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Full Length Article

Selection of Oil Palm Materials with Higher Water Use Efficiency using Carbon Isotope Discrimination

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Abstract

Carbon isotope discrimination (Δ) is a useful tool for screening planting material with higher water use efficiency (WUE). Growth, Δ and leaf gas exchange measurements were compared between five year-old clonal (PL68, PL104, PL106 and PL127) and commercial, tenera, Dura × Pisifera (D × P) palms grown on peat. Leaf area index (LAI) and standing biomass in clone PL104 palms were greater by 35% and 54%, respectively than D× P palms. Leaf gas exchange parameters such as photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (T_r), were lower in clonal palms than D × P palms due to low soil moisture content especially in August 2012. However, there were no significant difference in P_N and g_s between planting materials in February 2013. Subsequently, higher instantaneous WUE was found in clone PL106 palms with lower T_r, g_s and intercellular CO_2 concentration (c_i) in February 2013. Clone PL106 palms had higher leaf Δ values (5.04%) than D×P palms. The Δ in leaf tissues was positively correlated with WUE. Clone with a higher WUE had the greater leaf Δ value. In contrast, leaf Δ was negatively correlated with the T_r, g_s and ratio of intercellular CO_2 concentration and ambient CO_2 concentration (c_i/c_a). Our study suggested that selection for high WUE planting materials using Δ technique to provide greater value in oil palm breeding programs. PL106 was the most water use efficient material with better growth performance and could be a good potential planting material for areas prone to drought.

Keywords: Carbon isotope composition; Leaf gas exchange; *Elaeis guineensis*

Introduction

Oil palm (*Elaeis guineensis Jacq*.) is the world's largest oil producing crop and contributes 35.1% of the global vegetable oil output in 2015. Malaysia is one of the largest palm oil producers after Indonesia, with its production in 2015 standing at 19.96 million tonnes or 31.5% of the world's total palm oil production (MPOB, 2016). High yielding oil palm ensures the crop's sustainablity. Thus, crop improvement in oil palm can be achieved through clonal propagation. In Malaysia, oil palm clonal propagation is a technology with commercial output of clonal plantlets of about 5–10% from the annual oil palm planting material requirement (Soh, 2012). Moreover, mass clonal propagation is an inexpensive method to multiply the best genetic stock and can be used much faster (Denis, 2007). The demand for clonal palms is increasing due to increasing replanting and need for high yielding clones (Mohd Roslan *et al*., 2010).

Oil palm is a perennial and monocotyledonous crop having a single woody stem without secondary growth (Corley, 1977). Its vegetative growth and development are continuous and constant under favourable conditions, despite strong seasonal variations of reproductive growth (Corley and Tinker, 2003; Henson, 2007). One of the climatic elements that affect growth and yield of oil palm is rainfall (Lim *et al*., 2008). Oil palm requires even distribution of rainfall throughout the year. The impacts of climate change due to changes of rainfall will have adverse effects on rain fed crops, such as oil palm (Seng and Hasan, 2009). The adverse impact of climate change on agriculture, can be reduced through adaptation measure considered a vital component of any policy response to climate change (Brooks and Adger, 2005; Deressa *et al*., 2009; Gbetibouo, 2009). The selection of crop with high water use efficiency (WUE) traits would be one of the adaptation measures for climate change. Therefore, there is a pressing need to improve WUE of oil palm varieties in a sustainable manner and environmentally friendly methods. Under the Malaysian Second National Communication (2011), it was reported that oil palm breeding programmes are needed to develop new varieties of oil palm with high WUE traits.Water use efficiency can be defined as plant dry matter production per unit of water loss at the whole-plant level and at the

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leaf level, WUE is defined as instantaneous ratio between photosynthesis and stomatal conductance to water vapour (Farquhar *et al*., 1989).

Plant water uptake under limited water supply conditions could be improved through agronomic management and breeding program with different approaches including physiology and molecular marker approaches (Brito *et al*., 2014).

Breeding programs for long-term agricultural sustainability by improving water use efficiency in all crops play a crucial role in the future when faced with increasing demand for water supply and global climate change (Cattivelli *et al.*, 2008; Mohgaddam *et al.*, 2013). However, selection of high yielding crops grown under unfavorable condition with high WUE trait using molecular approach is time consuming (Brito *et al.*, 2014). Selection of high WUE plant materials could be done at the leaf level using photosynthetic gas exchange but this method is expensive in capital equipment; requires more than one gas exchange equipment for measurement, laborious and unreliable method for long-term WUE (Johnson and Rumbaugh, 1995; Canavar *et al.*, 2014). Carbon isotope discrimination (Δ) is an indirect technique for selecting high WUE in C3 plants like oil palm. Carbon isotope discrimination could reduce measurement time required by conventional breeding for selecting high yielding crops in water-deficient plants.It has been used widely on other crops such as alfalfa, wheat, tomatoes and cocoa (Carelli *et al*., 1999; Erice *et al*., 2011; Mohgaddam *et al*., 2013; Wei *et al*., 2016).

The effects of climate change on physiological factors such as P_N and g_s will be reflected in the plant tissues. Leaf Δ has been used as an integrated measure of photosynthetic gas exchange to evaluate changes in environmental conditions (Farquhar *et al*., 1982; Meinzer *et al*., 1990; Zimmerman and Ehleringer, 1990). Leaf Δ was found to be strongly correlated with WUE in many crops (Meinzer *et al*., 1990; Zimmerman and Ehleringer, 1990; Brito *et al*., 2014). The relationship between Δ and WUE is incorporated with intercellular CO2 concentration (ci) (Farquhar *et al*., 1989). Although many studies reported that Δ has been suggested as a tool for high WUE in many crop species, but there is no information available for oil palm.

Therefore, this study was carried out to evaluate the growth, gas exchange and relationship between leaf Δ and WUE in different clonal palms and D × P palms as well as to identify oil palm planting material with better growth characteristics and high water use efficiency using leaf Δ.

Materials and Methods

Experimental Site and Planting Materials

This experiment was conducted at an oil palm plantation in Teluk Intan, Perak, Peninsula Malaysia. The plantation was planted with second generation oil palm on shallow and compacted peat, which was classified as part of the Penor series with sub-group level as Terric Sulfisaprist in the keys to soil taxanomy, 11^{th} Edition,or as *Sapric Topogambrist* in the Malaysian Soil Taxanomy (Paramanathan, 2012). The planting materials were used in this experiment consisted of one commercial tenera, (Dura × Pisifera) (D × P) and four clonal (i.e., PL68, PL104, PL106 and PL127) palms. Clonal palm seedlings were grown from tissue culture by the Breeding and Tissue Culture Unit, Malaysian Palm Oil Board, and planted in December 2007 at a density of 136 ha^{-1}. Each planting material was replicated by three palms. The study was initiated in February 2012 and continued till February 2013 when the palms were about five-years old.

Meteorological Condition

Meteorological conditions during the experiment were recorded at hourly intervals by an automated weather station situated in an open area in the plantation. Monthly rainfall from January 2012 until February 2013 and mean daily changes of air temperature and VPD were taken from 9:00–13:00 h are given in Fig. 1.

Soil Condition

Soil temperature and moisture were measured at a depth of 12 cm at three different locations near the palm base which averaged for each palm. A digital soil thermometer was used to measure soil temperature and soil moisture content by a soil moisture meter (Hydrosense, Campbell Scientific, USA).

Plant Growth

Growth parameters including palm height and total number of green fronds according to the standard procedures as described by Corley and Breure (1981) were recorded. The palm height measurement was taken from the base of the frond 33 to the ground level.

Other growth parameters such as total leaf area was measured at the end of the experimental period in February 2013 using non-destructive method (Fairhurst and Hardter, 2003). The amount of leaf area in a canopy could be expressed by determining the leaf area index (LAI). The LAI measures the total one-sided (or half of the total two-sided) green leaf area per unit toground surface area (Corley and Breure, 1981). It is defined as leaf area that interacted with solar radiation and responsible for carbon absorption and exchange with the atmosphere (Mohd Roslan, 2006). The LAI of palms were measured according to the following formula:

$$LAI \frac{= total\ leaf\ area\ per\ palm\ (m^2)\ \times\ planting\ density\ (palms\ ha^{-1})}{10\ 000}$$

Where, leaf area is based on vegetative measurements using frond 17.

Oil Palm Standing Biomass

The oil palm standing biomass was determined from the combination of fronds and trunk dry weight using non-destructive methods. Frond dry matter production is the product of frond production and mean weight of the fronds. The total dry weight of fronds was estimated by multiplying the total number of green fronds and mean frond dry weight. The weight of the trunk was estimated from measurements of trunk increment, trunk diameter and an estimate of dry matter per unit trunk volume (Corley and Breure, 1981).

Leaf Gas Exchange

Leaf gas exchange parameters such as photosynthetic rate (P_N), stomatal conductance (g_s), transpiration rate (T_r) and intercelullar CO_2 concentration (c_i) were measured on intact leaflets attached to frond 17 with a portable photosynthesis system (LI-6400XT, Li-COR, Inc., USA). Conditions inside the leaf cuvette were selected as follows: ambient temperature, ambient humidity, photosynthetically active radiation (PAR) of 1000 μmol photon $m^{-2} s^{-1}$ (red/blue light source), and reference CO_2 of 400 μmol mol^{-1} air. Instantaneous water use efficiency (WUE), the ratio of intercellular CO_2 and ambient CO_2 concentrations (c_i/c_a) were calculated as P_N/T_r and c_i/c_a, respectively. Photosynthetic gas exchange characteristics were determined from the five planting materials and measurements were taken at 3 month intervals over a 13 month period.

Leaf Relative Chlorophyll Content

Relative chlorophyll content was measured on the same intact leaflets attached to frond 17 used for gas exchange measurement. The measurement was done using a portable chlorophyll meter (SPAD 502, Minolta Camera Co., Osaka, Japan).

Carbon Isotope Discrimination

Carbon isotope ratios ($\delta^{13}C$) were determined from the leaflets of frond 17. The leaves samples were taken in February 2013. Samples were oven dried at 70°C for 48 h and grounded to fine powder. Carbon isotope composition was determined with the use of an Isotopic Ratio Mass Spectrometer at the Malaysian Institute of Nuclear Technology (MINT). Results were expressed as $\delta^{13}C$ (‰) using the equation given below (Farquhar *et al*., 1989):

$$\delta^{13} C = \left[\frac{R_P}{R_S} - 1\right] \times 1000 ‰$$

Where R_p is the $^{13}C/^{12}C$ ratio measured in the planting material and R_s is the $^{13}C/^{12}C$ ratio in the standard. The unit is expressed as part per thousand (‰). A secondary standard calibrated against Pee Dee Belemnite (PDB) carbonate was used for comparison. The following formula was used to calculate Δ value (Farquhar *et al*., 1989):

$$\Delta (‰) = \frac{\delta_a - \delta_P}{1 - \delta_P} \times 1000$$

Where, δ_p is the $\delta^{13}C$ of the sample and δ_a is the $\delta^{13}C$ of atmospheric CO_2. On the PDB scale, atmospheric CO_2 has a current deviation of approximately −8‰ (Farquhar *et al*., 1989).

Statistical Analysis

Results were statistically analyzed as a randomized complete block design with three replications. The influence of different planting materials was analyzed using analysis of variance. Regression analysis was used to determine the relationships between leaf Δ with WUE and gas exchange between different oil palm planting materials. The comparison of means was done using Tukey's method at P<0.05. All analysis were performed using the Statistical Analysis System (SAS) version 9.0.

Results

Soil Moisture Content and Soil Temperature

Seasonal changes influenced the soil moisture content and soil temperature of planting material grown on peat (Fig. 2). There was no significant difference between planting material and soil moisture in February and May 2012. However, the soil moisture was significantly different between planting materials on August and November 2012, and February 2013. The highest soil moisture content throughout the measurement period was recorded from clonal palm PL104 (61.7%) and the lowest (28.89%) was clonal palm PL106. The soil temperature was significantly different between planting materials, but the soil temperature was not significantly different between measurement times. The minimum and maximum soil temperature are 26.15 and 28.98°C, respectively.

Growth and Dry Matter Production

Growth characteristics of 5-year old oil palm such as leaf area index (LAI), total leaf area and standing biomass were significantly ($P \leq 0.05$) affected by the different planting materials measured in February 2013 (Table 1 and 2). The LAI in PL104 palm was significantly greater than D × P palm and other planting materials, but significantly different with PL106 palm. The LAI in PL127 palm was significantly lower than the other planting materials. The total leaf area was significantly greater (35%) in clone PL104 palms than D × P palms. However, there was no significant difference in frond number between planting materials.

PL104 had significantly higher frond dry weight, trunk dry weight and standing biomass of about 42, 80 and 54% respectively, than D × P palm.

Table 1: Growth characteristics of five year-old oil palm planting materials.Distinct letters in the row indicate significant differences according to Tukey’s test ($P≤0.05$)

Planting material	Leaf area index	Green Frond (no/palm)	Total leaf area (m^2/palm)	Palm Height (cm/palm)
D × P	4.58bc	38 a	285.99 bc	156.33 a
PL68	4.32bc	33 a	270.12 bc	142.67 a
PL104	6.02a	40 a	387.28 a	168.00 a
PL106	5.38ab	36 a	336.02 ab	156.33 a
PL127	3.61c	38 a	225.60 c	91.00 b

Table 2: Dry matter production of five year-old oil palm planting materials. Distinct letters in the row indicate significant differences according to Tukey’s test ($P≤0.05$)

Planting material	Frond dry weight (kg/palm)	Trunk dry weight (kg/palm)	Standing biomass (kg/palm)
D × P	103.85 ab	56.72 b	160.57 bc
PL68	79.62 b	50.61 b	130.23 c
PL104	128.22 a	98.64 a	226.84 a
PL106	144.61 a	68.31 ab	212.92 ab
PL127	79.05 b	40.27 b	119.31 c

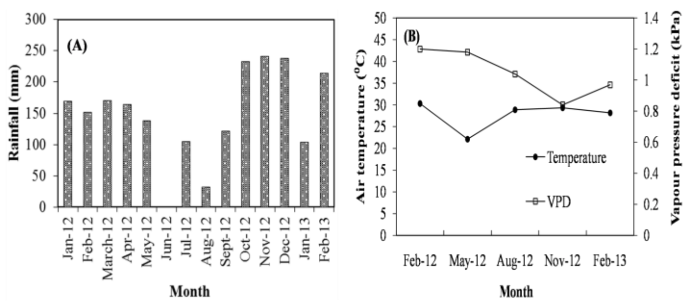

Fig. 1: (A) Monthly rainfall and mean (B) daily changes of air temperature and vapor pressure deficit

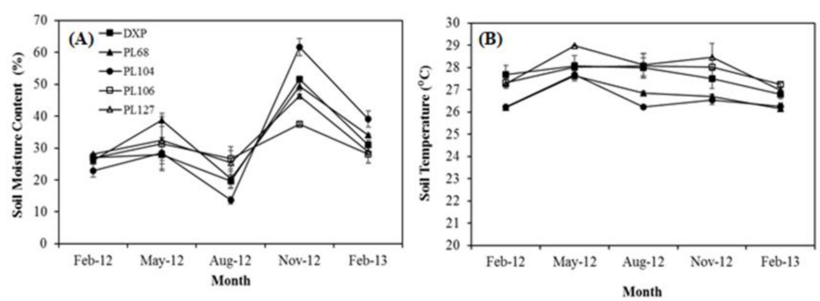

Fig. 2: Seasonal changes of (A) soil moisture content and (B) soil temperature of different oil palm planting materials throughout the growing season. Vertical bars represent the standard error of means

However, the dry matter production such as frond and trunk dry weights and standing biomass between PL104 and PL 106 palms were not significantly different (Table 2).

Gas Exchange and Leaf Characteristic:

The P_N was significantly different ($P≤ 0.05$) between planting materials, but was not significantly different between planting materials only in February 2013. The D × P palm had the highest P_N in February, August and November 2012 compared to the other planting materials. However, P_N in PL127 was higher than D × P palm in May 2012. Intercellular CO_2 concentration between planting materials was significantly different in May 2012 and February 2013 (Fig. 3B). The c_i in the PL127 palm (348.66 μmol m^{-2} s^{-1}) was higher in May 2012, but was not significantly different between PL68 (343.27 μmol m^{-2} s^{-1}), PL106 (324.06 μmol m^{-2} s^{-1}) and D × P (335.99 μmol m^{-2} s^{-1}) in February 2013 (Fig. 3A).

The g_s was significantly different ($P≤ 0.05$) between planting materials in February, May and August 2012 (Fig. 3C). Meanwhile, T_r was significantly influenced by the planting materials throughout the year, except in November 2012 (Fig. 3D). Both g_s and T_r were higher in D × P palms in February and August 2012.

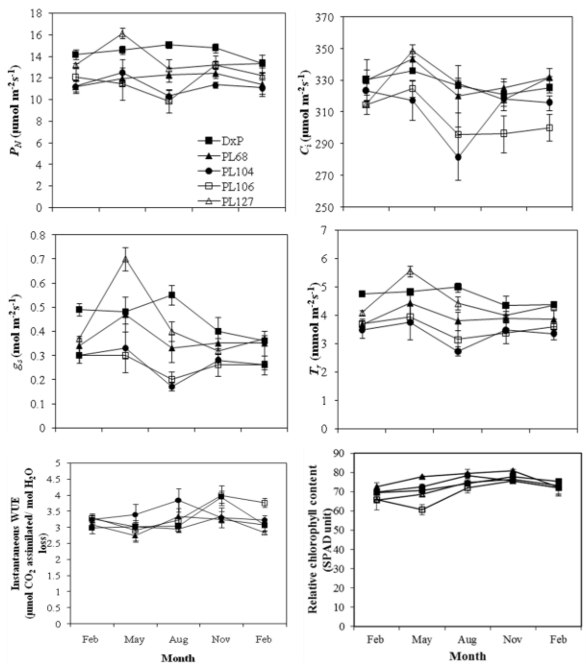

Fig. 3: Gas exchange parameters and chlorophyll content of different oil palm planting materials throughout the growing season; Photosynthetic rate, P_N (A), Intercellular CO_2 concentration, c_i (B), Stomatal conductance, g_s (C), Transpiration rate, T_r (D), Instantaneous water use efficiency, WUE (E) and Relative chlorophyll content (F). Verticals bar represent the standard error of means

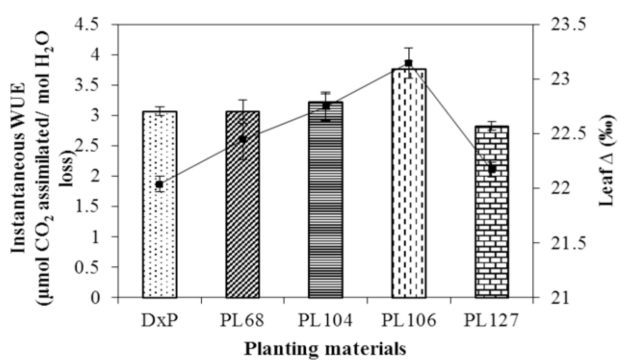

Fig. 4: Instantaneous water use efficiency (bar chart) and leaf carbon isotope discrimination (line chart) of different oil palm planting materials in February 2013. Verticals bar represent the standard error of means

In general, seasonal gas exchange pattern in D × P palms was higher than in clonal palms throughout the year (Fig. 3). There was slightly decrease in PN, gs and ci during months in clonal palms with low rainfall (Fig. 1A) and soil moisture content (Fig. 2A) with high VPD (Fig. 1B) in August 2012. However, in November 2012, the gas exchange parameters were slightly increased in clonal palms during the monthly rainfall and soil moisture content both had high and low VPD.

Instantaneous water use efficiency and relative chlorophyll content between planting materials were significantly different in February 2013 (Fig. 3E and Fig. 3F). The WUE in clone PL106 was about 22% higher than D × P palm. However, WUE between clones PL106 and PL104 were not significantly different. The high relative chlorophyll content was from clone PL68 palm with a value of 77.87, while therelative chlorophyll content was 10% greater in PL68 than D × P palm. Therefore, relative chlorophyll content between clones PL68 and PL104 palm were not significantly different.

Carbon Isotope Discrimination

The WUE and leaf Δ values in February 2013 were significant ($P \leq 0.05$) between planting materials (Fig. 4). The PL106 clone had the highest mean leaf Δ value (23.15‰) and D × P palm the lowest (22.04‰). The relationship between WUE and leaf Δ of five oil palm planting materials in February 2013 was a positive quadratic (Fig. 5). There was a significant correlation between leaf Δ, T_r (Fig. 6), g_s (Fig. 7) and c_i/c_a (Fig. 9). However, leaf Δ and P_N was not significant (Fig. 8).

Discussion

The LAI of oil palm was estimated based on leaf area, the number of green leaves and planting density (Gerritsma and Soebagyo, 1999; Corley and Tinker, 2015). The LAI in clone PL104 was greater than D × P palm. The higher LAI was largely due to the higher leaf area of the mature opened leaf (Table 1). The leaf area is determined by the leafiness and photosynthetic surface of crops and it depends on the leaf growth, number of leaves, plant density and leaf senescence (Khan, 1981). However, frond number had no influence for the higher LAI in PL104 and PL106 palms since there was no significant difference in frond number between planting materials.

Frond and trunk dry matter in clone PL104 palm was greater than D × P palm. This indicated that the higher dried weight might be due to the higher accumulation of dry matter in the leaf, petiole and also in the trunk. Higher standing biomass of oil palm planting materials in clone PL104 might be contributed by the higher dry matter accumulation from the trunk and greater palm height. Corley and Tinker (2015) reported that the main standing biomass accumulation is from the trunk. This result was consistent with a previous study done by Khalid *et al*. (1999) on mature oil palm where the weight of oil palm was significantly correlated with the palm height. Frond dry mass is also one of the factors of higher standing biomass in the clone PL104 since leaf bases were included in the standing biomass (Corley and Tinker, 2015).

The planting materials had a significant influence on the photosynthetic rate of palm. The higher P_N in D × P palms was related to higher g_s value.

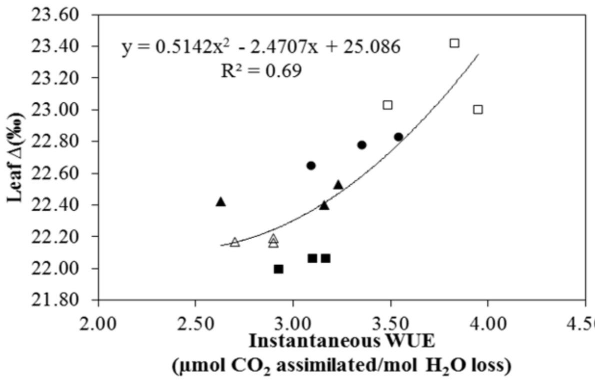

Fig. 5: Relationship between instantaneous water use efficiency and leaf carbon isotope discrimination of different oil palm planting materials measured in February 2013. Symbol ■ represents the D × P palm, ▲represents the PL68 palm, ● represents the PL104 palm, □ represents PL106 palm and Δ represents PL127 palm

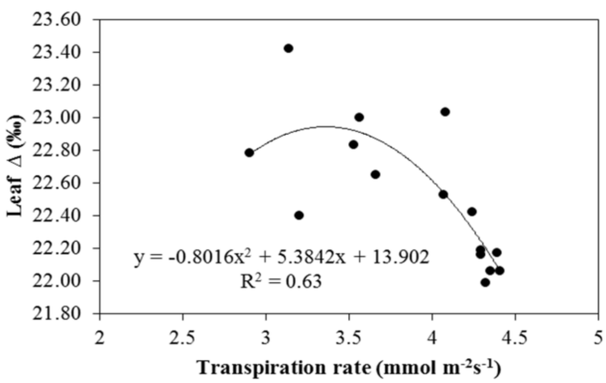

Fig. 6: Relationship between leaf carbon isotope discrimination and transpiration rate measured in February 2013

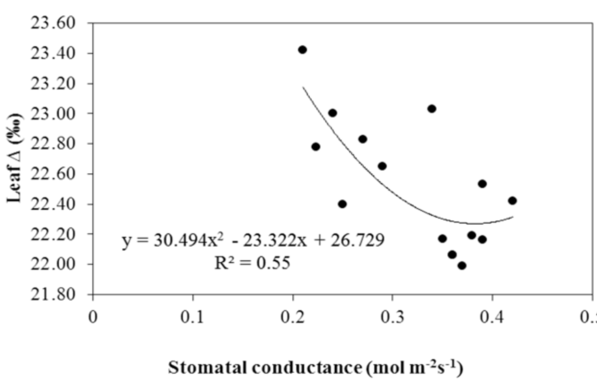

Fig. 7: Relationship between leaf carbon isotope discrimination and stomatal conductance measured in February 2013

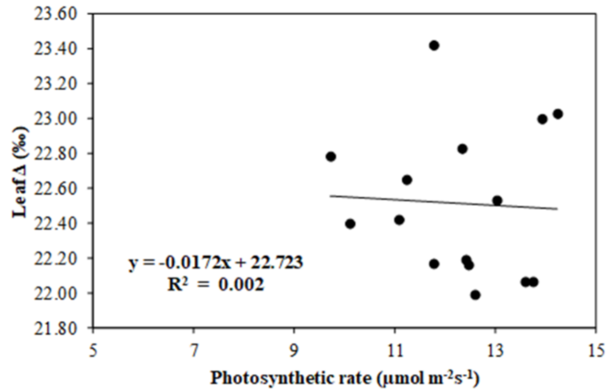

Fig. 8: Relationship between leaf carbon isotope discrimination and photosynthetic rate measured in February 2013.

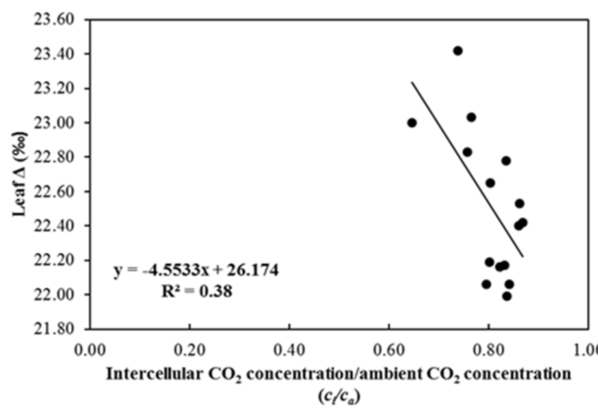

Fig. 9: Relationship between leaf carbon isotope discrimination and ratio of intercellular CO_2 concentration and ambient CO_2 concentration measured in February 2013

The D × P palms tend to sustain the P_N and g_s during higher and lower rainfall distribution in February (152.1 mm) and August 2012 (31 mm) respectively.

In general, P_N, g_s and T_r pattern in all clonal palms throughout the period were lower than D × P palm. The seasonal gas exchange pattern such as P_N, g_s and c_i in all clonal palms were more sensitive to the changes in soil moisture in August than in November due to low soil moisture content during the dry period. This result is consistent with the finding in natural holk oak species (Asensio *et al*., 2007) that soil moisture was one of the main factors driving CO_2 gas exchange in oak trees.

Moving on, the higher WUE in clone PL106 indicated that it is the best water saver. This might be attributed to the lower T_r, g_s and c_i values. Farquhar *et al*. (1989) suggested WUE was negatively correlated with c_i. A similar study in *Phaseolus vulgaris. L* found that WUE could be improved either through lowering g_s, raising photosynthetic capacity or both (Guo *et al*., 2006).

The relationship between WUE and leaf Δ of the different planting materials was positively quadratic (Fig. 5). This indicated that palm with higher WUE had greater leaf Δ value. Leaf Δ was found to be negatively correlated with T_r and g_s (Fig. 6 and Fig. 7). The result was inconsistent with findings by Zhang *et al*. (1994) in a forest tree, Monneveux *et al*. (2006) in durum wheat, and Yasir *et al*. (2013) in bread wheat cultivars. They reported that WUE had a strong negative correlation with leaf Δ. This might arise because of the negative association between leaf Δ and c_i/c_a (Fig. 8). It was contracted with the principle of Farquhar *et al*. (1982), Farquhar and Richards (1984) that leaf Δ positively correlated with c_i/c_a and negatively with WUE. Farquhar *et al*. (1989), Condon *et al*. (2002) had reported lower g_s or greater photosynthetic capacity or a combination of both factors leading to lower c_i/c_a and hence lower Δ. In this study, Δ is might be driven by gs than by photosynthetic rate. The strong association between Δ and g_s could be explained by the fact it strongly depended on g_s. This is similar with the principle findings by Morgan *et al*. (1993) in wheat.

Conclusion

Clone PL104 and PL106 palms exhibited better growth and dry matter characteristics such as LAI, frond and trunk weights and standing biomass. Carbon isotope discrimination was found to be a suitable tool for screening oil palm planting materials for WUE. The Δ value in palm leaf tissue was positively correlated with WUE. Higher WUE palm was associated with increased in leaf Δ value. Consequently, the clone PL106 had the highest value in WUE, leaf Δ and better growth performance, thus may have good potential planting material for areas prone to drought.

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