Journal of Oil Palm Research Vol. 29 (2) June 2017 p. 242 – 250

VARIATIONS OF $\delta^{13}C$ AND $\delta^{15}N$ IN OIL PALM TREE ORGANS: AN INSIGHT INTO C AND N DISTRIBUTION

SYAHIDAH AKMAL MUHAMMAD*; MOHD OMAR AB KADIR*; AINOLSYAKIRA MOHD RODHI and HASNURI MAT HASSAN‡**

ABSTRACT

Many studies have shown that seasonal variation in fruit bunch production occurs in relation to climatic factors and internal trophic conditions. As the annual fruit bunch production is important for planters, there is a need to get some insights into the carbon (C) and nitrogen (N) distribution in oil palm tree which are key factors in delineating plant C fluxes and nutrient cycling dynamics. Hence, this study utilises stable C and N isotope analyses to assess possible variations in the $\delta^{13}C$ and $\delta^{15}N$ signature in oil palm organs. Our study found that the average $\delta^{13}C$ of each oil palm tree organs are; root: –27.1‰, frond: –27.9‰, leaflets: –29.1‰, mesocarp: –28.6‰ and kernel: –28.7‰. The $\delta^{13}C$ results showed an enrichment of ^{13}C in heterotrophic organs compared to leaflets. Whilst the average $\delta^{15}N$ values of oil palm tree organs are; root: 4.4‰, frond: 8.9‰, leaflets: 1.6‰, mesocarp: 10.3‰ and kernel: 6.8‰. The $\delta^{15}N$ results also showed ^{15}N enrichment in heterotrophic organs compared to leaflets. In conclusion, there were clear isotopic differences between autotrophic leaflets and heterotrophic organs. This study demonstrates the applicability of stable isotope analysis (SIA) in providing novel insights into C and N distribution in oil palm tree whereby both factors are key in the understanding of oil palm physiology which in turn is crucial in maintaining a productive fruit bunch yield annually.

Keywords: carbon distribution, *Elaeis guineensis*, isotope fractionation, nitrogen distribution, stable isotope analysis.

Date received: 8 August 2016; **Sent for revision:** 10 October 2016; **Received in final form:** 2 March 2017; **Accepted:** 20 April 2017.

INTRODUCTION

Oil palm tree (*Elaeis guineensis*) originates from West Africa and was first introduced to Malaya in early 1870's as an ornamental plant. Later in 1917, it has its first commercial planting which took place in Tenammaran Estate in Selangor (MPOC, 2016). Oil palm has now become one of the most important crops globally and considered to be the most efficient oilseed crop in the world. One hectare of oil palm plantation is able to produce up to 10 times more oil than other leading oilseed crops with a maximal oil yield of 12.2 t ha^{-1} yr^{-1} (world average about 4 t $ha^{-1}yr^{-1}$) far beyond rapeseed (*Brassica napus*) or sunflower (*Helianthus annus*) (2.3 and 1.5 t $ha^{-1}yr^{-1}$, respectively) (Lamade *et al*., 2016). Among the 10 major oilseeds, oil palm accounted for only 5.5% of global land use for cultivation but producing palm oil that represents nearly 32.0% of global oils and fats output in 2012 (Ahmad *et al*., 2009). The demand for palm oil has increased tremendously in the last few decades worldwide which made

* Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia.
E-mail: syahidah.muhammad@usm.my

** Analytical Biochemistry Research Centre, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia.

‡ Agrobiology Division, School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Pulau Pinang, Malaysia.

oil palm an essential plantation crop in Malaysia since Malaysia is one of the leading producers and exporters of palm oil (Ahmad *et al*., 2009). However, there are rising concerns regarding the environmental sustainability of this expansion as well as the pressure to maintain a productive fruit bunch yield. Therefore, increasing efforts are being made to study and get a better understanding of key factors in sustaining a high yield of fruit bunch production such as the assimilated C distribution within plants during and after photosynthesis (Lamade *et al*., 2016), reserve carbon pool (Legros *et al*., 2009b), plant responses to environmental conditions (Legros *et al*., 2009a,b; Rivera-Mendez *et al*., 2012; Cros *et al*., 2013), the functional relationship and dependency between autotrophic sources and heterotrophic sink tissues (Lamade *et al*., 2009) as well as C transfer and allocation pathways at the tree scale (Lamade *et al*., 1996; 2009; Melling *et al*., 2008). These studies had helped immensely in our understanding of the oil palm physiology but more data are needed on carbon (C) distribution and especially on nutrients cycling dynamics in oil palm tree which are still lacking.

Stable isotope analysis (SIA) is a technique that has been proven to give novel insights into metabolic pathways and complex C and nitrogen (N) fluxes in the natural systems by measuring the isotope fractionation of the natural abundances of C and N isotopes at different stages of the pathway. The environmental and physiological information encoded in the isotope signature due to fractionation processes, usually caused by kinetic reaction, allows in principle to link changes in C and N metabolism on the biochemical scale (Brüggemann *et al*., 2011). Hence, oil palm physiology and its key factors can be understood and their mechanism(s) and pathways delineated by monitoring and tracking C and N distribution among plant organs either through the application of isotope labelling or taking advantage of natural isotope abundance using stable isotope analysis. Many studies have been carried out on oil palm physiology using SIA, but the focus is mainly on C fluxes and not much on $\delta^{15}N$ composition in oil palm which is a critical factor in understanding plant nutrients cycling dynamics. To the best of our knowledge, the only study that utilised N isotope analysis involving oil palm is Carvalho *et al*. (2008). However, their study was not on the understanding of the oil palm organs interactions but more on evaluating biological N fixation dependence by oil palm ecotypes. Others are applying the technique on other plant species *e.g*. N uptake, assimilation and translocation in ferns (Werth *et al*., 2015), $\delta^{15}N$ as an indicator of environmental stress in peach trees (Pascual *et al*., 2013), N dynamics in pine (Choi *et al*., 2005), $\delta^{15}N$ in wild barley (Robinson *et al*., 2000), $\delta^{15}N$ in peanut (*Arachis hypogaea* L.) leaf tissue (Rowland and Lamb, 2005), $\delta^{15}N$ in soyabeans (Bergersen *et al*., 1988), N isotopes to describe the movement of N within wheat plants (Yoneyama *et al*., 1997), $\delta^{15}N$ relationship to water status and N supply in grapes (Stamatiadis *et al*., 2007) and *etc*. The scarcity of available data on N cycling in oil palm tree system highlights the fact that our knowledge of $\delta^{15}N$ composition in oil palm and its interpretation towards the understanding of the plant organs interaction is not as clear as that of $\delta^{13}C$ (Robinson *et al*., 1998; Robinson, 2001).

Hence, the present study was carried out to add more data on C distribution in oil palm organs and at the same time give an insight into N uptake and assimilation in a plant system that grows in tropical climates and environmental conditions like Malaysia. Both are key elements towards understanding the oil palm physiology which is subsequently crucial in maintaining a productive fruit bunch yield annually.

MATERIALS AND METHODS

Sampling Location

This study was carried out in Peninsular Malaysia which is also known as West Malaysia. Peninsular Malaysia has a tropical climate with an annual average temperature of 20°C to 30°C, annual rainfall of 2500 mm, and the average relative humidity from 70% to 90%.

Sampling was carried out in April 2015, at a private plantation in Kulim, Kedah. The monthly rainfall data during that particular time was in the range of 200 – 250 mm obtained from the nearest meteorology station (in Bayan Lepas, Pulau Pinang, Peninsular Malaysia) which indicates normal precipitation values, hence the season was neither too dry nor too wet. The location coordinates are 5° 26′ 25″ N and 100° 37′ 46″ E. The oil palm trees in that plantation were planted since the year 2002. During the sampling, the oil palm trees were mostly around 13 years old and thus considered to be mature oil palm trees. Other information obtained from the planter with regards to soil and planting materials and regimes were as follows: 1) soil type was classified as Xanthic Kandiudo and generally considered deep and well-drained fertile fine sandy to clay loams; 2) commercial plant type: *tenera* hybrid (*dura* x *pisifera*) palms; 3) planting density was 143 palms ha^{-1} which was achieved by triangular planting adopting 9 m x 9 m x 9 m spacing; 4) fertiliser used was compounds in granular form and the fertiliser was applied normally by standard dosage; 5) the rate of fertiliser application was twice a year as per normal practice.

Plant Material

Based on accessibility and physiological conditions of oil palm trees at the sampling location such as similar height and maturity and showing no symptoms of diseases, 10 trees were identified to be the most suitable for our experiment. Samples of roots, fronds, leaflets, and oil palm fruits were taken from each tree. These samples were then stored in clean zip-lock plastic bags before being transported to the laboratory. Oil palm frond 9 and 17 and ripe fruit bunch samples were collected for this work. The ripeness of the fruit bunch was determined by colour intensity as well as looseness of fruitlets. Root samples were collected at 15 cm depth with an auger around the tree base and frond sample consisted of a small area between the petiole and the rachis and was cut radially from a leaf. When it comes to sampling the leaflets from the leaves for our experiment, we took the leaflets that grow out of the central nerve of the oil palm leaf rachis as indicated by Lamade *et al*. (2009). The leaflets were cut from two lower tier (ranked) leaflets and two upper tier leaflets from frond #9 and frond #17 and on the same day between 10 am and 1 pm.

Once in the laboratory, the different leaflets collected were mixed and cut into small pieces, individually, to facilitate milling into powder form. As for oil palm fruits, the samples were divided into two parts *i.e.* the mesocarp, the reddish pulp of the fruit of the oil palm where the palm oil is stored, and the kernel, the innermost layer of the oil palm fruit, or also known as palm seed. All samples were gently rinsed with distilled water to remove any dirt. The samples were then air-dried to remove excess water and stored in a –4°C freezer before milling. To prepare the samples for milling, the samples were freeze dried for 30 hr to remove moisture followed by liquid N addition to make grinding process easier. All samples were ground into fine powder using a blender or Spex SamplePrep 8000M mixer/mill. In order to avoid cross contamination, all the equipment used for drying and grinding were washed with detergent followed by a thorough rinse with acetone.

Stable Isotope Analysis

Prior to stable isotope analysis, ~0.5 mg of samples was weighed into small tin capsules in duplicates. The sample duplication was carried out to account for intra-sample variability in stable isotope analysis. These samples were then folded and compressed before being loaded into an auto-sampler for the analysis of stable C and N isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) using Flash 2000 elemental analyser (ThermoScientific, Waltham, MA) coupled to a Delta V Advantage isotope ratio mass spectrometer (Thermo, Milan, Italy). Raw isotope ratios from the analysis were then normalised to the international scales using IAEA reference materials, USGS-40 and -41 L-glutamic acid (~0.5 mg, respectively) assayed with the unknown samples. Urea (IVA-Analysentechnik GmbH & Co., Germany) was used as a quality control material to correct for drift and was measured for every 12 samples with known values of $\delta^{13}C$ = -40.81‰ and $\delta^{15}N$ = -0.49‰.

The typical precision for the duplicated samples was ±0.3‰ for $\delta^{13}C$ and ±0.3‰ for $\delta^{15}N$. Variations in stable isotope ratios were reported as permil (‰) deviations from internationally accepted standards which are Vienna Pee Dee Belemnite (VPDB) for carbon, atmospheric nitrogen (AIR) for nitrogen, in the delta (δ) notation. The δ notation is defined using the following Equation (1).

$$\delta\ (‰) = (R_{sample} / R_{standard} - 1) \qquad \text{Equation (1)}$$

where R_{sample} is the isotope ratio ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of the sample, and $R_{standard}$ is the isotopic ratio of the international reference materials.

Statistical Analysis

The average difference of $\delta^{13}C$ and $\delta^{15}N$ in oil palm organs was established using the analysis of variance (ANOVA) while post-hoc analyses were performed by Tukey's multiple comparison tests at $P \leq 0.01$. Both statistical analyses were carried out using IBM SPSS Statistics v21.0 (IBM®).

RESULTS AND DISCUSSION

Natural C Distribution Pattern in Oil Palm Organs

Figure 1 shows the $\delta^{13}C$ values ranged from –29.1‰ to –27.1‰, which are in the range of $\delta^{13}C$ values of a C_3 plant, the type of plant that follows the C_3 photosynthetic pathway (Calvin-Benson cycle) during photosynthesis. The root appeared to be the most ^{13}C-enriched with a $\delta^{13}C$ of –27.1‰, while the leaflets were ^{13}C-depleted ($\delta^{13}C$ = –29.1‰) when compared to the other plant organs. The enrichment of ^{13}C in oil palm root further highlights the classification of oil palm tree as a C_3 plant because previous study by Cernusak *et al*. (2009) found that the roots of herbaceous C_4 plant, another type of plant, are generally not ^{13}C-enriched when compared with the $\delta^{13}C$ value of its leaves. Similar pattern has been highlighted elsewhere (Yoneyama *et al*., 1997; Hobbie and Werner, 2004). As for the $\delta^{13}C$ values in oil palm fruit components, the mesocarp and kernel, the difference between the two values (–28.6‰ and –28.7‰, respectively) was just 0.1‰ which is less than the standard error of the instrument, hence the difference is considered insignificant (*Table 1*).

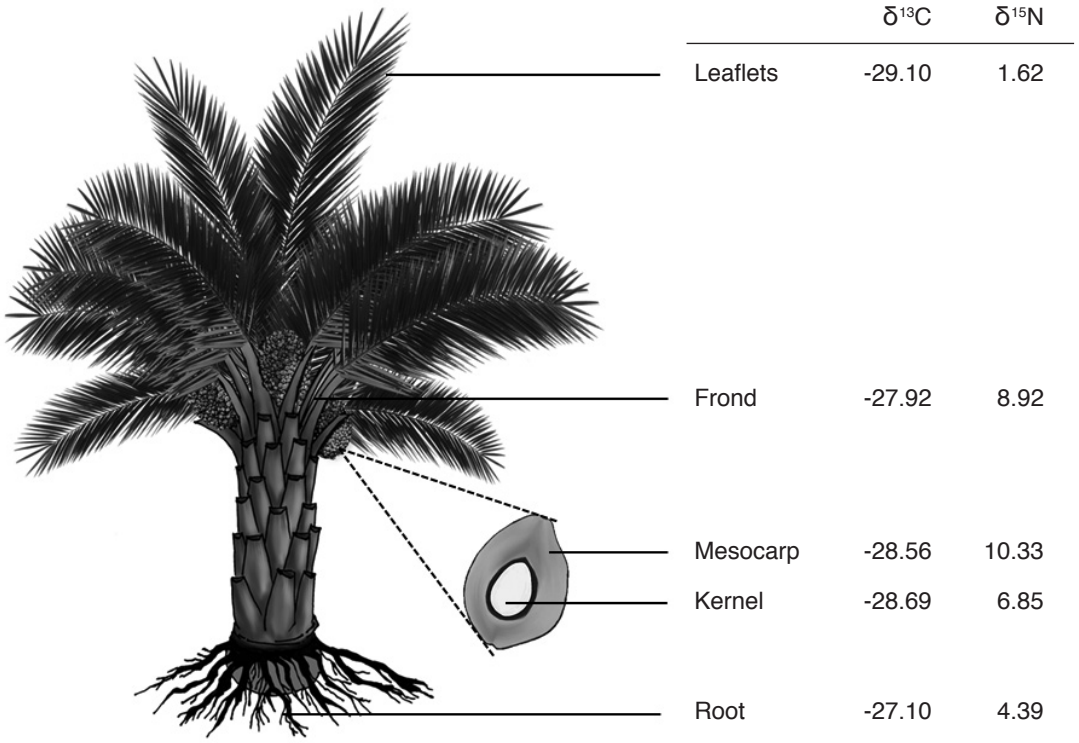

*Figure 1. Carbon and nitrogen isotope composition in oil palm tree organs, namely root, frond, leaflets and fruit components (mesocarp and kernel). Values are expressed as mean ± S.D. (*n *= 30 for each organ sampled from 10 trees).*

The more ^{13}C-depleted oil palm leaflets value is not unusual, which are in agreement with other previous findings by (Lamade *et al*., 2009; 2016). This pattern is due to the leaflets being autotrophic and supplies the heterotrophic plant organs (such as root, frond, and fruit) with photosynthate which are usually ^{13}C-enriched. More detailed explanations can be found in Badeck *et al*. (2005), Bowling *et al*. (2008), Cernusak *et al*. (2009) and Brüggemann *et al*. (2011) on the isotopic variations among plant organs whereby the biochemical compositions between autotrophic and heterotrophic plant organ play an important role coupled with the exportation of carbon which give rise to isotopic fractionation due to equilibrium and kinetic processes occurring inside the plant. They further proposed several mechanisms to explain the differences in isotopic signatures of plant organs in general and not limiting their discussion specifically to a C_3 type plant. One of the reasons for the differences in ^{13}C content between autotrophic and heterotrophic plant organs is the export activity of metabolites such as sucrose which are enriched in ^{13}C with respect to photosynthetic products in autotrophic organs. The outgoing ^{13}C-enriched metabolites will reside in heterotrophic organs making it more enriched with the heavier carbon isotope thus leaving the autotrophic organ such as leaves ^{13}C-depleted.

TABLE 1. AVERAGE DIFFERENCE OF $\delta^{13}C_{VPDB}$(‰) IN OIL PALM ORGANS

Plant organ	$\delta^{13}C_{VPDB}$(‰)[a]	*P*
Root	-27.10 ± 0.15 (d)	<0.01
Frond	-27.92 ± 0.08 (c)	<0.01
Leaflets	-29.10 ± 0.19 (a)	<0.01
Mesocarp	-28.56 ± 0.21 (b)	<0.01
Kernel	-28.69 ± 0.26 (b)	<0.01

Note: [a]Mean values with different letter(s) indicate significant differences at $p \leq 0.01$ by Tukey's multiple comparison tests (n = 30 for each organ sampled from 10 trees). Values are expressed in mean ± standard deviation.

Other possible reasons on the isotopic variations among plant organs would be the seasonal asynchrony of growth of photosynthetic and autotrophic tissues, with corresponding variation in photosynthetic discrimination against ^{13}C due to different environmental and ontogenetic conditions (Bathellier *et al*., 2008; Salmon *et al*., 2011), developmental variation in photosynthetic fractionation against ^{13}C during leaf expansion (Le Roux *et al*., 2001; Terwilliger *et al*., 2001; Li *et al*., 2007), seasonal variations in starch storage and remobilisation (Gessler *et al*., 2009), day versus night translocation of sucrose between leaves and sink tissues, with daytime sucrose being relatively ^{13}C-depleted and night-time sucrose ^{13}C-enriched (Tcherkez *et al*., 2004). Moreover, the respiratory fractionation can cause carbon isotopic values to differ among tree organs (Bathellier *et al*., 2008) in which case the respiring autotrophic organs tend

to respire ^{13}C-enriched carbon compounds thus leading to a ^{13}C-depletion in their tissues. The suggested explanations outlined in the literature corroborated our findings in which case show a trend of more positive $\delta^{13}C$ values in oil palm leaflets when compared to the $\delta^{13}C$ values in oil palm heterotrophic organs. Additionally, a study by Berveiller and Damesin (2008) has proven that phosphoenolpyruvate (PEP) carboxylase fixes HCO_3^- at a greater rate in heterotrophic organs such as root, frond and fruit thus a ^{13}C enrichment of organic material in such heterotrophic tissues would occur faster than in an autotrophic oil palm leaflets. The study goes on to reveal that the activity of PEP carboxylase was several-fold higher in current year tree stems than in leaves which in principle is similar with the pattern observed in this study. Similarly, the activity of PEP carboxylase was found to be about twice as high in roots than in leaves (Gessler *et al*., 2009).

On the other hand, among the heterotrophic organs in oil palm trees studied, there is a trend of ^{13}C-depletion in the fruit components, the mesocarp and kernel, which showed $\delta^{13}C$ values of –28.6‰ –28.7‰, respectively, relative to –27.1‰ in root and –27.9‰ in frond. This may be due to the fruit containing oleochemicals which consists of fatty acids, a type of lipid, produced after a series of enzymatic reactions that caused the compounds to be ^{13}C-depleted and remained in the mesocarp and kernel (Brüggemann *et al*., 2011, for detailed review of the process). In retrospect, the more negative $\delta^{13}C$ values found in oil palm fruit components in relative to other heterotrophic organs in this study are in line with previous published data by Lamade *et al*. (2016).

To further understand and deduce the different transformation pathways in the distribution and transportation of carbon throughout the whole oil palm tree, compound-specific isotope analysis should be conducted on sucrose or amino acids either incorporating the usage of ^{13}C-labelled tracer or taking advantage of the differences in the natural isotopic abundance of the compound of interests.

Natural N Distribution Pattern in Oil Palm Organs

Figure 1 shows that the $\delta^{15}N$ values ranged between 1.6‰ to 10.3‰. The autotrophic leaflets showed the least positive $\delta^{15}N$ value (^{15}N-depleted) with 1.6‰ when compared to the $\delta^{15}N$ values of heterotrophic organs analysed in this study. Meanwhile, among the heterotrophic organs, the mesocarp, the pulp of the fruit showed the most ^{15}N-enriched value ($\delta^{15}N$ = 10.3‰) while the kernel, the seed inside the fruit, appeared to be less ^{15}N-enriched in relative to the mesocarp with $\delta^{15}N$ of 6.8‰. Overall, the $\delta^{15}N$ results showed ^{15}N enrichment in all heterotrophic organs were significant at $P<0.01$ (*Table 2*) compared to the autotrophic leaflets, by 2.8‰ in root, by 7.3‰ in frond and up to 8.7‰ in fruit (mesocarp). As far as we are concerned, the present study is the first to report on the natural N distribution pattern in oil palm organs based on $\delta^{15}N$ values. Hence, our observations will rely on the explanations highlighted by previous work which carried out experimental studies on other plant species.

A review by Evans (2001) suggested that the intra-plant variation in N isotope composition can be caused by multiple assimilation events, *i.e.* organ-specific loss of N (Bergensen *et al*., 1988; Yoneyama *et al*., 1989; Evans *et al*., 1996) and reallocation of N. During plant growth period, the reallocation of N involves many enzymatic reactions such as NO_3^- reaction (Yoneyama and Kanek, 1989; Evans *et al*., 1996), glutamine synthetase-glutamine oxoglutarate aminotransferase (GS-GOGAT) (Yoneyama *et al*., 1993), transaminations (Macko *et al*., 1986; Yoneyama *et al*., 1998). All of these processes have been proven to discriminate against ^{15}N isotope which resulted in the products depleted in ^{15}N than the original source. For example, samples of trunkwood of *Prosopis glandulosa* in the Sonoran Desert of California was depleted in ^{15}N between 3‰-4‰ comparing to the rest of the plant. The depletion of ^{15}N seen in trunkwood in the study is an example of the generalisation that N sinks are often depleted in ^{15}N compared to their source (Shearer *et al*., 1983).

However, on the contrary, our results on the variations of $\delta^{15}N$ in plant organs showed just the opposite whereby the leaflets were more ^{15}N-depleted than the sink organs. So far, we could not suggest a good explanation in our results but several studies on other plant species did observe lower $\delta^{15}N$ values in leaf compared to $\delta^{15}N$ in sink organs (Yoneyama *et al*., 2000; Pascual *et al*., 2013) whilst Yang *et al*. (2015) observed no clear direction or trend in the $\delta^{15}N$ of different plants organs in a variety of alpine plants. In all of the studies mentioned, the strikingly similar factor in their work was the environmental induced

TABLE 2. AVERAGE DIFFERENCE OF $\delta^{15}N_{AIR}$(‰) IN OIL PALM ORGANS

Plant organ	$\delta^{15}N_{AIR}$(‰)[a]	*P*
Root	4.39 ± 0.14 (b)	<0.01
Frond	8.92 ± 0.19 (d)	<0.01
Leaflets	1.62 ± 0.30 (a)	<0.01
Mesocarp	10.33 ± 0.14 (e)	<0.01
Kernel	6.85 ± 0.34 (c)	<0.01

Note: [a]Mean values with different letter(s) indicate significant differences at $p \leq 0.01$ by Tukey's multiple comparison tests (n = 30 for each organ sampled from 10 trees). Values are expressed in mean ± standard deviation.

variations such as extreme dry weather, water stress episodes, N availability and to a certain extent, genotypic variation. Thus, the anomaly observed in the present study may arise due to the above mentioned factors.

Several authors have insinuated the difficulties of correlating and explaining N cycling dynamics in plants. Data and explicit demonstrations of possible contribution of compartmentation and transport phenomena within the plant to N isotope discriminations are scarce (Werner and Schmidt, 2002). N isotope discriminations have always been attributed to plant intrinsic transports of compounds between compartments or organs and possible mechanism processes were implied. However, in actual sense these processes are depending on actual flux conditions (Werner and Schmidt, 2002). For example, metabolic fluxes between different plant organs can change their direction in the course of the development of the plant (Masclaux *et al*., 2000), and the ^{15}N-abundance of amino acids in these plant organs can vary with the development state (Yoneyama *et al*., 1997). Moreover, according to Kolb and Evans (2002), the variations of $\delta^{15}N$ in plant tissues among tree species were interpreted to mean that major sites (roots, leaves) of nitrate reduction may differ among plant species or that the variations may be related to the cycling of N in plants and the differential contributions of N sources (newly absorbed N and re-allocated N) for tissue N accumulation. Robinson *et al*. (1998) also stated that it is difficult to measure the export of N from shoot to root in many species whilst obtaining good data for N influx and efflux is also problematical. So on the basis of inter-variation of $\delta^{15}N$ among plant organs; we can only conclude that the analysis of $^{15}N/^{14}N$ ratios at natural abundance ($\delta^{15}N$ levels) has clarified the occurrence of isotopic fractionation during uptake, assimilation and distribution of N in plants. Yoneyama *et al*. (2003) has stated clearly in their review, the $\delta^{15}N$ values in whole plants are determined by the $\delta^{15}N$ values of acquired N whilst the $\delta^{15}N$ values in different plant organs (parts) are determined by the $\delta^{15}N$ values of influx N and efflux N after metabolism in the organ. They added that cultivated crops (such as the oil palm in the present study) have different available N sources and N in various forms, which have different $\delta^{15}N$ values, thus the $\delta^{15}N$ values of field-grown crops can reflect both the $\delta^{15}N$ of their source N with changes in $^{15}N/^{14}N$ ratios due to isotopic fractionation during uptake, metabolism and distribution.

In future, the theory and mechanisms that determine $\delta^{15}N$ values of individual N metabolites and their individual N atoms in different plant organs and compartments in oil palm should be further studied, but most likely using compound-specific isotope analysis. The incorporation of ^{15}N-labelled substrates such as NH_4^+, NO_3^-, NO_2^-, NO_2 (gas) and amino acids to plants as tracers and following its fate can be an effective way to differentiate between external and internal plant sources and to quantify the importance of the internal cycling of N to support new growth (Proe *et al*., 2000). Additionally, the incorporation of dual-isotope (^{13}C and ^{15}N) labelling which is widely regarded as a very powerful tool may be the key to elucidate the relationship between internal N stores and recently fixed photosynthate (Dyckmans and Flessa, 2001). The efforts by Robinson *et al*. (1998), Hobbie *et al*. (1999) and Comstock (2001) in providing theoretical models to identify processes that could control plant $\delta^{15}N$ are valuable and timely, but more thorough, theoretical models such as those developed for plant carbon isotope composition are still necessary.

More detailed discussions on how isotope measurements were interpreted and associated with critical plant resources, *i.e.* C, water, and N which have helped to deepen our understanding of plant-resource acquisition, plant interactions with other organisms, and the role of plants in ecosystem studies can be found in Dawson *et al*. (2002).

CONCLUSION

In the present study, the natural C distribution pattern in oil palm organs were successfully demarcated by the different organ functions, the heterotrophic and the autotrophic organs. The heterotrophic or non-photosynthetic organs, *i.e.* root, frond and fruit components (mesocarp and kernel) analysed in this study showed the more positive values of $\delta^{13}C$ compared to the autotrophic organ, the leaflets with a difference of 2‰. Additionally, among the oil palm heterotrophic organs, the fruit components were found to be the most ^{13}C-depleted. The natural N distribution patterns in oil palm organs were also successfully demarcated by the different organ functions, the heterotrophic and the autotrophic organs, in the present study. The heterotrophic or non-photosynthetic organs, *i.e.* root, frond and fruit components (mesocarp and kernel) showed the more positive values of $\delta^{15}N$ compared to the autotrophic organ, the leaflets with a difference of up to 8.7‰. The pulp of the fruit, the mesocarp was found to be the most ^{15}N-enriched among the heterotrophic oil palm organs. These results were the first to report on the variations of the oil palm organs based on $\delta^{15}N$ values. Interestingly, our results on the variations of $\delta^{15}N$ in plant organs showed that the leaflets were more ^{15}N-depleted than the sink organs. The pattern observed in the present study was different from the generally accepted pattern of N distribution in plant organs whereby

an autotrophic organ like the leaflets normally is ^{15}N-enriched than the heterotrophic organs. So far, we could not suggest a good explanation in our results but the anomaly observed could be due to environmental induced variations.

Conclusively, despite the work being at a preliminary stage and the limitation in the small sample size, our objective for this work which was to add more data on C distribution in oil palm organs and at the same time give an insight into N uptake and assimilation in an oil palm plant system that grows in tropical climates and environmental conditions like Malaysia has been achieved.

ACKNOWLEDGEMENT

This work was funded by the Ministry of Higher Education Fundamental Research Grant Scheme (FRGS) (Grant No: 203/PDOPING/6711388).

REFERENCES

AHMAD, K; AZMAN, I and ESNAN, A G (2009). *Industri sawit di Malaysia*. *Perusahaan Sawit di Malaysia: Satu Panduan* (Esnan Ab Ghani and Idris Omar eds.). Third edition, MPOB, Bangi. p. 3-11.

BADECK, F-W; TCHERKEZ, G; NOGUE´S, S; PIEL, C and GHASHGHAIE, J (2005). Post-photosynthetic fractionation of stable carbon isotopes between plant organs a widespread phenomenon. *Rapid Commun. Mass Spectrom., 19*: 1381-1391.

BATHELLIER, C; TCHERKEZ, G; BLIGNY, R; GOUT, E; CORNIC, G and GHASHGHAIE, J (2008). Metabolic origin of the $\delta^{13}C$ of respired CO_2 in roots of *Phaseolus vulgaris*. *New Phytol., 181*: 387-399.

BERVEILLER, D and DAMESIN, C (2008). Carbon assimilation by tree stems: potential involvement of phosphoenolpyruvate carboxylase. *Trees, 22*: 149-157.

BERGERSEN, F J; PEOPLES, M B and TURNER, G L (1988). Isotopic discriminations during the accumulation of nitrogen by soybeans. *Aust. J. Plant Physiol., 15(3)*: 407-420.

BOWLING, D R; PATAKI, D E and RANDERSON, J T (2008). Carbon isotopes in terrestrial ecosystem pools and CO_2 fluxes. *New Phytol., 178(1)*: 24-40.

BRÜGGEMANN, N; GESSLER, A; KAYLER, Z; KEEL, S G; BADECK, F; BARTHEL, M; BOECKX, P; BUCHMANN, N; BRUGNOLI, E; ESPERSCHUTZ, J; GAVRICHKOVA, O; GHASHGHAIE, J; GOMEZ-CASANOVAS, N; KEITEL, C; KNOHL, A; KUPTZ, D; PALACIO, S; SALMON, Y; UCHIDA, Y and BAHN, M (2011). Carbon allocation and carbon isotope fluxes in the plant-soil-atmosphere continuum: a review. *Biogeosciences, 8*: 3457-3489.

CARVALHO, A L V; ALVES, B J R; BALDANI, V L D and REIS, V M (2008). Application of ^{15}N natural abundance technique for evaluating biological nitrogen fixation in oil palm ecotypes at nursery stage in pot experiments and at mature plantation sites. *Plant Soil, 302(1)*: 71-78.

CERNUSAK, L A; TCHERKEZ, G; KEITEL, C; CORNWELL, W K; SANTIAGO, L S; KNOHL, A; BARBOUR, M M; WILLIAMS, D G; REICH, P B; ELLSWORTH, D S; DAWSON, T E; GRIFFITHS, H G; FARQUHAR, G D and WRIGHT, I J (2009). Why are non-photosynthetic tissues generally ^{13}C enriched compared with leaves in C3 plants? Review and synthesis of current hypotheses. *Funct. Plant Biol., 36*: 199-213.

CHOI, W J; CHANG, S X; ALLEN, H L; KELTING, A L and RO, H M (2005). Irrigation and fertilization effects on foliar and soil carbon and nitrogen isotope ratios in a loblolly pine stand. *For. Ecol. Manage., 213(1-3)*: 90-101.

COMSTOCK, J P (2001). Steady-state isotopic fractionation in branched pathways using plant uptake of NO_3 as an example. *Planta, 214*: 220-234.

CROS, D; FLORI, A; NODICHAO, L; OMORÉ, A and NOUY, B (2013). Differential response to water balance and bunch load generates diversity of bunch production profiles among oil palm crosses (*Elaeis guineensis*). *Trop. Plant Biol., 6*: 26-36.

DAWSON, T E; MAMBELLI, S; PLAMBOECK, A. H; TEMPLER, P H and TU, K P (2002). Stable isotopes in plant ecology. *Annu. Rev. Ecol. Syst., 33*: 507-559.

DYCKMANS, J and FLESSA, H (2001). Influence of tree internal N status on uptake and translocation of C and N in beech: a dual ^{13}C and ^{15}N labeling approach. *Tree Physiol., 21*: 395-401.

EVANS, R D; BLOOM, A J; SUKRAPANNA, S S and EHLERINGER, J R (1996). Nitrogen isotope composition of tomato (*Lycopersicon esculentum* Mill. cv. T-5) grown under ammonium or nitrate nutrition. *Plant Cell Environ., 19*: 1317-1323.

EVANS, R D (2001). Physiological mechanisms influencing plant nitrogen isotope composition. *Trends Plant Sci., 6(3)*: 121-126.

GESSLER, A; BRANDES, E; BUCHMANN, N; HELLE, G; RENNENBERG, H and BARNARD, R

L (2009). Tracing carbon and oxygen isotope signals from newly assimilated sugars in the leaves to the tree-ring archive. *Plant Cell Environ., 32*: 780-795.

HOBBIE, E A and WERNER, R A (2004). Intramolecular, compound specific, and bulk carbon isotope patterns in C3 and C4 plants: a review and synthesis. *New Phytol. 161(2)*: 371-385.

HOBBIE, E A; MACKO, S A and SHUGART, H H (1999). Interpretation of nitrogen isotope signatures using the NIFTE model. *Oecologia, 120(3)*: 405-415.

KOLB, K J and EVANS, R D (2002). Implications of leaf nitrogen recycling on the nitrogen isotope composition of deciduous plant tissues. *New Phytol. 156(1)*: 57-64.

LAMADE, E; DJEGUI, N and LETERME, P (1996). Estimation of carbon allocation to the roots from soil respiration measurements of oil palm. *Plant Soil., 181*: 329-339.

LAMADE, E; SETIYO, I E; GIRARD, S and GHASHGHAIE, J (2009). Changes in 13C/12C of oil palm leaves to understand carbon use during their passage from heterotrophy to autotrophy. *Rapid Commun. Mass Spectrom., 23*: 2586-2596.

LAMADE, E; TCHERKEZ, G; DARLAN, N H; RODRIGUES, R L; FRESNEAU, C; MAUVE, C; LAMOTHE-SIBOLD, M; SKETRIENÉ, D and GHASHGHAIE, J (2016). Natural ^{13}C distribution in oil palm (*Elaeis guineensis* Jacq.) and consequences for allocation pattern. *Plant Cell Environ., 39*: 199-212.

LE ROUX, X; BARIAC, T; SINOQUET, H; GENTY, B; PIEL, C; MARIOTTI, A; GIRARDIN, C and RICHARD, P (2001). Spatial distribution of leaf water-use efficiency and carbon isotope discrimination within an isolated tree crown. *Plant Cell Environ., 24(10)*: 1021-1032.

LEGROS, S; MIALET-SERRA, I; CALIMAN, J P; SIREGAR, F A; CLEMENT-VIDAL, A and DINGKUHN, M (2009a). Phenology, growth and physiological adjustments of oil palm (*Elaeis guineensis*) to sink limitation induced by fruit pruning. *Ann. Bot., 104*: 1183-1194.

LEGROS, S; MIALET-SERRA, I; CALIMAN, J P; SIREGAR, F A; FABRE, D and DINGKUHN, M (2009b). Role of transitory carbon reserves during adjustment to climate variability and source-sink imbalances in oil palm (*Elaeis guineensis*). *Tree Physiol., 29*: 1199-1211.

LI, S-G; TSUJIMURA, M; SUGIMOTO, A; DAVAA, G; OYUNBAATAR, D and SUGITA, M (2007). Temporal variation of $\delta^{13}C$ of larch leaves from a montane boreal forest in Mongolia. *Trees, 21(4)*: 479-490.

MACKO, S A; ESTEP, M F; ENGEL, M H and HARE, P E (1986). Kinetic fractionation of nitrogen isotope during amino acid transamination. *Geochim. Cosmochim. Acta., 50*: 2143-2146.

MASCLAUX, C; VALADIER, M; BRUGIÈRE, N; MOROT-GAUDRY, J and HIREL, B (2000). Characterization of the sink/source transition in tobacco (*Nicotiana tabacum* L.) shoots in relation to nitrogen management and leaf senescence. *Planta., 211*: 510-518.

MELLING, L; GOH, K J; BEAUVAIS, C and HATANO, R (2008). Carbon flow and budget in young mature oil palm agrosystem on deep tropical peat. *The Planter, 84*: 21-28.

MPOC (2016). Malaysian Palm Oil Council. http://www.mpoc.org.my/Palm_Oil.aspx., accessed on 17 June 2016.

PASCUAL, M; LORDAN, J; VILLAR, J M; FONSECA, F and RUFAT, J (2013). Stable carbon and nitrogen isotope ratios as indicators of water status and nitrogen effects on peach trees. *Sci. Hortic., 157*: 99-107.

PROE, M F; MIDWOOD, A J and CRAIG, J (2000). Use of stable isotopes to quantify nitrogen, potassium and magnesium dynamics in young Scots pine (*Pinus sylvestris*). *New Phytol., 146*: 461-469.

RIVERA-MÉNDEZ, Y D; CHACÓN, L M; BAYONA, C J and ROMERO, H M (2012). Physiological response of oil palm interspecific hybrids (*Elaeis oleifera* H.B.K. Cortes versus *Elaeis guineensis* Jacq.) to water deficit. *Braz. J. Plant Physiol., 24*: 273-280.

ROBINSON, D; HANDLEY, L L and SCRIMGEOUR, C M (1998). A theory for $^{15}N/^{14}N$ fractionation in nitrate-grown vascular plants. *Planta, 205*: 397-406.

ROBINSON, D; HANDLEY, L L; SCRIMGEOUR, C M; GORDON, D C; FORSTER, B P and ELLIS, R P (2000). Using stable isotope natural abundances ($\delta^{15}N$ and $\delta^{13}C$) to integrate the stress responses of wild barley (*Hordeum spontaneum* C. Koch.) genotypes. *J. Exp. Bot., 51*: 41-50.

ROBINSON, D (2001). $\delta^{15}N$ as an integrator of the nitrogen cycle. *Trends Ecol. Evol., 16(3)*: 153-162.

ROWLAND, D L and LAMB, M C (2005). The effect of irrigation and genotype on carbon and nitrogen isotope composition in peanut (*Arachis hypogaea* L.) leaf tissue. *Peanut Sci., 32(1)*: 48-56.

SALMON, Y; BARNARD, R L and BUCHMAN, N (2011). Ontogeny and leaf gas exchange mediate the carbon isotopic signature of herbaceous plants. *Plant Cell Environ., 34*: 465-479.

SHEARER, G; KOHL, D H; VIRGINIA, R A; BRYAN, B A; SKEETERS, J L; NILSEN, E T; SHARIFI, M R and RUNDEL, P W (1983). Estimates of N_2-fixation from variation in the natural abundance of ^{15}N in Sonoran Desert ecosystems. *Oecologia, 56*: 365-373.

STAMATIADIS, S; CHRISTOFIDES, C; TSADILA, E; TASKOS, D; TSADILAS, C and SCHEPERS, J S (2007). Relationship of leaf stable isotopes ($\delta^{13}C$) and ($\delta^{15}N$) to biomass production in two fertilized merlot vineyards. *Am. J. Enol. Vitic., 58(1)*: 67-74.

TCHERKEZ, G; FARQUHAR, G; BADECK, F and GHASHGHAIE, J (2004). Theoretical considerations about carbon isotope distribution in glucose of C-3 plants. *Funct. Plant Biol., 31*: 857-877.

TERWILLIGER, V J; KITAJIMA, K; LE ROUX-SWARTHOUT, D J; MULKEY, S and WRIGHT, S J (2001). Intrinsic water-use efficiency and heterotrophic investment in tropical leaf growth of two Neotropical pioneer tree species as estimated from $\delta^{13}C$ values. *New Phytol., 152(2)*: 267-281.

WERNER, R A and SCHMIDT, H-L (2002). The in vivo nitrogen isotope discrimination among organic plant compounds. *Phytochemistry, 61*: 465-484.

WERTH, M; MEHLTRETER, K; BRIONES, O and KAZDA, M (2015). Stable carbon and nitrogen isotope compositions change with leaf age in two mangrove ferns. *Flora, 210*: 80-86.

YANG, Y; SIEGWOLF, R T W and KÖRNER, C (2015). Species specific and environment induced variation of $\delta^{13}C$ and $\delta^{15}N$ in alpine plants. *Front. Plant Sci., 6*: 423. DOI: 10.3389/fpls.2015.00423.

YONEYAMA, T and KANEKO, A (1989). Variations in the natural abundance of ^{15}N in nitrogenous fractions of Komatsuna plants supplied with nitrate. *Plant Cell Physiol., 30*: 957-962.

YONEYAMA, T; KAMACHI, K; YAMAYA, T and MAE, T (1993). Fractionation of nitrogen isotopes by glutamine synthetase isolated from spinach leaves. *Plant Cell Physiol., 34(3)*: 489-491.

YONEYAMA, T; HANDLEY, L L; SCRIMGEOUR, C M; FISHER, D B and RAVEN, J A (1997). Variations of the natural abundances of nitrogen and carbon isotopes in *Triticum aestivum*, with special reference to phloem and xylem exudates. *New Phytol., 137*: 205-213.

YONEYAMA, T; FUJIHARA, S and YAGI, K (1998). Natural abundance of ^{15}N in amino acids and polyamines from leguminous nodules: unique ^{15}N enrichment in homospermidine. *J. Exp. Bot., 49(320)*: 521-526.

YONEYAMA, T; FUJIWARA, H and ENGELAAR, W M (2000). Weather and nodule mediated variations in $\delta^{13}C$ and $\delta^{15}N$ values in field-grown soybean (*Glycine max* L.) with special interest in the analyses of xylem fluids. *J. Exp. Bot., 51(344)*: 559-566.

YONEYAMA, T; ITO, O and ENGELAAR, W M (2003). Uptake, metabolism and distribution of nitrogen in crop plants traced by enriched and natural ^{15}N: progress over the last 30 years. *Phytochem. Rev., 2(1)*: 121-132.