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Photosynthetic efficiency of PSII and growth of young rubber tree (Hevea brasiliensis) planted with Mucuna *(Mucuna bracteata)* cover crop

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Abstract

Mucuna bracteata is a legume crop recommended for use as a cover crop to plant between rows of young rubber trees in an intercropping system. It has many advantages as a cover crop including its rapid growth rate, deep root system, drought tolerance and high nitrogen fixation rate. However, there is little information regarding the physiological roles, particularly in regards to enhancement of photosynthetic efficiency and growth performance, in which *M. bracteata* plants provide for the young rubber trees. Photosynthesis parameters including Photosystem II efficiency, chlorophyll content, the greenness or relative chlorophyll content of leaves (SPAD values) and growth of two-year-old rubber trees planted with or without *M. bracteata* were evaluated. The rubber plantation was situated at Khon Kaen University (NE Thailand) and the measurements were performed during the dry (March) and rainy (July) seasons in 2015. The results showed that the soil temperature in the experimental plot with cover crop was significantly lower than that in the plot without the cover crop. In contrast, soil moisture content in the plot with the cover crop was significantly higher than that without the cover crop. In the dry season, the effective quantum yield of *PSII*, $\Delta F/F_m'$, and the maximal fluorescence in the light-adapted state (F_m') of young rubber trees growing in the plot with the cover crop were found significantly higher than those without the cover crop. However, in the rainy season, $\Delta F/F_m'$, and F_m' of the rubber trees planted with the cover crop were significantly lower than those without the cover crop, whereas other fluorescence parameters were not different. In both seasons, the contents of Chlorophyll a, Chlorophyll b and total Chlorophyll, SPAD values, leaf area and girth of rubber trees planted with the cover crop were significantly higher than those without the cover crop. This suggested that the use of *M. bracteata* as cover crop for the rubber plantation was beneficial for growth of young rubber plants. *M. bracteata* provided a favorable environment for the plantation leading to higher chlorophyll content, increased photosynthetic performances and hence better growth of the young rubber trees

Keywords : Photosynthesis, *PSII* efficiency, Rubber tree, Mucuna, Chlorophyll content, SPAD

Introduction

Rubber tree is an important economic crop in Thailand which is the second exporter country of rubber in the world after Indonesia. Rubber trees are traditionally planted in the South of Thailand where the environments were favourable for growth of rubber plants with generally very high rainfall and fertile soils. During the last decade a large area of rubber plantation has been established in the Northeast of Thailand where the soils are of low fertility, temperatures are higher, rainfalls are much less and many areas occasionally faces drought. These results are in low production potential of rubber in this part of the country. At present, applying fertilizers is the only practice that farmers use to enhance plant growth and increase rubber production. Planting cover crops in between rows of young rubber trees (intercropping system) has been suggested as another practice to increase growth and productivity of rubber tree plantation. Many researchers have reported the use of several crop species as cover crop for young rubber trees. These include upland rice, mung bean, soybean and peanut (1); papaya, tobacco, water melon (2); cassava and peanut (3). Moreover, cover legumes intercropped with rubber trees are also recommended for soil erosion control, improving soil fertility and soil quality, weed and pest control (4,5,6,7). The cover legumes such as *Pueraria phaseoloides*, *Centrosema pubescens* and *Calopogonium caeruleum* are commonly used as ground cover in rubber cultivation. However, these cover legumes are not always successful against weed growths during initial rubber cultivation (6). Thus, new cover legumes such as *Mucuna pruiens*, *Mucuna cochinchinesis* and *Mucuna bracteata* have been introduced. They are widely used in Malaysia, Indonesia and South America (6,8,9). Mucuna species are fast growing legumes, with deep root system, drought tolerant, have high nitrogen fixation rates and produce high biomass. They can reduce soil temperature and protect from soil erosion (6,8,9). In intercropping or cover cropping systems total light use efficiency in the major crops as well as the associated crops can be enhanced. Higher yield of intercropped plants can be attributed to higher light use efficiency (7). The incident light in the mixture canopies is intercepted by each plant species (7). However, the photosynthesis characteristics in the mixed canopies, i.e. the efficiency from light interception to the conversion of energy into biochemical energetic compounds, are not well understood and documented. Chlorophyll fluorescence is a powerful technique widely used to study photosynthesis in the field because it is a sensitive, real-time and non-destructive method (10). Chlorophyll pigments absorb light energy and then drive photosynthetic process (photochemistry), release heat energy or re-emit fluorescence. The original fluorescence measurements under natural condition are from pigments of photosystem II (PSII) (11) and it relates to light use efficiency of photosynthesis. The PSII efficiency of photosynthesis is expressed in mol of CO_2 converted or of carbohydrate formed (11). Therefore, our hypothesis is that the cover crops supply nitrogen to soil and that this nitrogen will be up taken by the rubber trees. Consequently leaf nitrogen, chlorophyll pigments and rubber tree photosynthesis can increase. High photosynthesis relates with plant growth. Thus, this research aims 1) to compare the PSII efficiency and plant growth of young rubber trees (Hevea brasiliensis) planted in the experimental plots with and without the legume, Mucuna bracteata as cover crop, and 2) to evaluate the benefit of M. bracteata planted in between rows of young rubber trees.

Materials and Methods

Site and Plant material

This research was conducted in the Fruit Tree division at Faculty of Agriculture, Khon Kaen University, Thailand in 2015. The spacing of rubber tree was 3 x 7 m. M. bracteata was planted in double row (spacing 3 x 3 m) between rows of rubber trees (Figure 1). The trees were two years old when the measurement started. We used a randomized complete block design (RCBD) with 4 replications (plots). The area of 1 plot was 21 x 14 m with 21 rubber trees and 24 M. bracteata plants. Soil type of the experimental plot was loamy sand. Fertilizer (N-P-K : 20-8-20) was applied at a rate of 100 g/plant twice a year in both treatments (with or without M. bracteata as cover crop). Tillage and weeding control were managed twice a year in both of treatments.

Microclimate measurement

In the experimental site, microclimate was measured during photosynthesis and plant growth measurement in March (dry season) and July (rainy season) 2015. Soil temperatures at 0-10 cm depth were measured using thermometer. Soil humidity was measured by weighing soil method. Soil samples were collected at 10 cm depth by soil core (5 cm diameter) and then weighed for fresh weight (FW). Soil sampled was oven dried at 150 ^{o}C until constant weight or dry weight (DW). Soil moisture content was calculated following the equation [(FW-DW)/FW] x 100 and unit reported as percentage. Air temperatures and air humidity above ground surface at 1 m height were measured with Indoor digital thermometer-hygrometer (Minnesota Measurement Instruments LLC, USA). Soil and air temperature, soil moisture content and air humidity were sampled at 2 points, one at 30 cm and another at 2 m from tree line (See Figure 1).

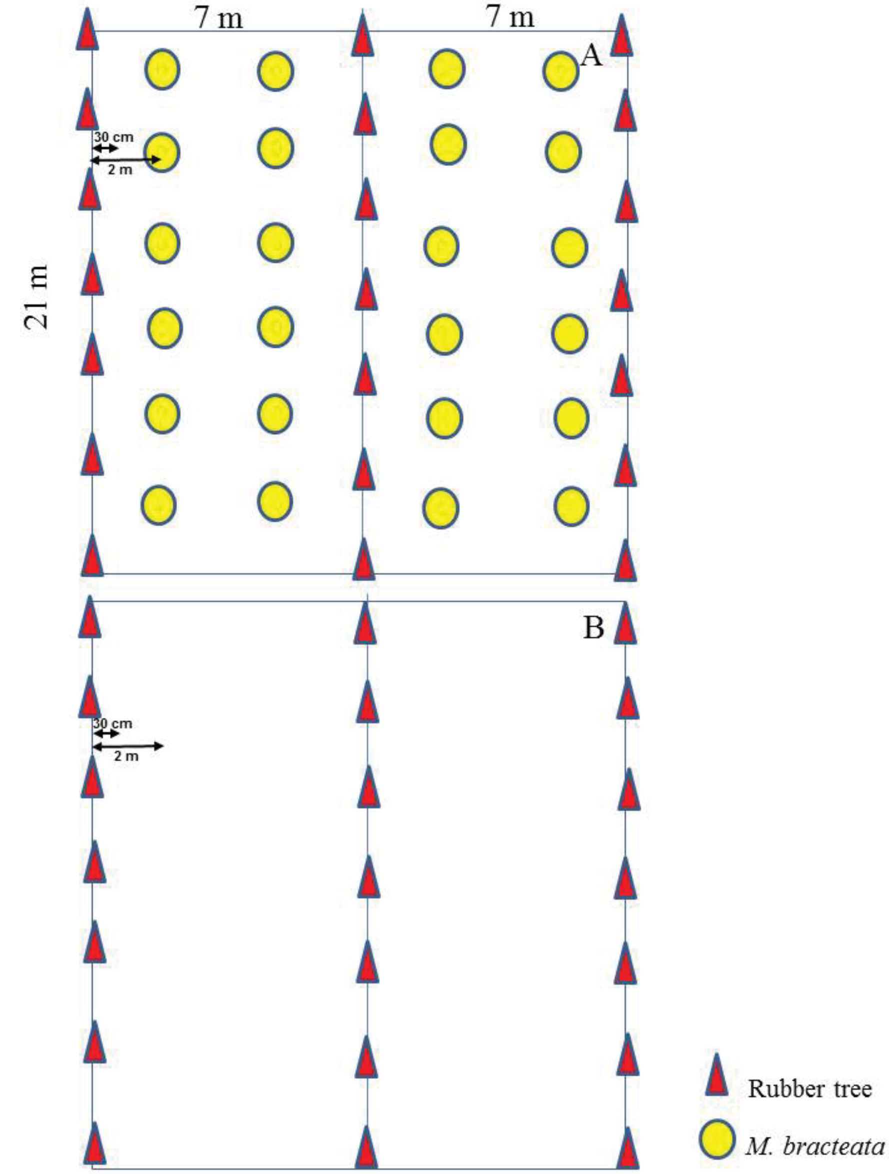

Figure 1. Schematic diagram of the experimental plots showing plant spacing of rubber trees with (A) or without (B) *M. bracteata* as the cover crop.

Determination of chlorophyll fluorescence

Chlorophyll fluorescence in the middle leaflet of attached leaves of rubber trees (3 leaves per plant) and mature leaves of *M. bracteata* (3 leaves per plant) was measured in the experimental field by using a Mini-PAM fluorometer (Walz, Effeltrich, Germany). In our experiment, we used two rubber tree plants/plot and two *M. bracteata* plants/plot. As shown in equation (1) and (2), The following parameters of chlorophyll fluorescence were measured: F_0, minimal fluorescence in the dark-adapted state; F_s, steady state fluorescence under a natural irradiation; F_m, the maximal fluorescence in the dark-adapted state, and F_m', the maximal fluorescence in the light-adapted state (natural irradiation). Maximal quantum yield of *PSII* photochemistry in dark condition (maximal photosynthetic efficiency), F_v/F_m [equation (1)] and effective quantum yield of *PSII* photochemistry in light condition (photosynthetic efficiency in natural condition), $\Delta F/F_m'$ [equation (2)] were then determined as described by Schreiber (12).

$F_v/F_m = (F_m - F_o) / F_m$................................(1)

$\Delta F/F_m' = (F_m' - F_s) / F_m'$...........................(2)

Plant growth

On the same leaves used for photosynthetic measurement, leaf area of rubber trees and *M. bracteata* was measured using leaf area meter (LI-3100 area meter, LI-COR, Inc., USA). Girth of rubber trees was measured in March (dry season) and July (rainy season) 2015 at 1 m above ground surface.

Determination of chlorophyll content

On the same leaves used for photosynthetic measurement, leaf chlorophyll contents were measured as described by Arnon (13). Leaf samples (0.1 g) were extracted with 80% acetone and filtered with Whatman#2 filter papers. The total extract volume was added to 10 ml by 80% acetone. The absorbance of extraction was measured with a spectrophotometer at 645 and 663 nm using 80% acetone as a blank.

Chl a (mg g^{-1}) = [12.7(A_{663}) – 2.69(A_{645})] x (V / 1000W)……………...........................…...(3)

Chl b (mg g^{-1}) = [22.9(A_{645}) – 4.68(A_{663})] x (V / 1000W)……………...........................…(4)

Total chlorophyll (mg g^{-1}) = [20.2(A_{645})+ 8.02(A_{663})] x (V / 1000W)....................…(5)

A_{645} and A_{663} represent the absorbance of extraction at 645 and 663 nm, respectively. V and W represent the total extract volume and the leaf fresh weight, respectively.

Leaf chlorophyll was also estimated using a SPAD meter (SPAD 502 Plus Chlorophyll meter, Spectrum Technologies, Inc., USA). SPAD unit is a measure of the greenness or relative chlorophyll content of leaves.

Statistical analysis

The data per leaf (3 leaves/tree) and trees (2 trees/blocks) were averaged and the ANOVA done on 4 replications (blocks). The significant differences of photosynthesis and plant growth parameters between rubber trees planted with *M. bracteata* and only rubber tree plantation and also the significant differences of season in each species were analyzed by t-test at $P < 0.05$ using SPSS version 17.0.

Results

Microclimatic condition in the intercropping system

Microclimate information of the experimental plot of rubber trees planted with and without *M. bracteata* in dry and rainy seasons is shown in Figure 2. The maximum soil temperature in dry season was measured at 30 cm (32.5 ± 0.4 ^{o}C) and the minimum was measured at 2 m (26.7 ± 0.1 ^{o}C) from the tree line (Figure 2A-B). Moreover, in both dry and rainy seasons, soil temperature of the experimental plot with cover crop at 2 m from tree line (Figure 2B) was significantly lower compared with that without the cover crop. In rainy season, soil moisture contents in the experimental plot with the cover crop at 30 cm and 2 m from the tree line were 10.5% (Figure 2C) and 11.8% (Figure 2D), respectively which were significantly higher than those in the plot without the cover crop. However, air temperature and air humidity did not significantly differ between the two experimental plots (Figure 2E-H).

Photosynthetic characteristics in the intercropping system

Photosynthetic characteristics of rubber tree planted with and without *M. bracteata* were indicated by the efficiency of photosystem II (*PSII*) ($\Delta F/F_m'$, F_v/F_m, F_o, F_m, F_s and F_m'), *chl a*, *chl b* and total chlorophyll contents, and SPAD values. As shown in Figure 3, in dry season, $\Delta F/F_m'$ and F_m' of rubber tree with cover crop (0.652 ± 0.01 and 655 ± 26, respectively) were significantly higher than those without the cover crop (0.56 ± 0.02 and 478 ± 60, respectively). Conversely, in rainy season, the values of $\Delta F/F_m'$ and F_m' were significantly lower with (0.573 ± 6.7 and 1540 ± 64, respectively) than without the cover crop (0.622 ± 0.02 and 1758 ± 45) as shown in Figure 3A and 3C. Other fluorescence parameters such as F_v/F_m, F_o, F_m and F_s did not differ significantly between treatments as shown in Figure 3B, 3D, 3E and 3F. Fluorescence parameters such as F_o, F_m, F_s and F_m' in *M. bracteata* were significantly higher in the rainy compared to the dry season (Figure 4C-F). Contents of *chl a*, *chl b* and total chlorophyll in leaves of rubber trees growing with the cover crop in both dry and rainy seasons were significantly higher than those grown without the cover crop (Figure 5A-C). For *M. bracteata*, higher total chlorophyll content was observed in the dry season compared to that in the rainy season (Figure 5F). In addition, in both dry and rainy seasons, SPAD values in leaves of rubber trees planted with the cover crop were significantly higher than those without as shown in Figure 6A. The mean SPAD values of *M. bracteata* were higher in the rainy season compared with that in the dry season (Figure 6B).

Plant growth in the intercropping system

Plant growth was expressed by leaf area of the rubber trees and *M. bracteata*, and girth of the rubber trees as shown in Figure 7 and 8. Leaf area of the rubber trees planted with *M. bracteata* was 53.4 ± 5.8 cm^2 in dry season and 54.6 ± 3.3 cm^2 in rainy season. These values were significantly higher than leaf area of rubber trees planted without cover crop (33.48±1.38 cm^2 in dry season and 32.83±3.23 cm^2 in rainy season) as shown in Figure 7A. *M. bracteata* had significantly higher leaf area in rainy season (91.3 ± 4.5 cm^2) than in dry season (67.2 ± 2.8 cm^2) as shown in Figure 7B. Girth of the rubber trees was higher when planted with than without cover crop (Figure 8).

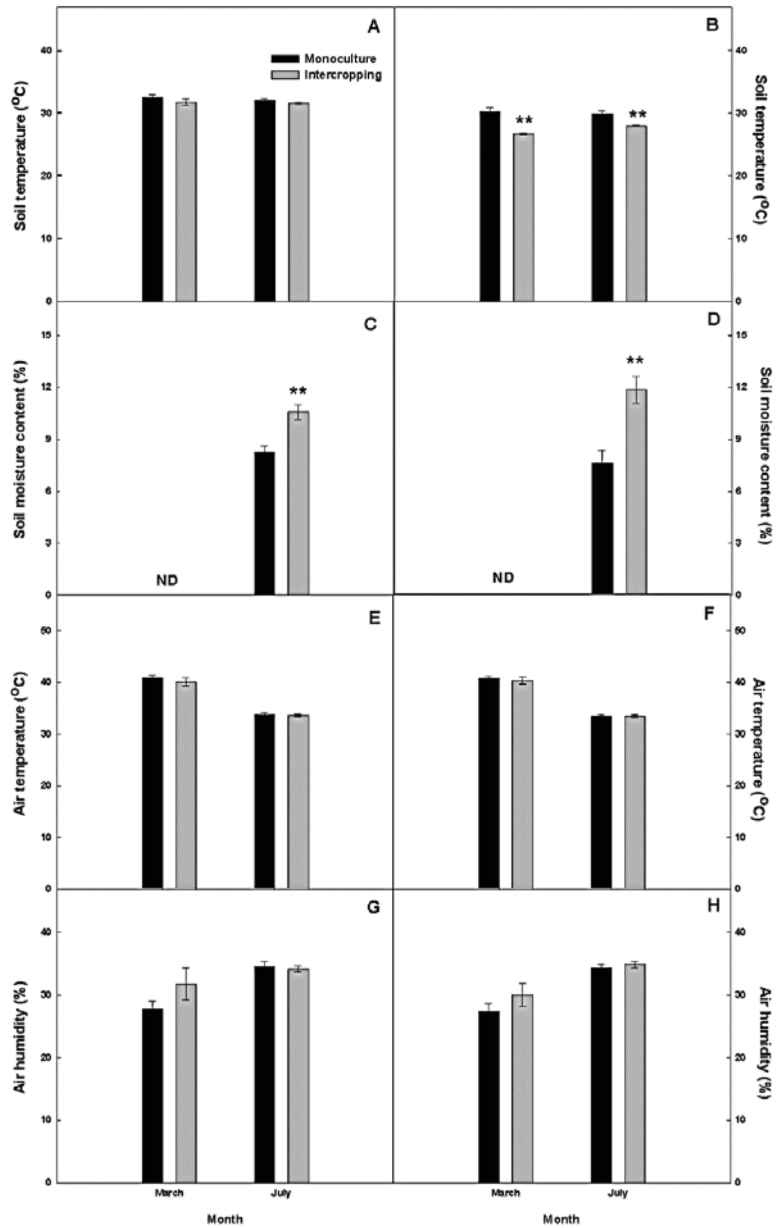

Figure 2. Microclimate condition, soil temperature, soil moisture content, air temperature and air humidity in dry season (March) and wet season (July) 2015. (A) Soil temperature at 30 cm from tree line, (B) soil temperature at 2m from tree line, (C) soil moisture content at 30 cm from tree line, (D) soil moisture content at 2 m from tree line, (E) air temperature at 30cm from tree line, (F) air temperature at 2 m from tree line, (G) air humidity at 30 cm from tree line and (H) air humidity at 2 m from tree line. Black vertical bars show data in plots of rubber trees without *M. bracteata* and grey vertical bars show data in plots of rubber tree with *M. bracteata*. The values are means ± SE (n = 7-8). (ND means not determined). ** represented the means significantly different at $p \leq 0.05$.

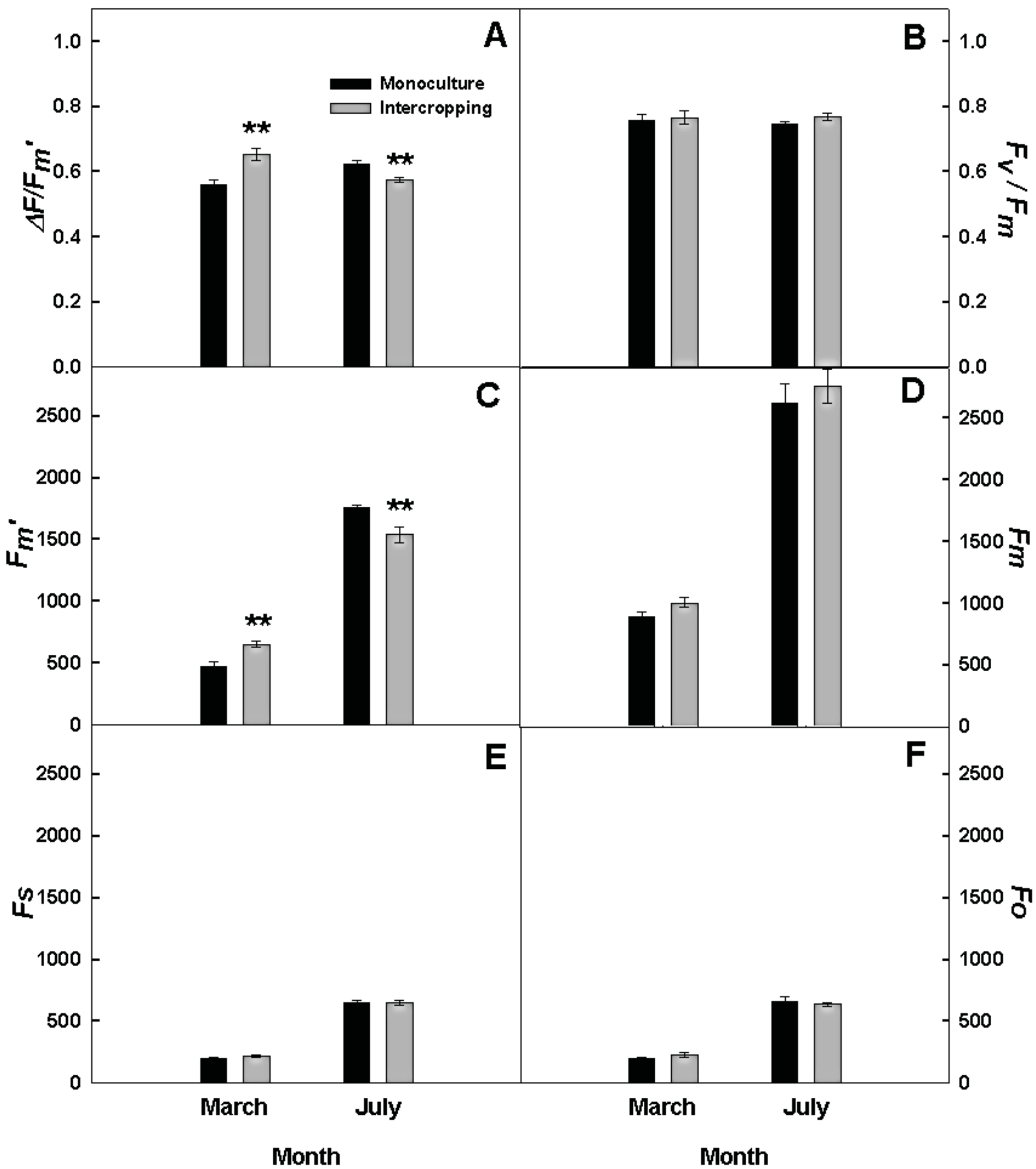

Figure 3. Chlorophyll fluorescence parameters for *PSII* in the leaves of rubber trees planted without (black vertical bar) and with *M. bracteata* (grey vertical bar) in dry season (March) and rainy season (July) 2015. (A) Effective quantum yield of *PSII* efficiency; $\Delta F/F_m'$, (B) maximum quantum yield of *PSII* efficiency; F_v/F_m, (C) maximum fluorescence in light-adapted state; F_m', (D) maximum fluorescence in dark-adapted state; F_m, (E) steady state fluorescence in light-adapted state; F_s and (F) basic fluorescence in dark-adapted state; F_o. The values are means ± SE (n=4). **represented the means significantly different at $p \leq 0.05$.

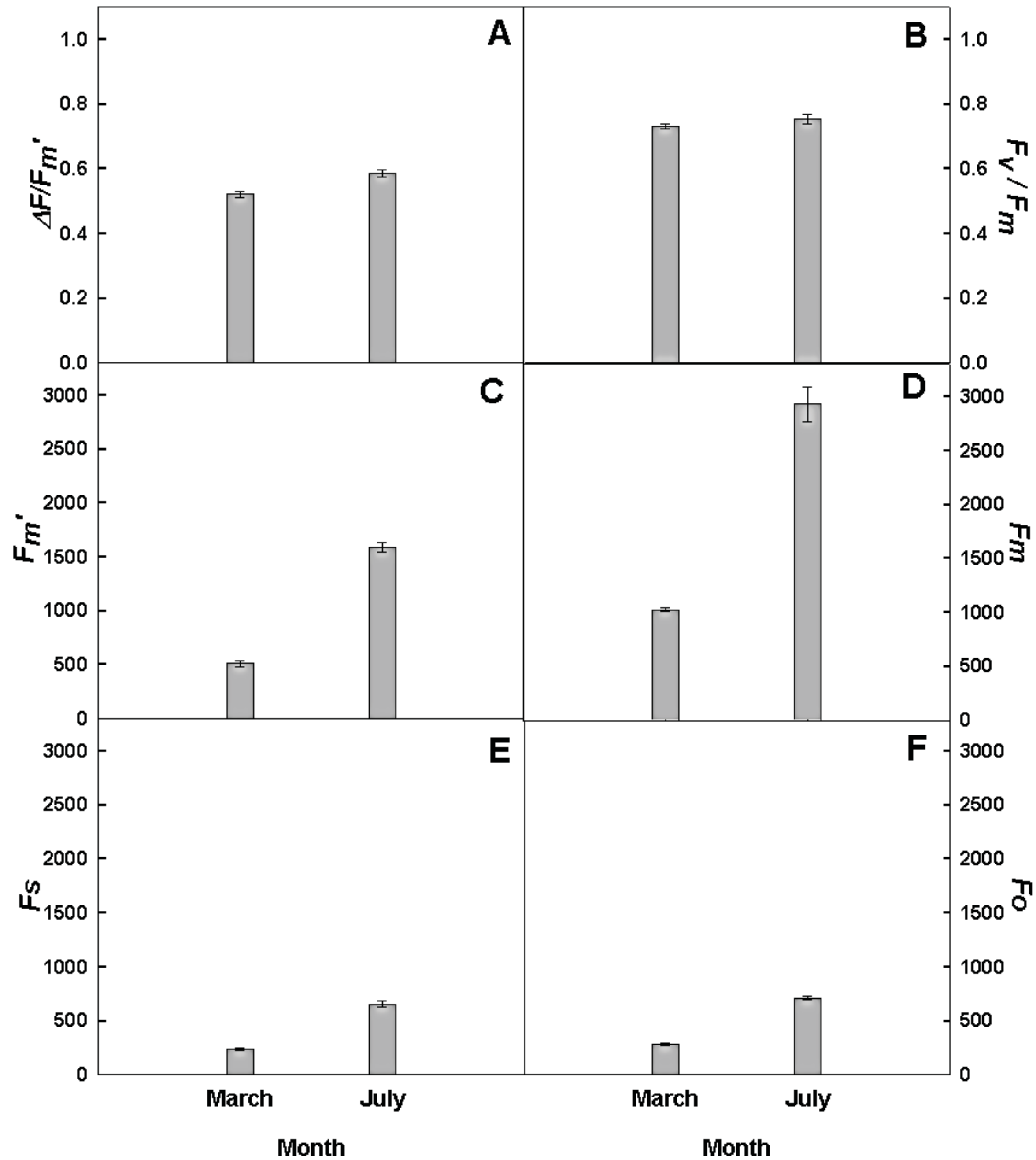

Figure 4. Chlorophyll fluorescence parameters for *PSII* in the leaves of Mucuna (*M. bracteata*) planted in between rows of rubber trees in dry season (March) and rainy season (July) 2015. (A) Effective quantum yield of *PSII* efficiency; $\Delta F/F_m'$, (B) maximum quantum yield of *PSII* efficiency; F_v/F_m, (C) maximal fluorescence in light-adapted state; F_m', (D) maximal fluorescence in dark-adapted state; F_m, (E) steady state fluorescence in light-adapted state; F_s and (F) basic fluorescence in dark-adapted state; F_o. The values are means ± SE (n=4).

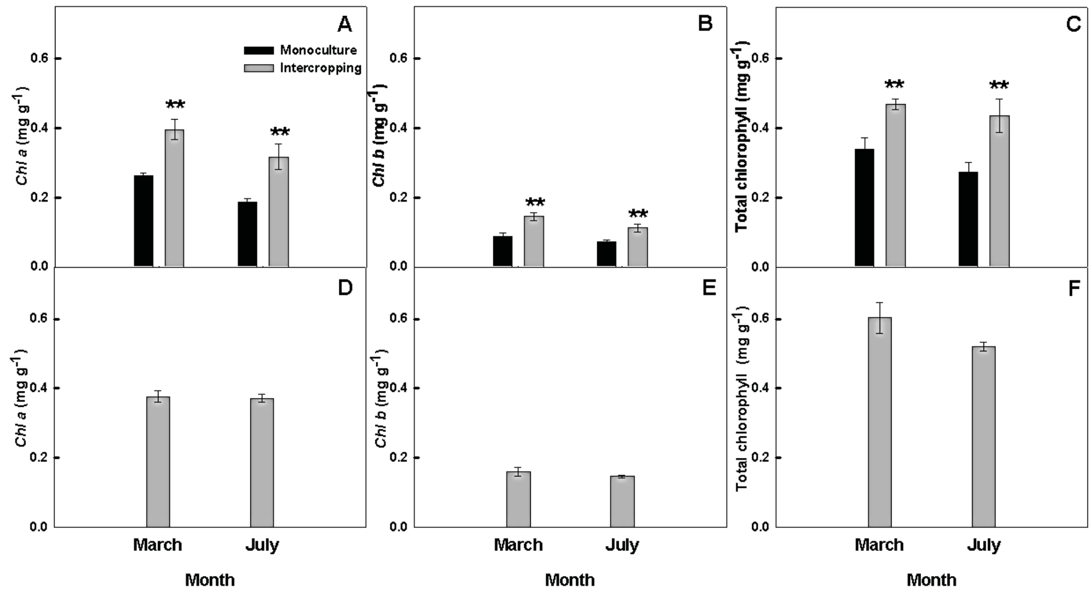

Figure 5. Contents of (A) *Chl a*, (B) *Chl b* and (C) total chlorophyll in the leaves of rubber trees planted without (monoculture system; black vertical bar) and with *M. bracteata* (intercropping system; grey vertical bar). Contents of (D) *Chl a*, (E) *Chl b* and (F) total chlorophyll in the leaves of Mucuna (*M. bracteata*) planted in between rows of rubber trees. The leaves were collected for chlorophyll measurement in dry season (March) and rainy season (July) 2015. The values are means ± SE (n=4). **represented the means significantly different at p≤0.05.

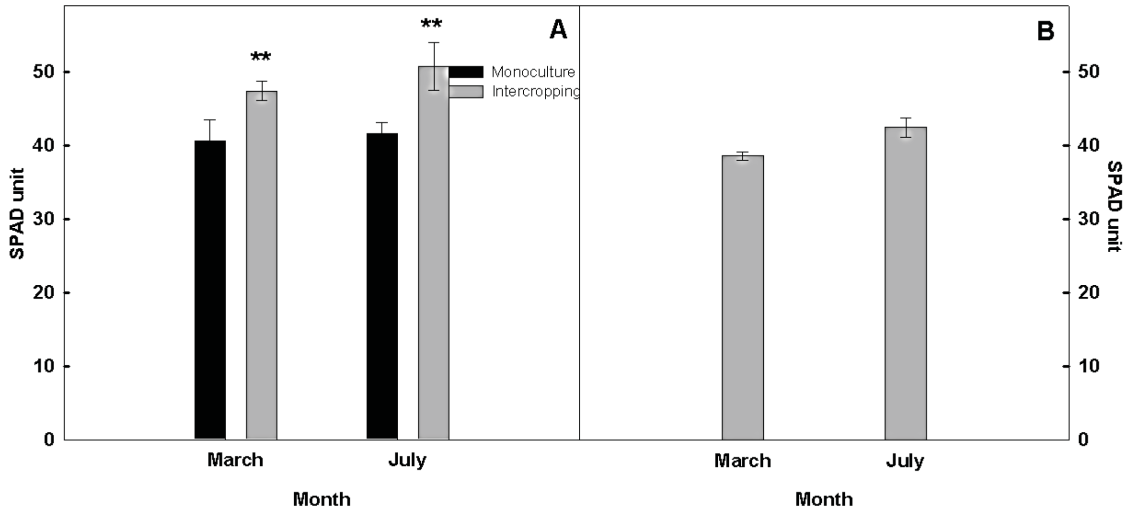

Figure 6. SPAD values of (A) leaves of rubber trees planted without (monoculture system; black vertical bar) and with *M. bracteata* (intercropping system; grey vertical bar). (B) leaves of Mucuna (*M. bracteata*) planted in between rows of rubber trees. SPAD values were determined in dry season (March) and rainy season (July) 2015. The values are means ± SE (n=4). **represented the means significantly different at p≤0.05.

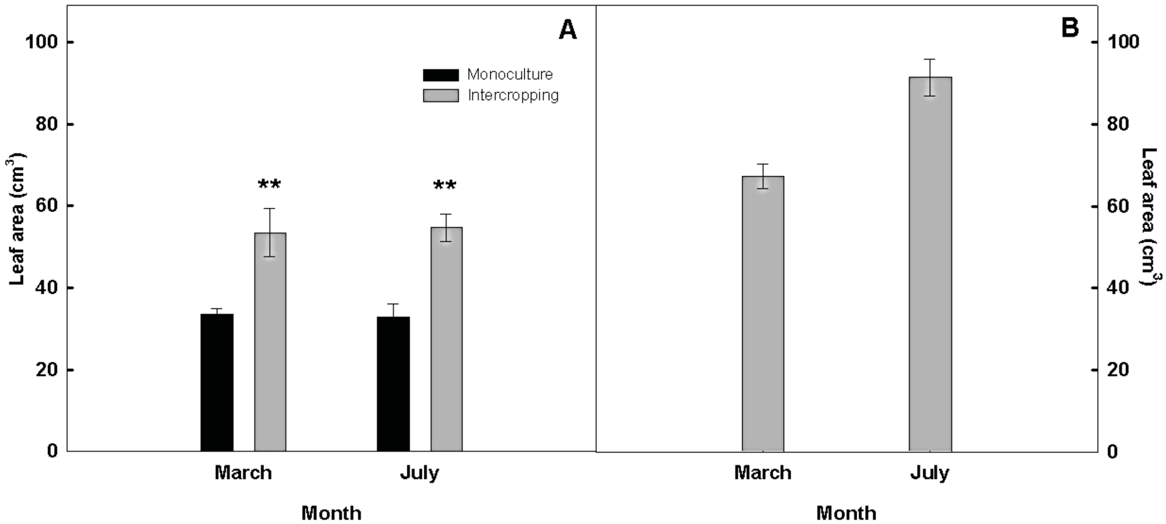

Figure 7. (A) Mean leaf area per leaf of rubber trees planted without (black vertical bar) and with Mucuna (grey vertical bar). (B) Leaf area per leaf of Mucuna (*Mucuna bracteata*) planted in between rows of rubber trees. The measurements were taken in March (dry season) and July (rainy season) 2015. The values are means ± SE (n=4). **represented the means significantly different at p≤0.05.

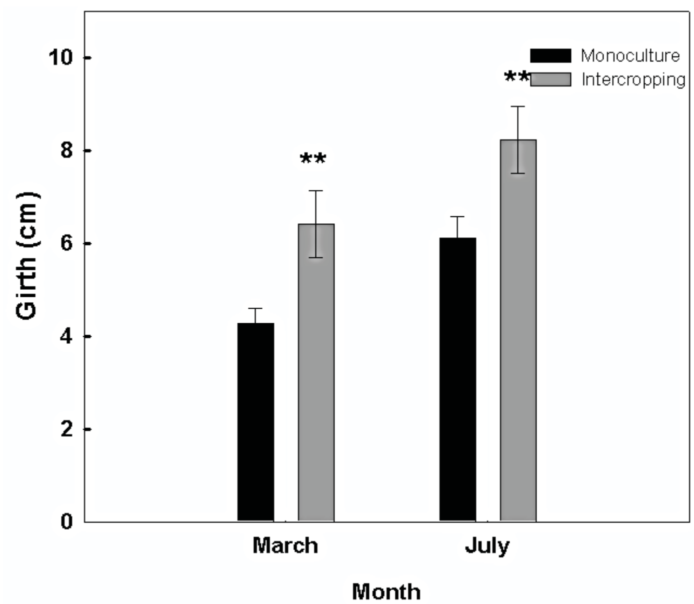

Figure 8. Girth of two-year-old rubber trees measured at 1 m from ground. The rubber trees are planted with (black vertical bar) or without (grey vertical bar) the cover crop Mucuna. The measurements were taken in March (dry season) and July (rainy season) 2015. The values are means ± SE (n=4). ** represented the means significantly different at p≤0.05.

Discussion

The objective of this present study was to compare physiological parameters such as photosynthesis and growth between rubber trees planted with or without cover legumes and to assess the advantage of leguminous cover crop, M. bracteata planted with young rubber tree.

First, in regards to microclimatic condition, the intercropping cultivation of rubber trees with M. bracteata reduced soil temperature and increased soil moisture content. The differences were statistically significant both dry and rainy seasons particularly in the area where the grounds are covered with M. bracteata canopy (2 m from the tree line). Chariachangel (6,8) reported that leguminous cover plants planted with main plants function to improve soil structure that leads to increase aeration and maintain moisture content. Sungthongwisate (9) also suggested that ground covers increase soil moisture retention. Such improvement of soil conditions may improve rubber physiological status and growth. Photosynthetic characteristics of the rubber trees planted with M. bracteata showed high performance in overall PSII efficiency, chlorophyll content and SPAD value compared to those of the single plant stands. The high values of effective quantum yield of PSII efficiency, $\Delta F/F_m$', indicated the high efficiency of light use of rubber trees in natural conditions. $\Delta F/F_m$' of leaves of the rubber trees growing with M. bracteata, showed significantly higher performance than those of the trees growing without the cover crop in the dry season, but not in rainy season. The levels of $\Delta F/F_m$' which indicates the efficiency of PSII under light condition varies depending on light intensity and temperature (14,15). Moreover, $\Delta F/F_m$' values of plants grown in the field also vary depending on the environmental conditions at the time of measurement whether it is the full sunlight or partly cloudy, whether the measurement is done outside or under canopy, the light intensity and the leaf temperatures (10). In our studies, we found that air temperature and light intensity near the canopy of the rubber trees without cover crop at 30 cm from tree line were 42 ^oC and 1,571 µmol photon $m^{-2}s^{-1}$; and at 2 m from tree line 41.1 ^oC and 1,433 µmol photon $m^{-2}s^{-1}$, respectively, in dry season. In the rainy season, air temperature and light intensity near the canopy of the rubber trees with cover crop at 30 cm from tree line were 33.4 ^oC and 460 µmol photon $m^{-2}s^{-1}$, respectively; and at 2 m from tree line 33.5 ^oC and 582 µmol photon $m^{-2}s^{-1}$, respectively. It can be seen that the air temperature in the plot with and without M. bracteata in both seasons were not significantly different. However, there was significant difference in light intensity near the canopy of the rubber trees growing with and without M. bracteata. The difference in light intensity in the canopy of rubber trees growing with and without the cover crop resulted in the difference in $\Delta F/F_m$' values particularly in the dry season. This study showed that the $\Delta F/F_m$' values are positively related with the chlorophyll content in the leaves of rubber trees. Higher contents of Chl a, Chl b and total chlorophyll were found in the rubber trees with M. bracteata compared to those in the single crops.

Chlorophyll is a typical pigment in photosynthetic organisms and Chl a and Chl b are major pigments that are found in green plants (16). Gitelson (17) reported that the chlorophyll fluorescence ratios are linearly proportional to chlorophyll content. As a result, the chlorophyll content and chlorophyll fluorescence (as an indicator of photosynthesis of PSII efficiency) are related and both of them are related to leaf nitrogen content. Saarinen (18) showed that nitrogen content in needles of Pinus sylvestris is positively correlated with chlorophyll contents. In addition, soil nitrogen content also relates to leaf nitrogen content. Yahan et al. (19) reported that concentration of leaf nitrogen and phosphorus in 386 woody species at 14 forest sites across eastern China exhibited positive correlation with the concentration of soil nitrogen and phosphorus. Generally, leguminous cover crops supply the amounts of nitrogen needed for various plants including rubber trees (20). Cover legumes improves soil physical properties, increases soil organic matter and also influence rubber productivity. M. bracteata, is a legume used as a cover in rubber tree or oil palm plantations to enhance soil organic matter, supply nitrogen, control soil erosion and increase biomass production (6,8,9). Kothandaraman et al. (21) showed an increase in the contents of total N, available K and soil organic carbon at 30 cm depth in three-year-old rubber tree intercropped with M. bracteata. Also, M. bracteata as a ground cover under oil palm increase soil fertility (22). Kaewjumpa et al. (23) found that soils under the rubber tree planted with M. bracteata exhibited higher N content and non-significant increase in organic matter, organic carbon and exchangeable K. Moreover, legume cover crops provide N_2-fixation and N transfer to the trees. N_2 fixation by legume cover crops depends on soil, climatic condition and legume species (24). Clermont-Dauphin et al. (25) reported that N_2 fixation by Pueraria phaseoloides transferred to rubber tree exhibited highly relationship with tree leaf N. Its N fixation varied from 39% to 46% of tree leaf N.

In addition, a large part of N in the leaves was also found in Rubisco enzyme. Rubisco is an enzyme link between photosynthetic carbon reduction (carboxylation) and photorespiratory carbon oxidation (oxygenation) cycle. The rate of carboxylation (V_c) and oxygenation (V_o) depends on the amount of activated enzyme that are indicated by the maximum velocity (V_{Cmax} and V_{Omax}) (26). Moreover, the maximum of electron transport rate (J_{max}) is also a factor to limit carboxylation rate at the ribulose-1,5-bisphosphate (RuP_2) saturated rate (27). It meant that M. bracteata provided the micronutrients e.g. nitrogen for the enhancing of growths in both leaf area and girth of rubber tree.

Conclusions

In this study, we report for the first time a direct evidence of the beneficial effects of a legume cover crop (M. bracteata) growing with young rubber trees on increasing photosynthesis performance as indicated by an increased PSII efficiency under light adapted condition. Moreover, higher photosynthetic capacity correlated well with higher chlorophyll content and better growth of rubber trees compared with those growing without M. bracteata. It may be concluded that this cover legume provides a favorable microenvironment and additional soil nitrogen for the young rubber trees. This intercropping system can be suggested as an economical and environmentally friendly method for enhancing growth of rubber trees in the Northeast of Thailand.

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