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Effect of fertilizer application on photosynthesis and oil yield of *Jatropha curcas* L.

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Abstract

The use of *Jatropha curcas* oil as a source of biofuel has been well-explored. However, the physiological and growth studies of *J. curcas* have received considerably lesser attention. In this study, leaf gas exchange measurements and leaf nitrogen content were determined for four varieties of *J. curcas*, grown in the field or in pots. Based on stable carbon isotope analysis (δ^{13}C) and gas-exchange studies, *J. curcas* is a C_3 sun plant and the range of leaf photosynthetic rates (or CO_2 assimilation rates, P_{Nmax}) were typically between 7 and 25 µmol(CO_2) m^{-2} s^{-1} and light saturation generally occurred beyond 800 µmol(quanta) m^{-2} s^{-1}. Higher rates of leaf photosynthesis were generally obtained with the mature leaves. In addition, increased foliar P_{Nmax} were recorded in potted *J. curcas* variety Indiana with increasing nitrogen (N) nutrition levels. These plants also showed greater growth, increased leaf N content, higher maximum CO_2 assimilation capacity ($P_{NhighCO2}$) and chlorophyll (Chl) content, indicating the potential of optimizing the growth of *Jatropha* by varying fertilizer nutrient levels. A rapid assessment for leaf N using a nondestructive and portable Chl meter had been established for *J. curcas*. This approach will allow repeated sampling of the same plant over time and thus enable the monitoring of the appropriate levels of soil fertility to achieve good *Jatropha* plantation productivity. High N nutrition improved the overall plant oil yield by increasing the total number of fruits/seeds produced per plant, while not affecting the intrinsic seed oil content.

Additional key words: biofuel; chlorophyll meter; *Jatropha curcas* L.; leaf nitrogen content; oil; photosynthetic rates; stable isotope.

Introduction

In recent years, the depletion of fossil fuel reserves, increasing and unstable prices of crude oil and environmental concerns have hasten the commercial exploration of using vegetable oils as alternative fuels. Biodiesel is made from renewable sources such as vegetable oil, and consists of the simple alkyl esters of fatty acids (Berchmans and Hirata 2008). Biodiesels, derived from either edible or inedible oils, have been increasingly widely used as an alternative to fossil fuels. Plant species that produce edible oil includes oil palm (*Elaeis guineensis*), canola (*Brassica napus*), Chinese pistache (*Pistacia chinensis*), yellow horn (*Xanthoceras sorbifolium*) and quandong (*Santalum acuminatum*), while inedible species comprise castor bean (*Ricinus communis*), Indian beech (*Pongamia pinnata*), and physic nut (*Jatropha curcas*) (Li *et al.* 2007, Fu *et al.* 2008, Gui *et al.* 2008, Scholz and da Silva 2008, Scott *et al.* 2008, Carels 2009, King *et al.* 2009, Murugesan *et al.* 2009, Ye *et al.* 2009). However, it is believed that large-scale production of biodiesel from edible oils may bring about global imbalance to the food supply and demand market. Arable land that would otherwise have been used to grow food would instead be used to grow biofuel plants, reducing food supply and causing food price to rise.

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Abbreviations: CCI – chlorophyll content index; CCM – chlorophyll content meter; Chl – chlorophyll; DMF – dimethylformamide; E – transpiration rates of leaves; g_w – leaf stomatal conductances to water; MAT – months after treatment; PFD – photon flux density; $P_{NhighCO2}$ – maximum CO_2 assimilation capacity; P_{Nmax} – CO_2 assimilation rate; T_{60} – treatment with 60 g of fertilizer; T_{120} – treatment with 120 g of fertilizer; T_{240} – treatment with 240 g of fertilizer.

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Hence, an inedible biofuel species, such as *Jatropha curcas* L., will be a better candidate to address this ongoing debate (Wood 2005, anonymous 2007).

Although *J. curcas* plants can survive on marginal land with minimal water requirements, it is not clear whether the plant productivity will still remain high under suboptimal growth conditions (Weyerhaeuser *et al.* 2007, Hoekstra *et al.* 2009, Jongschaap *et al.* 2009). Moreover, the current agricultural output of biofuels is not sufficient to replace fossil fuels completely due to the growing demand for fuel energy (Ruth 2008). Many countries have plans to increase their coverage of *Jatropha* biofuel feedstock plantation. One example is in southwest China where the current 70,000 ha of *Jatropha* plantation lands are being expanded to over one million ha (Weyerhaeuser *et al.* 2007). However, increasing the land acreage of *Jatropha* plantations without enhancing plant productivity will not improve the cost competitiveness of the *Jatropha* biodiesel industry. Thus, there is an urgent need to improve *Jatropha* plantation productivity.

Plant growth and productivity can be increased through fertilizer management. Detailed studies on how fertilizer application affects plant physiology and improve crop yield have been carried out for many commercial crops such as corn, wheat and rice (Scharf *et al.* 2002, Jiang *et al.* 2004, Stewart *et al.* 2005). However, information on *J. curcas* physiology and its response to a nutrient supplement in this context was generally lacking (Openshaw 2000). The issue on how to strike a fine balance between the utilization of marginal lands and obtaining satisfying yields through fertilization management is the key guiding principle to develop a sustainable and more efficient renewable biofuel energy source. Hence, research into understanding the basic physiology and plant productivity of *J. curcas* is much needed.

In this study, the various plant physiological parameters (leaf gas exchange, leaf Chl content, leaf N levels) of four varieties of *J. curcas* were examined, in order to understand its basic physiology under different fertility treatments. The $^{13}C/^{12}C$ isotopic content of *J. curcas* leaves was acquired to determine the type of carbon fixation pathway during leaf photosynthesis. This study also investigated the feasibility of using a hand-held assessment instrument as a rapid assessment tool to assess the physiological status of *J. curcas* plants. At the same time, *J. curcas* seed oil content data were evaluated to see whether there is a correlation between photosynthetic rates and seed oil content among different *Jatropha* varieties and whether different soil N supplement levels can affect fruiting and/or seed oil content.

Materials and methods

Plant materials: Four varieties of *J. curcas*: Indiana, Marilyn, Rachel, and Guangxi were grown from seeds obtained from their respective mother stock plants grown in different countries (India, Cambodia and China). They were transplanted to a field plot in Singapore for experimentation. The plot was on a hill, with about 30° slope. The average temperature during the experimental period was about 28°C with maximum irradiation of 2,200 μmol(quanta) $m^{-2} s^{-1}$. Plants were well maintained and regularly weeded once a week. No extra watering was supplied and plants received water from the regular rains during the period of experimentation. These field-grown plants were supplemented with 60 g of slow-release fertilizer (*Osmocote® Plus*, Australia; N:P:K ratio = 18:5:8) once a month. Measurements of the various parameters were taken after three to four months of growth.

One-month-old healthy *J. curcas* var. Indiana seedlings (with two adult leaves and two seed leaves) were repotted into large pots (soil volume 8 l, containing 2:1 soil-sand mixtures) and treated with three different fertilizer treatments: 60 g (T_{60}), 120 g (T_{120}), and 240 g (T_{240}) of *Osmocote® Plus*. Each treatment had ten replicate plants. There were ten other control plants without application of fertilizer (T_0). Fertilizer was applied once every month. Measurements of the various parameters were carried out 2 months after treatment (MAT), except leaf N content which was measured 5 MAT. The plants (four-month-old) were repotted into 35-l potting bags and detopped at 30 cm from the root-shoot junction (control plants were detopped at 15 cm to 20 cm as they were shorter) 3 MAT to allow branching. Photosynthetic rates and Chl content index (CCI) were continually monitored in 5, 7, and 9 MAT. Leaf stable carbon isotopic analysis was carried out using leaves of T_{60} plants harvested in 5 MAT (the same leaves which were subjected to gas-exchange measurements). Plants started to flower from 6 MAT and fruits were collected about two months later when ripen.

Leaves were classified as young, mature, or old leaves depending on their position along the shoot axis of the plant. Excluding the first few very young, rapidly expanding and delicate soft leaves, the three upper-most expanding (beyond 50% of maximum leaf area) leaves were categorized as “young” leaves; the three bottom-most leaves were deemed to be “old” leaves, and the three leaves along the mid-point of the shoot axis were considered to be “mature” leaves.

Field plot measurements were carried out using three replicate plants (three young, three mature, and three old leaves per plant) per var. of *J. curcas.* For the nutrient treatment experiments, two to five healthiest plants (two mature leaves per plant) were selected from each treatment for growth-parameter measurements. The selected leaves were subjected to leaf gas-exchange measurements, Chl content determination, and leaf N content analysis. The harvesting of leaves was done within the same day after gas exchange measurements.

Gas-exchange measurements were carried out on the selected leaves (Yong *et al.* 1997, 2000), using a conventional open-system portable photosynthesis system (*LI-6400, Li-COR Inc.*, Lincoln, NE, USA) equipped with an Infra Red Gas Analyzer (*IRGA*), and a *6400-O2B LED* light source installed above the leaf chamber. Photosynthetic rates (P_{Nmax}), stomatal conductances (g_w), and transpiration rates (E) of each leaf were generally measured on sunny days, with the following parameters: T_{block} of 31–32°C, photon flux density (PFD) of 1,800 µmol(quanta) m^{-2} s^{-1}. The CO_2 concentrations were maintained between 390 and 400 µmol(CO_2) mol(air)$^{-1}$ while the relative humidity (RH) was in the range of 46 to 71%. Ten readings were taken for each leaf selected for measurements. Light-response curves were obtained by varying the light intensity between 0–1,800 µmol(quanta) m^{-2} s^{-1}, while the CO_2 concentrations were maintained between 390–400 µmol(CO_2) mol(air)$^{-1}$ and RH was in the range of 46 to 71%. The CO_2-response curves of *J. curcas* were obtained using the default "A-C_i curve" program of the *Li-6400* system by varying the CO_2 concentrations within the leaf chamber [between 50–2,200 µmol(CO_2) mol(air)$^{-1}$], whilst keeping all the other parameters constant.

Carbon isotope discrimination: For $^{13}C/^{12}C$ ratio measurements (Farquhar *et al.* 1989), whole-leaf samples, oven-dried and ground, were combusted in a Carlo Erba elemental analyser coupled to an isotope ratio mass spectrometer (*Isochrom, Micromass*, Manchester, UK). The conventional 'delta' notation of expressing the isotopic composition of a material relative to that of a standard on a per mil deviation basis was used.

Chl content (non-destructive and destructive method): Chl content of leaves was assessed using a nondestructive and a destructive (chemical extraction) method. The nondestructive measurement of the leaf Chl content was carried out using a hand-held Chl content meter (*CCM-200, Opti-Sciences*, Hudson, NH, USA) on selected leaves (van den Berg and Perkins 2004) of *J. curcas*. The purpose was to develop a rapid nondestructive Chl content assessing method for field soil nutrition management. Consequently, the Chl content index (CCI) for designated points on each leaf was obtained and a correlation analysis with the destructive Chl content method was then carried out. The same leaf lamina used earlier to obtain CCM readings were excised for destructive Chl content measurement (Wellburn 1994). The total leaf Chl contents were extracted using dimethylformamide (DMF), and the Chl concentration of the leaf extracts were determined using a *UV-VIS* spectrophotometer (*UVmini-1240, Shimadzu*, Kyoto city, Japan).

Leaf N content was determined (Yong *et al.* 2000) using an elemental analyzer (*Vario EL Elemental Analyzer*, Elementar, Germany). Accurately-weighed 5-mg ground dried leaf samples were placed in tin capsules for combustion at 1,150°C in the elemental analyzer reactor.

Seed oil content: Fresh *J. curcas* seeds were obtained from the mother stock plants of the different varieties grown in different countries as stated in plant materials and also from experimentally-grown plants treated with different N nutritions. Oil content was determined based on the protocol published by the British Standard Institution (BSI 1999). *J. curcas* seeds were considered to be medium-size seeds and as such, the steps taken to extract its seed oil were done according to Section 8.3.4 of this standard protocol. Moisture content was calculated with reference to the equations stated in the standard protocol (BSI 1995a,b). The *Jatropha* seed oil was extracted with hexane (*Sigma-Aldrich*, USA), using a Soxhlet apparatus coupled with a rotary evaporator.

Statistical analysis: One-way Analysis of Variance (*ANOVA*) was performed with the *MINITAB* software (*MINITAB Inc.*, Release 14, 2003) to determine if the biological parameters of *J. curcas* differ significantly among the varieties and among different fertilizer treatments. Subsequently, the statistical significance of the mean differences was tested by *Tukey*'s multiple range comparison tests.

Results

Leaf photosynthesis and stable carbon isotope content: In general, field-grown *J. curcas* leaf photosynthetic rates (P_{Nmax}) varies between 9 and 18 µmol(CO_2) m^{-2} s^{-1} (Table 1, Fig. 1*A*) when measured in ambient air [CO_2 level of 400 µmol(CO_2) mol(air)$^{-1}$]. Among all the varieties, mature *J. curcas* leaves generally had higher P_{Nmax} and leaf N content while older leaves had lower values (Table 1). Therefore, for the other remaining experiments, all leaf data analyses were carried out using data obtained from mature leaves unless otherwise stated.

In addition, the $P_{NhighCO2}$ values (an estimate of the maximum CO_2 assimilation capacity of leaves) among the four varieties were similar (Fig. 1*B*). The $\delta^{13}C$ ratios of the leaves of T_{60} plants were in the range of –26.1‰ to –29.2‰, which coincided with the $\delta^{13}C$ ratios of C_3 plants (O' Leary 1981, Farquhar *et al.* 1989).

Stomatal conductances and transpiration rates: This current study showed that *Jatropha* g_w had an impact on the CO_2 assimilation rates of *Jatropha* plants. Among the

Table 1. Effects of leaf age on field-grown *Jatropha curcas* leaf CO_2 assimilation rates (P_{Nmax}), leaf nitrogen (N) contents, transpiration rates (E), stomatal conductances to water (g_w), and chlorophyll (Chl) content index (CCI). All values represent the means ± standard error of 3 to 5 replicates. Values with different small letters are significantly different when compared among different varieties and leaf ages within columns ($P\leq0.05$; *Tukey*'s multiple range comparison tests). N.D. = not determined.

Variety	P_{Nmax} [$\mu mol(CO_2)\ m^{-2}\ s^{-1}$]	N [%]	E [$mmol(H_2O)\ m^{-2}\ s^{-1}$]	g_w [$mol(H_2O] m^{-2}\ s^{-1}$]	CCI
Old leaves					
Guangxi	13.5 ± 1.6^{abd}	2.3 ± 0.3^{b}	3.8 ± 0.2^{b}	0.23 ± 0.02^{b}	38.0 ± 1.4^{b}
Marilyn	14.1 ± 1.1^{ab}	3.1 ± 0.2^{c}	3.8 ± 0.3^{abd}	0.25 ± 0.04^{bcd}	26.6 ± 1.1^{ad}
Rachel	9.5 ± 0.8^{d}	2.6 ± 0.2^{d}	2.9 ± 0.2^{d}	0.16 ± 0.02^{d}	30.6 ± 1.5^{d}
Mature leaves					
Guangxi	15.5 ± 0.9^{abc}	3.9 ± 0.1^{a}	4.5 ± 0.4^{abc}	0.37 ± 0.02^{a}	27.3 ± 1.0^{ad}
Marilyn	16.6 ± 0.7^{abc}	3.5 ± 0.1^{ac}	4.9 ± 0.2^{abc}	0.34 ± 0.02^{abc}	20.3 ± 1.3^{c}
Rachel	17.8 ± 0.6^{c}	3.4 ± 0.1^{a}	5.1 ± 0.1^{c}	0.36 ± 0.02^{ac}	23.5 ± 0.6^{ac}
Indiana	16.1 ± 0.8^{abc}	N.D.	4.5 ± 0.2^{abc}	0.33 ± 0.02^{abcd}	N.D..
Young leaves					
Guangxi	15.2 ± 1.1^{abc}	N.D.	4.7 ± 0.4^{abc}	0.34 ± 0.04^{ab}	N.D.
Marilyn	N.D.	N.D.	N.D.	N.D.	N.D.
Rachel	13.8 ± 0.7^{ab}	N.D.	4.7 ± 0.2^{abc}	0.35 ± 0.03^{abc}	N.D.

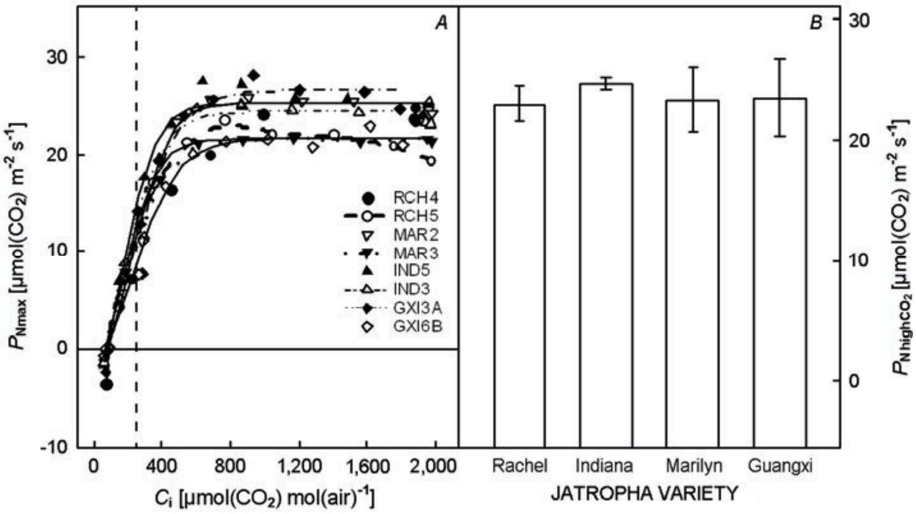

Fig. 1. *A*: The CO_2 assimilation curves of *Jatropha curcas* measured over a range of intercellular CO_2 (C_i) concentrations (*A*-C_i curves). *B*: The maximum photosynthetic capacity ($P_{NhighCO2}$) of the different varieties of *J. curcas* (RCH – Rachel, IND – Indiana, MAR – Marilyn and GX – Guangxi), responding to high CO_2. Two representative curves for each variety are presented here. *Dotted line* indicates the C_i value of 280 µmol(CO_2) mol(air)$^{-1}$ which corresponds to the ambient CO_2 of around 380–400 µmol(CO_2) mol(air)$^{-1}$. Data for maximum photosynthetic capacity in response to high CO_2 were the means ± standard error of the 2 replicate plants.

different varieties, P_{Nmax} and g_w were shown to be strongly correlated (R^2 = 0.82, Fig. 2) for field-grown plants. Our findings for *J. curcas* were similar to the findings of Wong *et al.* (1979). For the measurement of the potted *Jatropha* plants taken 2 MAT (8-l pot), P_{Nmax} of leaves generally increased with increasing nutrient levels (Fig. 3*A*, *left panel*) while g_w (Fig. 3*B*, *left panel*) and the transpiration rates (E) of leaves (Fig. 3*C*, *left panel*) showed smaller increases. In addition, plants grown in the large pots (T_{60} treatment) had similar P_{Nmax}, g_w and E values when compared to those in the field plot under the same level of the nutrient supplement (Fig. 3, *right panel*).

CCI: The results from this study (Fig. 4) showed that the obtained CCI values were significantly correlated with the actual foliar Chl content and leaf N level of the plants under fertilizer treatments (R^2 = 0.95 and 0.87, respectively, $P\leq0.05$), implying that CCI, obtained *via* nondestructive method, is an effective estimate of the actual values of the two physiological parameters.

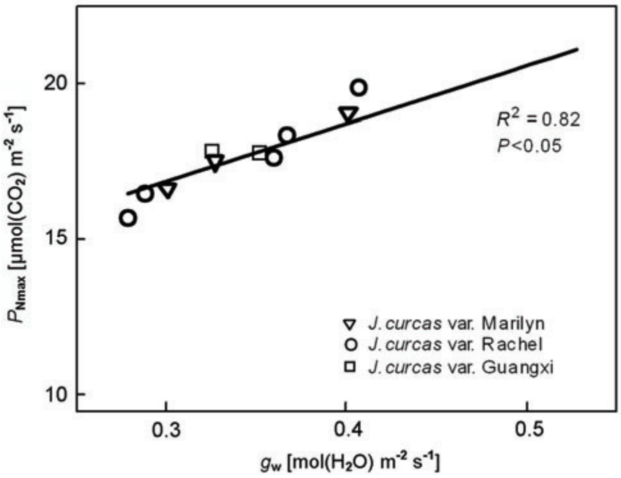

Fig. 2. The correlation between CO_2 assimilation rates (P_{Nmax}) and stomatal conductances to water (g_w) of field-grown *Jatropha curcas* leaves.

However, the CCI values only had weak correlation with the foliar Chl content and leaf N level in field-grown plants (R^2 = 0.45 and 0.48, respectively, $P \le 0.05$; Fig. 4*A*,*B*). Leaf P_{Nmax} of the plants undergone soil N treatments showed positive correlation with corresponding CCI values (R^2= 0.71, $P \le 0.05$, data not shown). However, it was observed that the P_{Nmax} and the CCI values of field plot plants were only weakly correlated (data not shown). Interestingly, when N nutrition is not limiting, the CCI values of *Jatropha* leaves were consistently higher than 13.

Effects of fertilizer treatments on the physiology: With increasing fertilizer application, the light saturation of *J. curcas* was shifted towards higher photon flux density (PFD), which was in the range of 750 to 1,500 µmol(quanta) m^{-2} s^{-1} (Fig. 5*A*). Similarly, it was observed that both $P_{NhighCO2}$ and P_{Nmax} increased with increasing fertilizer application (Fig. 5*B*, Table 2). T_0 had the lowest P_{Nmax}, while the highest P_{Nmax} was achieved for plants

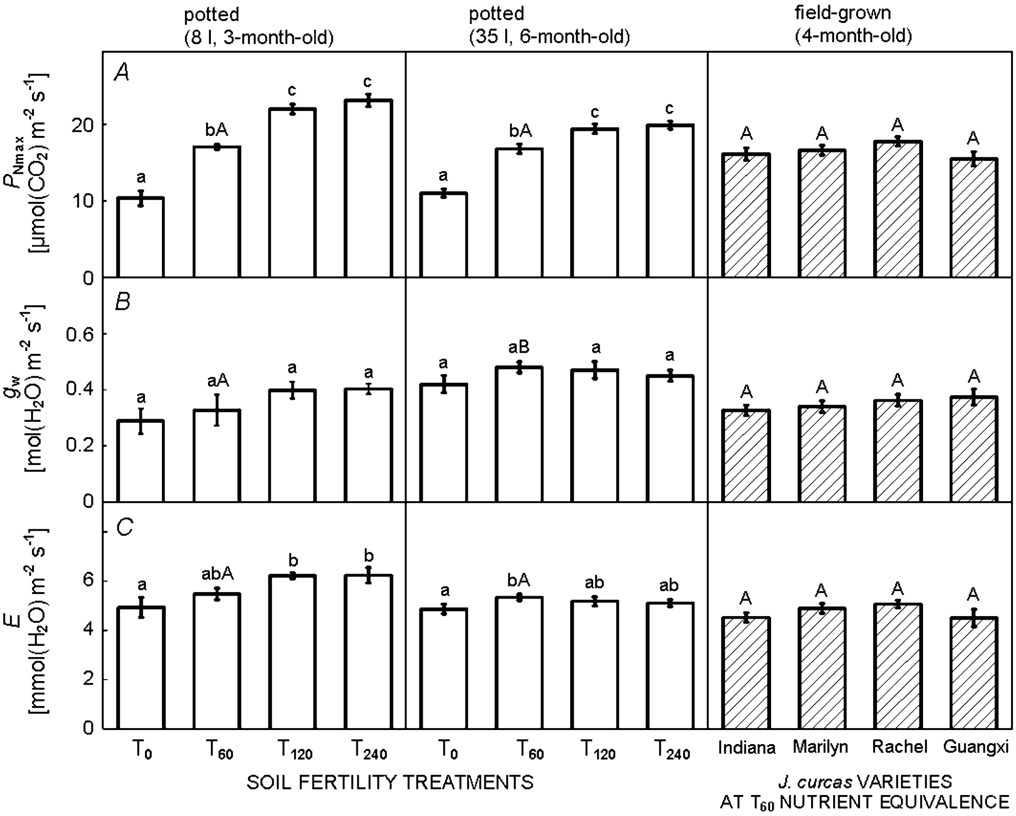

Fig. 3. A comparison between photosynthetic parameters of *Jatropha curcas* var. Indiana grown in pots under different nutrient treatments 2 and 5 months after treatment (*left* and *center panel*) and different *Jatropha* varieties (Indiana, Marilyn, Rachel and Guangxi) grown on a field plot (*right panel*, nutrient level was similar to T_{60} treatment). The photosynthetic parameters measured are: *A*: CO_2 assimilation rates (P_{Nmax}), *B*: leaf stomatal conductances to water (g_w), and *C*: transpiration rates (*E*). Data for *Jatropha* grown in pots represent the means ± standard error of 3 or 4 replicate plants; while data for field-grown *Jatropha* were the means ± standard error of 3 replicate plants. Means with different small letters are significantly different when compared among different fertilizer treatment levels for potted plants; values with different capital letters are significantly different when compared within the same fertilizer treatment level (*i.e.* T_{60} and field-grown plants, $P \le 0.05$; *Tukey*'s multiple range comparison tests). The equivalent amount of fertilizer added for field-grown plants is 60 g of *Osmocote* $\approx T_{60}$.

Table 2. Effects of four fertilizer treatments on photosynthetic rates (P_{Nmax}) and chlorophyll (Chl) content indexes (CCI) of *Jatropha curcas* var. Indiana grown in pots. Root volume was increased from 8 to 35 l when plants were four-month-old (3 MAT). All values represent the means ± standard error of 3 to 7 replicates. Values with different small letters are significantly different when compared among different fertilizer treatment levels; means with different capital letters are significantly different when compared with values within the same fertilizer treatment level over time ($P \leq 0.05$; *Tukey*'s multiple range comparison tests). MAT – months after treatment.

	2 MAT	5 MAT	7 MAT	9 MAT
	P_{Nmax} [µmol(CO_2) m^{-2} s^{-1}]			
T_0	10.4 ± 1.0^{aA}	11.0 ± 0.5^{aA}	13.8 ± 0.3^{aB}	16.4 ± 0.6^{aB}
T_{60}	17.1 ± 0.3^{bAB}	16.8 ± 0.6^{bA}	20.0 ± 0.7^{bcB}	17.9 ± 0.4^{acAB}
T_{120}	22.0 ± 0.6^{c}	19.4 ± 0.6^{c}	20.9 ± 0.3^{cd}	20.3 ± 0.5^{bd}
T_{240}	23.1 ± 0.8^{cAB}	19.9 ± 0.5^{cA}	22.6 ± 0.7^{dB}	20.4 ± 0.9^{cdAB}
	CCI			
T_0	8.3 ± 0.3^{aA}	7.3 ± 0.3^{aA}	8.1 ± 0.4^{aA}	18.3 ± 0.7^{aB}
T_{60}	20.9 ± 3.0^{bAB}	17.9 ± 0.7^{bA}	19.2 ± 1.2^{bAB}	23.1 ± 1.5^{bcB}
T_{120}	30.5 ± 1.8^{c}	26.8 ± 0.8^{c}	26.3 ± 0.8^{c}	26.3 ± 1.4^{cd}
T_{240}	32.9 ± 1.3^{c}	29.9 ± 0.9^{d}	28.1 ± 1.2^{c}	28.1 ± 1.6^{d}

under T_{120} and T_{240} treatments; P_{Nmax} reached saturation stage at T_{120} treatment, where its value did not alter much with higher fertilizer treatment (*i.e.* T_{240}). This increasing trend was generally maintained over time (Fig. 3*A*, *left* and *center panel*; Table 2). This pattern of increase was also observed in CCI measurements, where T_0 had the lowest value and T_{120} or T_{240} achieved the highest CCI. With increasing nutrient levels, the leaf N level increased linearly and strongly correlated with leaf Chl content and P_{Nmax}, R^2 were 0.87 and 0.77, respectively (Fig. 4*D,E*). T_{120}/T_{240} plants had the greatest height among the treatment groups and they also had the highest number of mature leaves and axillary leaves (Fig. 6). All the T_{240} plants flowered, while only 70% of T_{120} plants and 50% of T_{60} plants did (Table 3).

Effects of fertilizer treatments on seed oil content and plant yield: Oil content analyses showed that mother stock seed oil content investigated in this study was between 50% and 60% (Table 4). At this juncture, there was no significant correlation ($P > 0.05$, data not shown) between the seed oil content of the different varieties (Guangxi, Indiana, Marilyn and Rachel) and their corresponding P_{Nmax}. Analysis of seed oil content of plants (var. Indiana) treated with different fertilizer levels revealed that seed oil content was not affected by the amount of nutrients supplemented. There was no significant difference in seed oil content among the 3 different levels of fertilizer treatment (Table 3). However, T_{240} had the highest fruit yield (*i.e.* number of fruits) when compared to T_{60} and T_{120}. The number of produced fruits increased exponentially with increasing nutrient supplement level. The average seed mass (air-dried mass) of T_{120} and T_{240} are similar and are significantly larger than the seeds produced under T_{60}.

Discussion

This study showed that *J. curcas* is a sun-loving species and it achieved maximum photosynthetic capacity at PFD beyond 800 µmol(quanta) m^{-2} s^{-1} [*cf.*, full-sun PFD is around 2,200 µmol(quanta) m^{-2} s^{-1}]. *Jatropha* exhibited photosynthetic rates in the range of 7 to 25 µmol(CO_2) m^{-2} s^{-1} (measured under ambient CO_2 concentration). Higher rates of photosynthesis [between 15 and 25 µmol(CO_2) m^{-2} s^{-1}] were observed for mature leaves when N nutrition was not limiting. Under low N nutrition, the range for mature leaf photosynthesis was between 7 and 12 µmol(CO_2) m^{-2} s^{-1}. The $\delta^{13}C$ values of *Jatropha* leaves (between –26.1‰ and –29.2‰) coincided with the $\delta^{13}C$ values of C_3 plants (*cf.*, –13.5 ± 1.5‰ of C_4 plants; O'Leary 1981, Farquhar *et al.* 1989). This is the first report of $\delta^{13}C$ values for *J. curcas* and together with photosynthesis data, provided the scientific evidence that this biofuel plant species is a C_3 sun-loving plant species.

J. curcas growing in large pots or field plot under similar nutrient conditions did not show major differences in their leaf gas-exchange parameters. This implied that the nutrient treatment results obtained using potted plants were not affected by root volume restriction when the plants were planted in reasonably large-sized pots (*e.g.* 8 to 35 l of soil volume in this study, for young plants) and the results could generally be applicable for young field-grown plants. Artifacts like root volume restriction may result from using very small pots (Shi *et al.* 2008). Therefore, this approach could be helpful in developing an appropriate fertilizer application level, especially for young *Jatropha* plants, in order to optimize plant growth during the crucial first year of field cultivation for *J. curcas*.

Rapid and nondestructive methods for measuring leaf Chl content are available for estimating leaf Chl content changes under certain abiotic (*e.g.* soil, water status) and biotic (*e.g.* pest attacks, virus infections) conditions for different plant species (Rao *et al.* 2001, Wang *et al.* 2004, Pinkard *et al.* 2006, Samsone *et al.* 2007). However, the accuracy of Chl content meter depends on several factors, such as plant species, leaf variegation, leaf thickness, and leaf age (Pinkard *et al.* 2006, Biber 2007, Silla *et al.* 2010). In this study on *J. curcas*, the CCI-derived values showed a significant linear correlation with the total Chl content (van den Berg and Perkins 2004, Ruiz-Espinoza *et al.* 2010) when measured on leaves of plants under fertilizer treatments (Fig. 4*C*). However, some other studies done using different plant species demonstrated

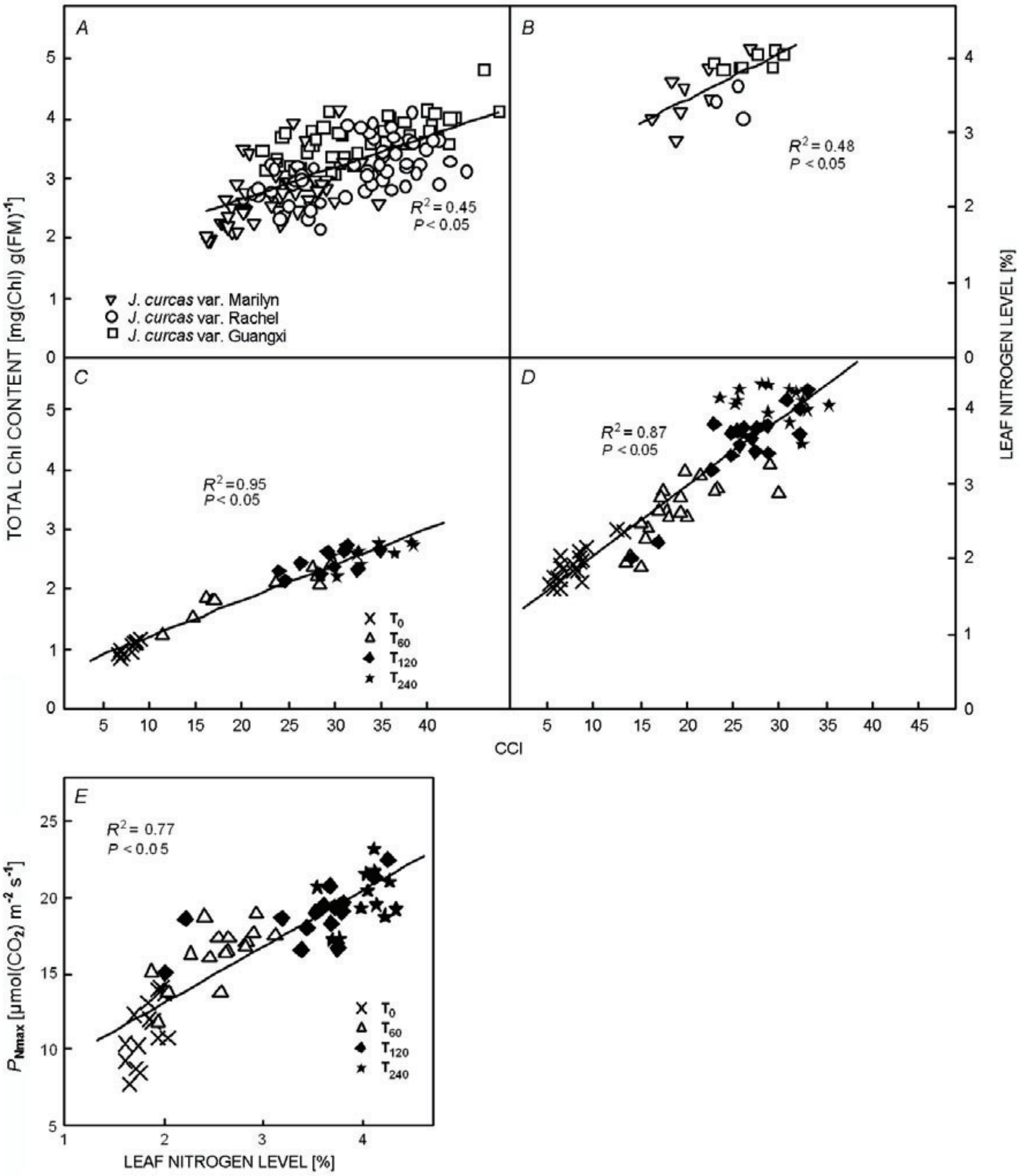

Fig. 4. *A*: The correlation between leaf total chlorophyll (Chl) content and Chl content index (CCI) of field-grown *Jatropha curcas*. *B*: The correlation between leaf nitrogen (N) levels and CCI of field-grown *Jatropha curcas*. *C*: The correlation between leaf total Chl content and CCI of *Jatropha curcas* var. Indiana treated with different fertilizer levels. *D*: The correlation between leaf N levels and CCI of *Jatropha* var. Indiana treated with different fertilizer levels. *E*: The correlation between leaf photosynthetic rates (P_{Nmax}) and leaf N levels of *Jatropha* var. Indiana treated with different fertilizer levels.

a nonlinear correlation between CCI and Chl content (Markwell *et al.* 1995, Richardson *et al.* 2002). The curvilinear nature of the correlation showed that Chl meter readings could not give accurate estimations when the actual Chl content is very high (Pinkard *et al.* 2006). Therefore, care should be taken when developing calibration curve using portable Chl meter to include sampling values of both extremes. Nevertheless, for moderate Chl content level, CCI can still provide a rather accurate estimation of the foliar Chl content based on a linear correlation model (van den Berg and Perkins 2004).

As predicted by Evans (1983), leaf N content serves as a better proxy for photosynthetic capacity. In our study, there was a significant correlation between leaf photosynthetic rates and leaf N contents of potted plants (R^2 = 0.77, Fig. 4*E*). N is the main element that

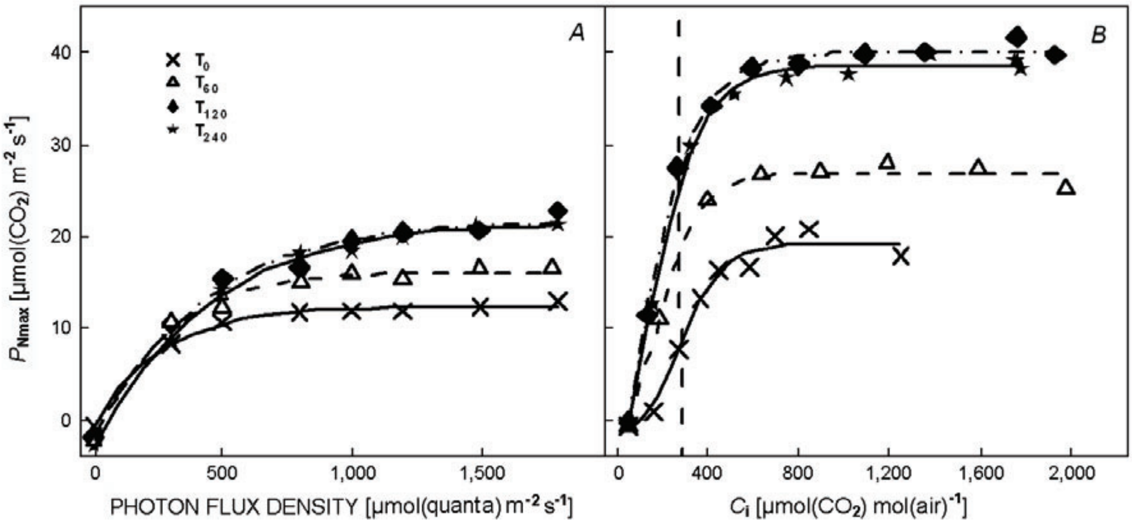

Fig. 5. The CO_2 assimilation rates of *Jatropha curcas* var. Indiana under different levels of fertilizer treatments. *A*: Light-response curves. *B*: CO_2 assimilation curves over a range of intercellular CO_2 (C_i) concentrations (*A*-C_i curves). *Dotted line* indicates the intracellular CO_2 concentration (C_i) value of 280 µmol(CO_2) mol(air)$^{-1}$ which corresponds to the ambient CO_2 of around 380–400 µmol(CO_2) mol(air)$^{-1}$.

Fig. 6. Aerial view of the *Jatropha curcas* plants (var. Indiana) grown under four different fertilizer treatments. *A*: Plants (three-month-old) in 8-l pots 2 months after treatment, rows from bottom to top: T_0, T_{60}, T_{120} and T_{240}. *B*: Plants (nine-month-old) in 35-l pots 8 months after treatment, rows from left to right: T_0, T_{60}, T_{120} and T_{240}.

constitutes proteins and higher N content generates more photosynthetic apparatus to support higher photosynthetic activities. There was also a significant correlation between leaf CCI values and N content. This provided the scientific basis for the use of portable Chl meters as a rapid assessment tool for estimating leaf N content, and thus the plant productivity status of *J. curcas*. Moreover, its nondestructive nature allows repeated sampling of the same plant undergoing various treatments. Several other studies also supported the favorable correlation between Chl index and leaf N level; and that Chl index was useful in monitoring fertilizer management to improve fertilizer-used efficiency of commercial crops (Peng *et al.* 1996, van den Berg and Perkins 2004, Hawkins *et al.* 2007). Interestingly, CCI reflected the corresponding changes in leaf Chl content and leaf N content better when there was a large difference in growth condition (Pinkard *et al.* 2006), *i.e.* nutrient supply. The correlation of the parameters of the plants grown under different nutrient supplement levels in pots was stronger than that of the field-grown plants treated similarly (*i.e.* Fig. 4*B*,*D*).

From this study, increased soil nutritional level was

Table 3. Effects of four fertilizer treatments on percentage of flowering plants, number of fruits produced per plant, average fresh seed mass and average seed oil content of *Jatropha curcas* var. Indiana grown under experimental conditions. The fruits were harvested starting from 6 months after treatment (for a period of 4 months). Seeds were air-dried before seed-mass measurement . Number of flowering plants for each fertilizer treatment (n = 10 replicate plants) was between 5 and 10. Control plants had not flowered throughout the entire experimental period. All values (except percentage of flowering plants) represent the means ± standard error of 4 to 9 replicates. Values with different small letters are significantly different when compared among different fertilizer treatment levels ($P \leq 0.05$; *Tukey*'s multiple range comparison tests). N. A. – not available.

	[%] of flowering plants	Average number of fruits per plant	Average seed mass [g]	Seed oil content [%]
T_0	0	N. A.	N. A.	N. A.
T_{60}	50	10 ± 3^a	0.66 ± 0.03^a	51.9 ± 1.5^a
T_{120}	70	29 ± 6^a	0.75 ± 0.02^b	52.4 ± 1.3^a
T_{240}	100	81 ± 9^b	0.75 ± 0.01^b	49.8 ± 1.2^a

Table 4. Seed oil content analysis of the different *Jatropha curcas* varieties grown in different countries (China, India, and Cambodia). There is no SE value as the various replicate samples had to be pooled together.

Variety	Oil content [%]
Guangxi (China)	55.3
Rachel (China)	60.0
Indiana (India)	53.1
Marilyn (Cambodia)	51.7

able to promote growth and enhance the photosynthetic capability of *J. curcas*. As shown in *J. curcas* var. Indiana, photosynthetic capacity was enhanced especially for T_{120} and T_{240} plants (Fig. 5*B*). Photosynthetic capacity of plants is primarily linked to the whole-plant N status, especially for N which is allocated into the chloroplasts. High nutritional supplement led to an increase in the leaf N level and the leaf N is later utilized in the manufacture of thylakoids and also the proteins involved in the photosynthetic Calvin cycle. With more N sources added to the plant, there is an increased N availability for biological functions thus promoting higher photosynthetic efficiency (Millard 1988, Evans 1989).

It is noteworthy that an optimal growth condition for *J. curcas* var. Indiana was achieved between T_{120} and T_{240} treatments. This information would be useful for fertilizer management in the large-scale planting of *J. curcas*, in an effort to maximize harvestable yield if the assumption that higher photosynthetic capabilities equates to higher oil yields (either more fruits and/or high oil content in seeds) is true. In our study, it was observed that the emerging leaves on young T_{240} (three-month-old, 2 MAT) had some marginal chlorosis. This demonstrated that excessive nutrient feeding had no further positive effect on *J. curcas* growth, especially for the young plants, where the leaf N and P_{Nmax} had already reached their maximum physiological capacity (Fig. 5*B*, Table 2). According to Marschner (1995), an elevation of nitrate content in certain plant species or organs beyond an optimal level would cause an imbalance between the supply and demand for growth, and in many instances, it could be detrimental to plant growth at very high levels.

The initial seed oil analyses carried out on the mother stock seeds of the three *J. curcas* varieties indicated that there was no significant correlation between the intrinsic seed oil content and photosynthetic rates among the varieties. There could be several reasons for this observation. One plausible explanation is that the current growth condition is different from the original growth conditions at which the seeds were produced. Thus, the physiology of the plants (producing the mother stock seeds) were somewhat altered, thereby affecting the seed oil content. To resolve this uncertainty, we selected and grew one of the chosen *J. curcas* var. (Indiana) under a series of soil N nutrition treatments. Subsequently, the seeds were harvested and analyzed. Interestingly, the results showed that there was no correlation between seed oil content and photosynthetic rates. This suggested a possible conservative genetic component in oil-synthesis regulation for *J curcas*. In our case, it is noteworthy that the var. Indiana has similar seed oil content values when grown in India (53.1%, Table 4), and later in Singapore (49.8% to 52.4%, Table 3). Moreover, Popluechai *et al.* (2009) recently showed that genetic diversity for *J. curcas* around the world is rather limited. The noticeable variability seen in the characteristic traits among different *J. curcas* accessions are suggested to have an environmental or epigenetic origin.

In several studies, it was found that seed oil content (oil concentration) decreased or responded weakly to increase in N supply, due to the concomitant increase in heavier protein production under high N nutrition (Abbadi *et al.* 2008, Ghasemnezhad and Honermeier 2008). This could be a result of the competition between protein synthesis and fatty acid synthesis for carbon building blocks. Ghasemnezhad and Honermeier (2008) also suggested that fatty acid synthesis has higher carbohydrate requirement than protein. Consequently, the increased N supply would enhance protein synthesis at the expense of fatty acid synthesis for primrose seeds. A negative correlation between seed oil content and protein content was established by Mason and Brennan

(1998) for canola.

Nevertheless, higher N level/photosynthetic capacity did improve *Jatropha* plant oil yield and this was achieved by increasing the number of fruits produced per plant (Table 3). On a broader prospective, the overall plant oil production is determined by two main factors: absolute oil yield per seed (oil content within a seed and seed size) and the total number of seeds (attributed to the number of fruits). *Jatropha* plants with lower intrinsic seed oil content can match the other *Jatropha* plants, in terms of oil yield per unit area, with higher intrinsic seed oil content if the former can produce significantly more fruits. This aspect of *Jatropha* plantation oil yield management (whether by breeding *Jatropha* with higher intrinsic seed oil content, and/or selecting varieties with more fruit production) deserves more studies.

In addition, our data have shown that higher N nutrition caused earlier flowering (about one month earlier, data not shown) in the T_{240} plants when compared to the other lower fertilizer treatments. Flowering induction is generally associated with the availability of N and/or carbohydrate. Leleu *et al.* (2000) showed that rapeseed with N supplements flowered earlier than the nonfertilised plants. N-deficiency study on lupin also demonstrated that flowering time was delayed in low-N-treated plants (Ma *et al.* 1997). Ma *et al.* (1997) suggested that the delay in flowering time could be due to the slower leaf emergence, as the nonfertilized plants had lower growth rate. However, the mechanisms underlying the flower induction effect had not been determined, although some studies proposed that N and carbohydrate may contribute to floral induction by an osmotic effect on water uptake (McIntyre 1997).

Conclusion: In conclusion, this present study showed that *J. curcas* is a typical C_3 sun-loving plant species. Like most other plants, its leaf photosynthetic rates decreased with age or attributed to a reduction in soil N nutrition. Growth observations of plants grown in large pots (*e.g.* 8 to 35 l of soil volume) can be extrapolated to field-grown young plants, which may assist in developing various plantation management strategies especially for the first year. For *J. curcas*, it was established that CCI is a good indicator of the leaf total extractable Chl and N content. This supports the use of portable Chl meter as a valuable tool for the rapid assessment of N content in *J. curcas* leaves in the field. In this study, increased N nutrition for *J. curcas* led to the production of more leaves, and these leaves have higher leaf Chl content, total N content, and leaf photosynthetic rates. Higher N nutrition enhanced overall plant oil yield by increasing fruit/seed production per plant. However, there was no correlation between the absolute seed oil content and N levels or photosynthetic rates. Our data indicated that an optimum nutrient treatment should be somewhere between T_{120} and T_{240}, in order to achieve a vibrant *Jatropha* plantation for maximal fruit production while balancing the operational cost in fertilizer applications and maintaining healthy *Jatropha* plant growth.

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