great promise for investigating and understanding the leaf metabolome, in order to associate metabolite biomarkers and specific phenotypes.

INTELLECTUAL PROPERTY

A patent has been filed-PI 2016700540 entitled '*A method for Identifying Oil Plants that are at least Partially Tolerant to* Ganoderma *based on the Leaves of the Oil Palm Plants*'.

ISSN 1511-7871
9 771511 787001

Head of Corporate Implementation and Consultancy Unit, Malaysian Palm Oil Board. 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.
Tel: 03-8769 4574 Fax: 03-8926 1337 E-mail: tot@mpob.gov.my Website: www.mpob.gov.my

MPOB

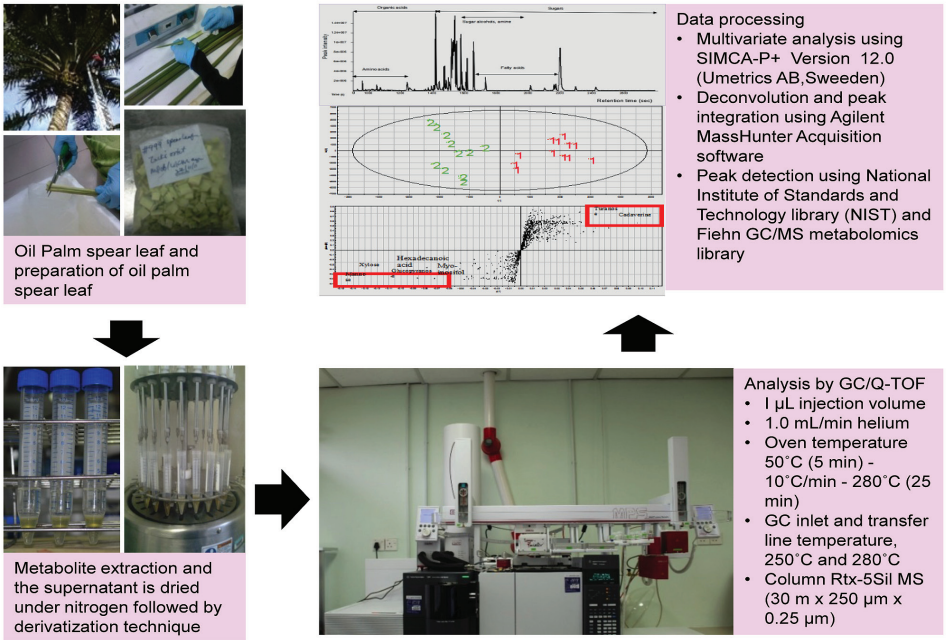

Figure 1. Metabolomics workflow of oil palm spear leaf metabolome using GC/Q-TOF.

SERVICES OFFERED

Identification of metabolites using GC/Q-TOF mass spectrometry. The service fee includes metabolite profiling with derivatisation MSTFA, fatty acid analysis and Automated Dynamic Headspace (DHS)-GERSTEL DHS/Headspace for volatile organic compounds (VOC) analysis. The price list of the services offered is in *Table 1*.

TABLE 1. COST FOR THE PROVIDED SERVICES

No.	Services	Charges per sample* (excluding 6% GST)	
		Academic (RM)	Industry (RM)
1.	Metabolite profiling with derivatisation MSTFA	200	250
2.	Fatty acid analysis	100	150
3.	SPME/Headspace for volatile organic compounds (VOC) analysis	150	200

Note: *Subject to change.

REFERENCES

NURAZAH, Z; IDRIS, A S; KUSHAIRI, A and RAMLI, U S (2016). A method for identifying oil palm plants that are at least partially tolerant to *Ganoderma* based on the roots of the oil palm plants. Malaysian Patent Application No. PI 2016700539.

RAMLI, U S; BENJAMIN, L Y C; TAHIR, N I; SYAHANIM, S; HASSAN, H; NURAZAH, Z; ROZALI, N L; DZULKIFLI, S B; ISHAK, N A and ABRIZAH, O (2016). Proteomics and metabolomics: Spearheading oil palm improvement and sustainability. *The Planter, 92 (1087)*: 727-737.

ROZALI, N L; IDRIS, A S; KUSHAIRI, A and RAMLI, U S (2017). Metabolomics differentiation of oil palm (*Elaeis guineensis* Jacq.) spear leaf with contrasting susceptibility to *Ganoderma boninense*. *Plant Omics. 10 (2)*: 45-52.

SUMNER, L W; MENDES, P and DIXON, R A (2003). Plant metabolomics: large scale phytochemistry in the functional genomics era. *Phytochemistry. 62*: 817-836.

SYAHANIM, S; ABRIZAH, O; MOHAMAD ARIF, A M; IDRIS, A S and MOHD DIN, A (2013). Identification of differentially expressed proteins in oil palm seedlings artificially infected with *Ganoderma*: A proteomics approach. *J. Oil Palm Res., 25 (3)*: 298-304.

TAHIR, N I; SHAARI, K; ABAS, F; PARVEEZ, G K A; ISHAK, Z and RAMLI, U S (2012). Characterisation of apigenin and luteolin derivatives from oil palm (*Elaeis guineensis* Jacq.) leaf using LC-ESI-MS/MS. *J Agr Food Chem, 60 (45)*: 11201-11210.

For more information, kindly contact:

Head of Corporate Implementation and Consultancy Unit, MPOB
6, Persiaran Institusi,
Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia
Tel: 03-8769 4574
Fax: 03-8926 1337
E-mail: tot@mpob.gov.my
www.mpob.gov.my