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Adaptive Ecophysiological Characteristics of Leaves and Root Distribution of Robusta Coffee Saplings as Affected by Age of Rubber Trees under an Intercropping System

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ABSTRACT

Robusta coffee has been grown traditionally in Southern Thailand. This study aimed to evaluate the adaptation in the growth development of sole Robusta coffee (*Coffea canephora*) saplings and rubber-Robusta coffee saplings intercropping (8- and 16-year-old rubber plantations) planted between the shaded and unshaded conditions. The results showed that capturing light by sole Robusta coffee saplings (100%) was greater than those recorded in the 8- and 16-year-old rubber plantations (93 and 78.22%) over the measurement period. Soil moisture content was markedly higher in both rubber sites (6.34 and 7.25%) throughout the growing season compared to the full sunlight condition. In addition to the rubber canopy, the Leaf Area Indexes (LAI) over the entire period varied from 0.35 to 3.29 and 0.38 to 2.33 for the 8- and 16-year-old rubber plantations, respectively. Results at 18 months after growing also indicated that Robusta coffee saplings grown in full sunlight had higher values of new leaves, and canopy width also had higher values than those of the other two treatments based on rubber intercropping. Meanwhile, no significant differences in photosynthetic pigments (Chl_a, Chl_b, Chl_a/Chl_b, Chl_{total}, and Car) were observed between the 8-year-old rubber plantations compared to the full sunlight coffee trees. For the root competition, there were significant differences in fine root length in the 20 and 40 cm layers, which were greatly found in 18 months old coffee plants under direct sunlight. Meanwhile, the older rubber trees (16 years) showed a high density of root spread penetrated to all soil depths compared to younger rubber trees (8 years). Thus, these results indicated that the growth potential of Robusta coffee saplings was strongly limited by a combination of shaded and root competition conditions under rubber plantation. Changes in the leaf and root structural traits of Robusta coffee saplings under the conditions of rubber ecological plantations would be valuable for manipulating the efficient growth of coffee for long-term rubber-based intercropping systems.

Keywords: Morpho-physiological traits, Root competition, Rubber ecosystems, Shade tolerance.

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INTRODUCTION

In rubber plantation, many weather conditions influence the intercrop production such as PAR (Photosynthetically active radiation), light transmission, air temperature, relative humidity, rainfall, wind, leaf wetness, and evapotranspiration (Ebi *et al.*, 2009). Meanwhile, light is probably the most limiting factor that controls the productivity of the intercrops at different patterns. As sunlight passes through, the tree canopy is altered by leaf absorption for the changing seasons. The proportion of direct and diffuse light might vary with the relative light transmission by increasing light intensity. For example, intercropping economically important crops in rubber plantation had

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limited access to sun light for growth when crop plants were not developed enough to capture available light sources under the heavy shade of rubber canopy (Rodrigo *et al*., 2001). Similarly, the fractional interception of radiation during five- to twenty-year-old sole rubber trees was approximately 20% (Wilson and Ludlow, 1990).

Also, the root competition among tree species was likely more important in the aspects of competitive intensity and soil resources related to cropping systems (Xu *et al.*, 2013). During lifespan, the structure and distribution of roots in the underground part of rubber plantation reflected individual competitive ability among trees and intercropping to soil moisture and nutrients, in particular at times when the availability of resources became limited (Wu *et al.*, 2016; Zribi *et al*., 2017). Generally, intercropping is one of the multiple cropping systems involving growing two or more crops in proximity. Meanwhile, the rubber-based intercropping system is also one of the major agroforestry systems in Southern Thailand because of the economic returns and sustainability aspect (Somboonsuke and Cherdchom, 2000). An intercropping system of smallholding rubber-based farming systems is also managed for the crop grown widely in Southern Thailand. Following the low production price, smallholders have had to adjust their attitudes towards their traditional ways of farming to improve farm efficiency and productivity, especially, rubber-intercropping-farming system (Somboonsuke and Shivakoti, 2001).

Coffee is one of the most widely consumed beverages throughout the world. Moreover, in Southern Thailand, Robusta coffee (*Coffea canephora*) is a major species of economic value that represents 70% of the coffee production in all regions of Thailand (Office of Agricultural Economics, 2018). Robusta coffee plant is a small understory woody crop and is naturally shade tolerant. Coffee plant evolves in the undergrowth of high tree density (Da Matta *et al*., 2010). Although low light transmission becomes more evident in vegetative growth which often grows lower than full sunlight, coffee has shown the high-quality yield to the shady environment (Da Matta, 2004).

This is particularly important for intercropping competition for similar growth resources such as light, water, or nutrients. However, the distribution of roots in association with competitive shade tree and crop species has received rather less attention because roots are usually underground and far more difficult to be studied than those of the above-ground parts e.g. between *Eucalyptus deglupta* and coffee (Schaller *et al*., 2003). Consideration of root system development should be an important issue for successful intercropping practices. This study aimed to focus on vigorous root growth and development concerning the distribution of rubber roots at different-age plantations. In this study, low light transmission and coffee trees intercropped with rubber trees might show effective similarity. Then, an experiment was established to evaluate the growth development in shaded coffee saplings compared with those under full sunlight based on rubber-intercropping-farming system in Southern Thailand.

Therefore, studying the light transmission and spatial distribution of fine root of both rubber and Robusta coffee saplings was important for a better understanding of the competitive mechanisms between those two components in the rubber-based-intercropping system and for taking further steps to minimize competitions in such rubber agroforestry system. To contribute more ecologically and economically sustainable coffee agroecosystems, this study aimed (i) To assess the growth performance of intercropping under immature and mature rubber plantations and (ii) To evaluate the consequences of phenotypic differences in the interactions between the intercropping and their environment at three field conditions under rubber-based production system of smallholders growing Robusta coffee.

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MATERIALS AND METHODS

Experimental Sites and Plant Materials

The experiment was carried out on the rubber plantation of rubber-based smallholders, located at Satun Province, Southern Thailand (latitude N 6° 50' 25.1, longitude E 100° 37' 10.6, altitude 42 m above sea level). The experiment was conducted under three different plots: sole Robusta coffee (T1), rubber-Robusta coffee intercropping (8-year-old rubber plantation) (T2), and rubber-Robusta coffee intercropping (16-year-old rubber plantation) (T3). The planting distance of rubber trees between the rows was 3.0×7.0 m for 8 and 16 years old rubber trees under a planting density of approximately 476 trees ha^{-1}. Robusta coffee saplings with 6 pairs to 8 pairs of leaves grown from seed were planted in September 2014 for 30 trees per plot in the rubber inter rows. Then, Robusta coffee trees were planted in a single row at a distance of 3.0 m.

Vegetative Growth Measurement

All the vegetative development records of the coffee saplings were taken in March, June, September, and December 2015; and in March 2016 at the growth periods of 6-, 9-, 12-, 15-, and 18-month-old coffee saplings, respectively. The vegetative characteristics of the coffee saplings were evaluated in five replicates per treatment. On these samples, height, canopy width, stem diameter, and several new leaves per plant were recorded.

Rubber Tree Measurements

For the growth data, twenty sample rubber trees were randomly selected in each site.

The trunk girth at breast height (at 130 cm above ground level) was 61.25±4.76 cm and 76.28±5.98 cm in the 8 and 16 years old rubber trees, respectively. Leaf Area Index (LAI) was estimated using the hemispherical photography and was photographed with a Nikon Coolpix 8400 camera with NikonFC-E8 fish-eye lens and captured vertically upward from beneath the canopies. All pictures were analyzed using the GLA software (Frazer *et al*., 1999). LAI variations were observed once a month during the shedding and flushing period.

Climatic and Soil Data Collection

The meteorological data were collected from the agricultural meteorological station located in Satun Province. Monthly datasets of air temperature (maximum and minimum temperatures), total rainfall, total evapotranspiration, and relative humidity were collected to observe the local weather at the experimental site from January 2015 to June 2016. The microclimate data were recorded in two rubber plantations with the varying influence of the environmental conditions on the Robusta coffee saplings. Under a plantation, variations of light intensity (μmol m^{-2} s^{-1}), temperature (°C), and relative humidity (%) were continuously recorded at the same location. The hourly datasets of air temperature (Temp), and Relative Humidity (RH) were monitored continuously by the data logger of a micro weather station (H21-002 Data Logger, Onset HOBO, Massachusetts, USA) with a 12-bit temperature smart sensor (S-TMB-M006). Also, the light intensities above the canopy of Robusta coffee saplings were recorded using a light meter (Sun system, USA) at 11.00-13.00 hours on a sunny day and 5 positions inter rows and were recorded every month. To determine soil moisture (%), the study randomly took five soil samples from each site once a month using a soil auger at interval depths of 20 cm. All soil samples were oven-dried at 105°C for 72 hours. The soil texture at the depth of 20 cm was classified as sandy clay loam. Mean soil total nitrogen, available phosphorus, available potassium, organic matter, and pH

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were 0.10±0.02%, 1.52±0.65 mg kg^{-1}, 75.50±22.13 mg kg^{-1}, 2.14±0.48%, and 4.34±0.35, respectively.

Leaf Traits Measurements

Six plants per treatment were used for the leaf traits measurements. Fully expanded leaf pairs were selected and extracted with *N,N*-DiMethylFormamide (DMF) to determine the individual "Chlorophyll a" (Chl_a), b (Chl_b), total Chlorophyll (Chl_{total}) (mg cm^{-2}), and total Carotenoids (Car) (mg cm^{-2}) calculated using equations developed by Inskeep and Bloom (1985) and Wellburn (1994). Leaf dry mass was determined after drying in an oven at 65°C for 48 hours. For nitrogen analysis, leaf tissues were assayed for total Nitrogen (N) on a leaf-area basis (N_a) using the Kjeldahl method. Leaf Mass per unit Area (LMA) was calculated as the ratio of leaf dry mass to the surface area.

Also, the fresh leaf sample residues were oven-dried at 65°C for 48 hours. Ground and dried tissues were used to analyze the amount of leaf carbohydrate content by the anthrone test according to analytical methods of total Non-Structural Carbohydrate (TNC) described by Osborne and Voogt (1978). The absorbance of the extracts was determined at wavelengths of 647 and 664 nm with a UV spectrophotometer (Ultraspec 3000 UV/Visible, Pharmacia Biotech Inc., USA)

Fine Root Measurements

The mini rhizotron technique (PSU-ARDA Minirhizotron, Thailand) was used for monitoring fine root distribution (< 5 mm diameter) dynamics of rubber-Robusta coffee interaction described by Saelim *et al.* (2019). The acrylic tubes (100 cm length and 10 cm outside diameter) were installed at a 45° angle from vertical alignment from soil surface level at distances of 40 cm from the Robusta coffee tree. Two months after installation, the root images were captured by a high-quality web camera (Logitech HD C905) that was inserted into the acrylic tube operated by using a USB cable connected with a laptop. The resolutions of 640×480 pixels were set for the digital root image. All root images were processed using root image analyzer (Rootfly) (Clemson University, Clemson, SC, USA) software for calculating root length per area and root diameter. Repeated observations were recorded for an individual plant in every acrylic tube. New roots and longevity were recorded.

Moreover, other sampled roots between Robusta coffee and rubber trees were also collected by the PVC core sampling technique. Root samples were taken from 3 plants per plot of Robusta coffee saplings. Three columns per tree of 40-cm-long PVC tubes (10 cm diameter) were set up on opposite sides. Finally, the root dry mass per soil volume of each coffee sapling was calculated every month at the same location surrounding the PVC tubes.

Statistical Analyses

Statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, USA). To estimate the differences among three different plots, one-way Analysis Of Variance (ANOVA) was used to compare the mean differences among three different plots for all measured parameters. All treatments were tested using ANOVA and LSD multiple comparisons tests ($P \leq 0.05$).

RESULTS

Climatic and Soil under Rubber Plantation Ecosystems

The climatic data at the experimental site during the study period from 2015 to 2016 (Figure 1) showed that there were two dry periods. First, a short dry period occurred from January to March 2015. Then, the second was followed by a longer dry period

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from December 2015 to April 2016. The heavy rainfall events occurred from July to November 2015 (ranging from 288.90 to 587.90 mm). Evapotranspiration reached a peak in March 2016 with a total monthly amount of 181.80 mm. However, there was a range of monthly maximum Temperature (Tmax), minimum Temperature (Tmin) and Relative Humidity (RH) from 29.78 to 37.40°C, 22.89 to 25.30°C, and 63.69 to 83.71%, respectively.

The monthly continuous incident PAR is shown in Figure 2. The high values of monthly PAR in T1 could be observed in 2015 (January, February, June, July, and November) and in 2016 (January to April, and June) with the maximum monthly PAR in the sunny day reaching up to 1,972.40 μmol m^{-2} s^{-1} (Figure 2a). PAR values in T2 ranged from 24.00 to 242.60 μmol m^{-2} s^{-1} during 2015, which were lower values than those from January to June 2016 (40 to 669.00 μmol m^{-2} s^{-1}). However, there were similar trends in T3, which were 68.60 to 839.80 and 142.80 to 652.00 μmol m^{-2} s^{-1} recorded from August 2015 to December 2015 and January 2016 to June 2016, respectively. Under rubber plantations, however, light intensities decreased to 7.00 and 21.78% in the 8- (T2) and 16- (T3) year-old rubber plantations compared with those of the sole Robusta coffee (T1) (100%).

During the same period, soil moisture declined through the summer (January to March 2015, and January to April 2016). Soil moisture was at higher levels in April and June to December 2015 (ranging approximately 20 to 40%) and then decreased gradually until March 2016, and then soil moisture was again higher in May and June 2016. Although slight differences were found among sites, soil moisture values were markedly lower in the sole Robusta coffee (T1) site (6.34 and 7.25% compared to under rubber plantations, respectively) than those of both the 8- (T2) and 16- (T3) year-old rubber sites throughout the growing season. The lowest values of monthly soil moisture were observed with similar patterns of soil moisture of all 3 sites (ranging from 6.13 to 14.32%).

The seasonal dynamics of the hourly air temperature and hourly relative humidity under rubber plantation (T2 and T3) throughout the growing season of 2015 to 2016 are shown in Figure 3. The hourly

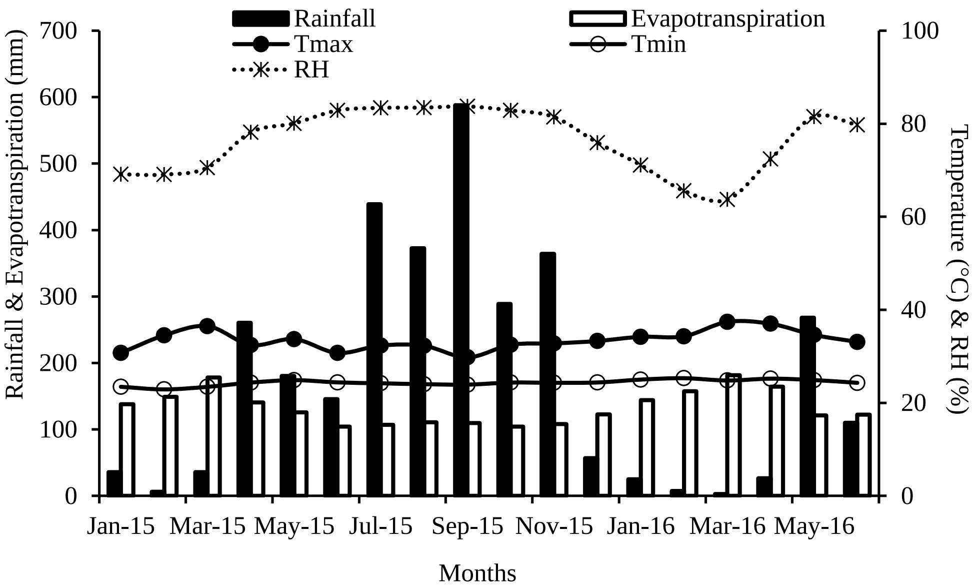

Figure 1. Monthly changes in total rainfall, total evapotranspiration, air temperature (maximum and minimum temperatures), and relative humidity in the experimental site at Satun Province, during January 2015 to June 2016.

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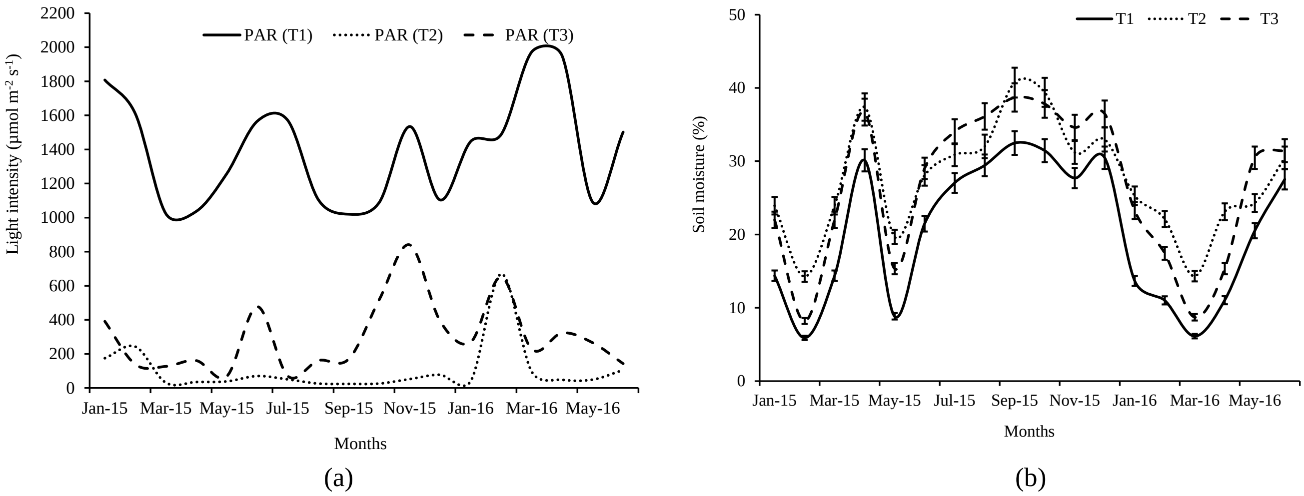

Figure 2. Monthly changes in light intensity (PAR) (a), and soil moisture at 0-20 cm soil depth (b) in Robusta coffee orchard (full sun) (T1), 8- (T2) and 16-year old (T3) rubber plantations during January 2015 to June 2016. Values represent the mean and standard derivation.

means of air temperature in T2 and T3 ranged from 20.96 to 43.30°C and 21.20 to 37.34°C from August 2015 to June 2016. Meanwhile, the ranges for the hourly means of RH were 30.10 to 100.00% and 38.79 to 100.00%, respectively (Figures 3-a and -b).

Changes in Growth of Robusta Coffee Saplings

Significant differences in the growth parameters of Robusta coffee saplings were found among the three different conditions (Table 1). The study found that height, canopy width, stem diameter, and new leaves increased steadily from 6 months to 12 months after growing. The total growth in the sole Robusta coffee (T1) planting had significantly greater canopy width and stem diameter than those of the other two treatments based on rubber intercropping (T2 and T3). However, no significant difference was found in height between full sunlight and shaded saplings. While new leaves were not significantly affected by rubber plantation conditions at 9, 12, 15, and 18 months after growing. The height of Robusta coffee saplings under 8-year-old rubber plantation (T2) showed similar trends with T1 from month 6 to month 18 (63.66±11.22 cm to 105.75±8.99 cm). Meanwhile, the study found that the canopy width and stem diameter had the highest values in the T1 by the end of this study.

Adaptive Physiology of Leaves in Robusta Coffee Saplings

In 2015, the highest values of photosynthetic pigments (Chl_a, Chl_b, Chl_{total}, and Car) were found in T1 (24.37±0.47, 13.49±0.37, 36.51±1.08, and 1.61±0.08 mg cm^{-2}), which were higher than those of the rubber plantation sites (Table 2). Also, the adaptive physiological parameters of FW, DW, LMA, N_a, Chl_a/Chl_b, and Chl_{total}/Car of Robusta coffee leaves were not significantly affected by different environmental conditions. However, these parameters were significantly different in 2016. For example, T2 showed the highest value of FW (3.00±0.24) compared to other treatments with a highly significant difference. Moreover, the lowest values of DW (0.29±0.10), N_a (0.08±0.03), and Chl_a/Chl_b (2.12±0.07) in T3 were significantly different compared to T1 and T2.

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Table 1. Comparative growth of Robusta coffee saplings in full sunlight (T1), under 8-year old (T2) and 16-year old (T3) rubber plantations during 6 to 18 months old.[a]

Treatment	Age (Month)	Height (cm)	Canopy width (cm)	Stem diameter (cm)	No of new leaves
T1	6	69.33±11.89ns	40.15±10.03a	0.87±0.05a	6.50±1.26ab
T2		63.66±11.22	28.45±9.21b	0.73±0.09ab	10.25±0.85a
T3		60.57±11.52	23.75±4.11c	0.45±0.27b	3.40±1.47b
T1	9	82.22±5.51a	67.33±12.29a	1.12±0.22a	46.40±12.44a
T2		79.63±4.56a	34.72±8.66b	0.71±0.16b	9.60±3.21b
T3		60.20±4.02b	28.86±7.61b	0.68±0.24b	7.00±1.87b
T1	12	101.80±15.10a	92.20±8.02a	1.62±0.24a	100.80±27.62a
T2		87.40±19.83ab	43.20±9.31b	1.09±0.24b	15.00±5.29b
T3		64.00±11.14b	26.40±6.77c	0.86±0.23b	9.60±3.85b
T1	15	104.00±5.09a	93.44±7.74a	1.65±0.44a	76.50±2.75a
T2		102.35±15.47a	44.57±5.82b	1.13±0.27ab	13.90±2.73b
T3		66.20±14.90b	28.98±11.97b	0.87±0.06b	9.22±3.09b
T1	18	105.12±8.77a	50.30±6.15a	1.79±0.43a	8.40±2.07a
T2		105.75±8.99a	26.00±4.24b	1.15±0.05b	4.80±2.77b
T3		67.14±7.07b	11.33±1.89c	0.89±0.12b	3.40±1.02b

[a] Means (±SD) followed by the same letter in each column have no significant difference by LSD ($P \leq 0.05$). ns= No significant difference.

Regarding 2015 - 2016, a decrease of Chl_a, Chl_b, Chl_{total}, total N, Chl_{total}/Car, and C/N ratio in Robusta coffee leaves of all treatments happened in the second year of growth. Comparing to the unshaded leaves (T1), the study found slight increases in total N and Chl_{total}/LA observed in 2016. However, Chlorophyll contents (Chl_a, Chl_b, and Chl_{total}) and TNC had lower values following the seasonal change in 2016. Meanwhile, the changing values of *Car* increased.

Seasonal Variations of Leaf Area Index in the Rubber Plantation

Figure 4 presents the monthly dynamic of LAI for the 8- and 16-year-old rubber plantations as measured by a fisheye lens for the whole period of measurement during 2015-2016. LAI measurement in T2 varied from 0.35 in February 2015 to 3.29 in September 2015. Meanwhile, the monthly LAI measurement in T3 ranged between 0.38 and 2.33. Variability in LAI dynamics of T2 decreased in the summer (February 2015) and sharply increased from March to September 2015. However, a dramatically reduced LAI was found in the rainy season (October to December 2015). Leaf fall was observed and followed by a distinct decline at the beginning of leaf flushing from February to March 2016. Meanwhile, monthly range of LAI was lower in T3 than those of T2 from August to December 2015. Leaf fall in T3 occurred in late December 2015 to January 2016. Leaf flushing in T3 appeared in February 2016 (0.38) and continued to increase rapidly in March 2016 after the leaf lamina had fully expanded.

Distribution of Fine Root Growth and Dry Weight in the Rubber Plantation

Vertical distributions of root lengths and diameters in Robusta coffee seedlings are shown in Figure 5. The total root lengths from the observation tubes occurred in the top 20 cm and 40 cm of the soil, which were greatly found in 18 months of all treatments. The root lengths of Robusta coffee were slightly higher in the sole plant than those in under-shade plants, which were similar

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Table 2. Comparative leaf traits of Robusta coffee saplings in full sunlight (T1), under 8-year old (T2), and 16-year old (T3) rubber plantations during 2015 and 2016.[a]

Parameters	2015			2016		
	T1	T2	T3	T1	T2	T3
LA (cm^2)	60.42±7.60b	88.03±13.46a	49.90±1.00b	70.88±27.06b	134.40±40.63a	74.37±48.15b
FW (g)	1.70±0.45ns	1.75±0.27	1.00±0.17	1.65±0.07b	3.00±0.24a	1.18±0.30b
DW (g)	0.46±0.12ns	0.48±0.07	0.27±0.01	0.42±0.01ab	0.60±0.05a	0.29±0.10b
Chl_a (mg cm^{-2})	24.37±0.47a	19.70±0.32b	14.45±0.19c	12.71±4.42ab	14.37±2.58a	7.94±1.09b
Chl_b (mg cm^{-2})	13.49±0.37a	10.22±0.28b	6.78±1.84c	5.06±1.74ab	5.66±0.95a	3.73±0.21b
Chl_{total} (mg cm^{-2})	36.51±1.08a	29.30±0.81b	21.29±5.42c	18.04±1.90a	20.37±2.32a	11.77±0.45b
Car (mg cm^{-2})	1.61±0.08a	1.30±0.05b	0.97±0.20c	2.70±0.29a	3.07±0.37a	1.79±0.05b
TNC (mg g^{-1} dry wt)	42.64±1.43a	32.86±1.84b	35.47±4.02b	28.03±3.41ns	12.35±0.85	20.99±5.69
Total N (%)	2.68±0.02b	3.04±0.02a	2.10±0.02c	4.30±0.03a	3.60±0.08b	1.64±0.01c
LMA	0.007±0.003ns	0.005±0.003	0.005±0.001	0.006±0.001ns	0.005±0.001	0.003±0.001
N_a	0.21±0.10ns	0.21±0.15	0.12±0.01	0.21±0.07a	0.14±0.08ab	0.08±0.03b
Chl_{total}/LA	0.23±0.03a	0.16±0.02b	0.14±0.02b	0.24±0.17ns	0.16±0.07	0.18±0.07
Chl_a/Chl_b	1.86±0.01ns	1.88±0.01	1.89±0.05	2.49±0.05 a	2.53±0.01a	2.12±0.07b
Chl_{total}/Car	16.23±0.15ns	16.94±0.11	17.00±0.16	6.66±0.10ns	6.68±0.06	6.56±0.09
C/N ratio	15.99±0.62a	10.83±0.67b	16.93±1.84a	6.50±0.75b	3.49±0.32b	12.82±3.31a

[a] Means (±SD) followed by the same letter in each column have no significant difference by LSD ($P\leq 0.05$). ns= No significant difference.

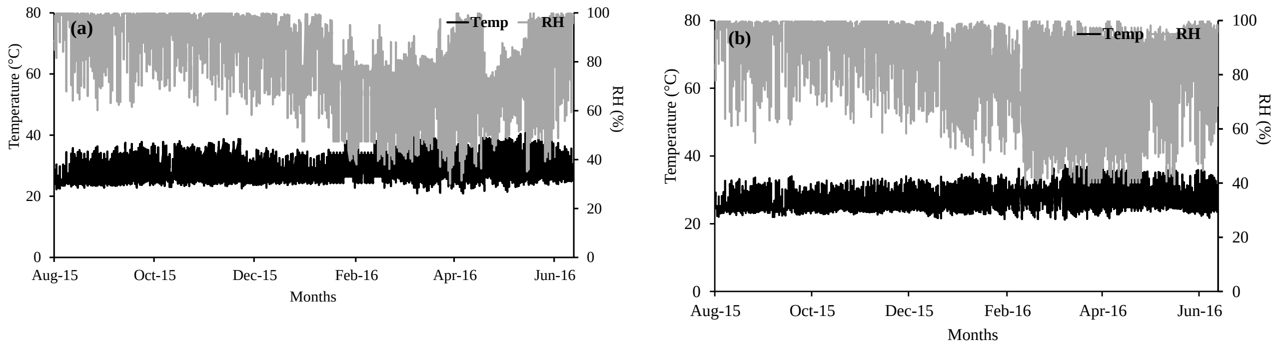

Figure 3. Changes in temperature and relative humidity in 8- (a) and 16-year old rubber plantations (b) during August 2015 to June2016.

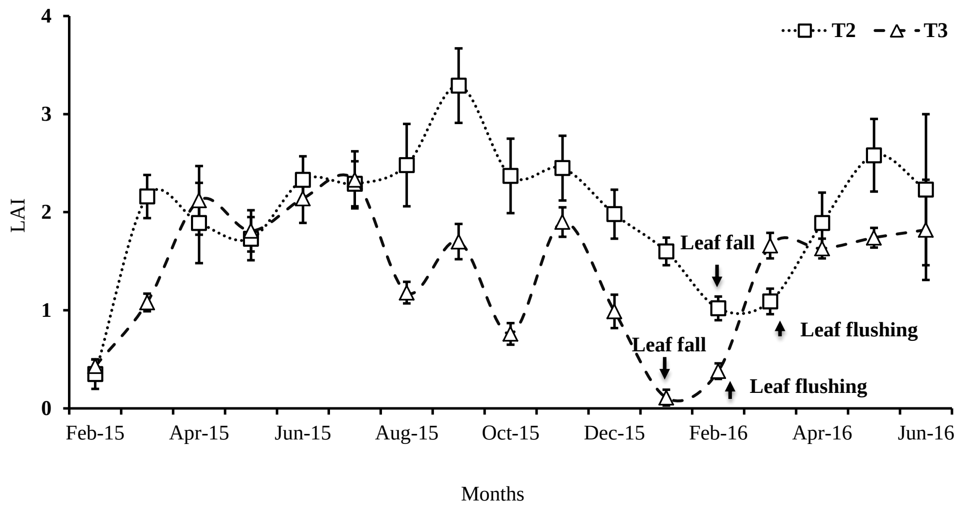

Figure 4. Monthly changes in Leaf Area Index (LAI) during periods of leaf fall and leaf flushing in 8- (T2) and 16-year old (T3) rubber plantations from February 2015 to June 2016. Values represent the mean and standard derivation.

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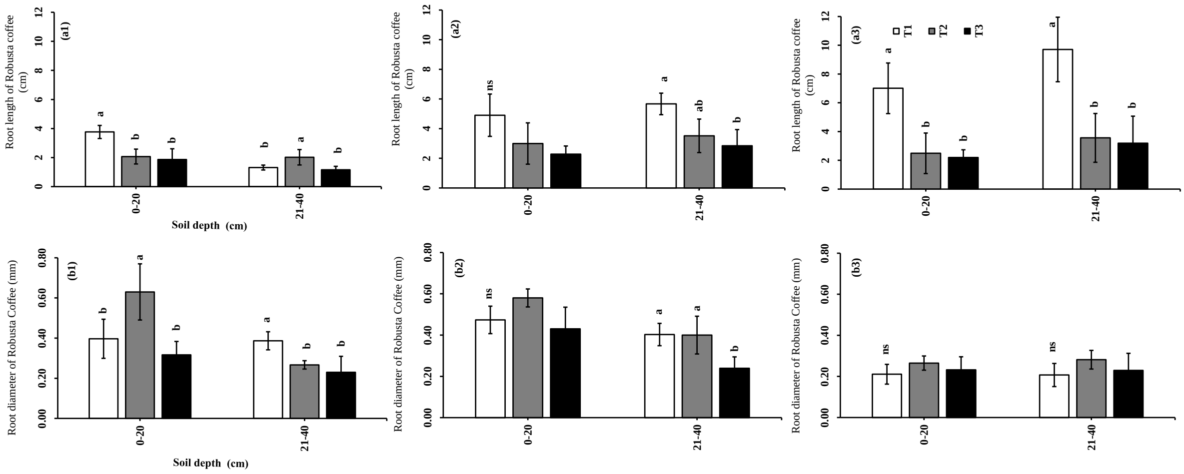

Figure 5. Root length and root diameter at 0-20 and 21-40 cm soil depth of Robusta coffee saplings under full sunlight (T_1) under 8- (T_2) and 16-year old (T_3) rubber plantations during 12- (a_1 and b_1), 15- (a_2 and b_2) and 18-month old (a_3 and b_3). Bars represent the mean and standard derivation followed by the same letter have no significant difference by LSD (P≤0.05). ns= No significant difference.

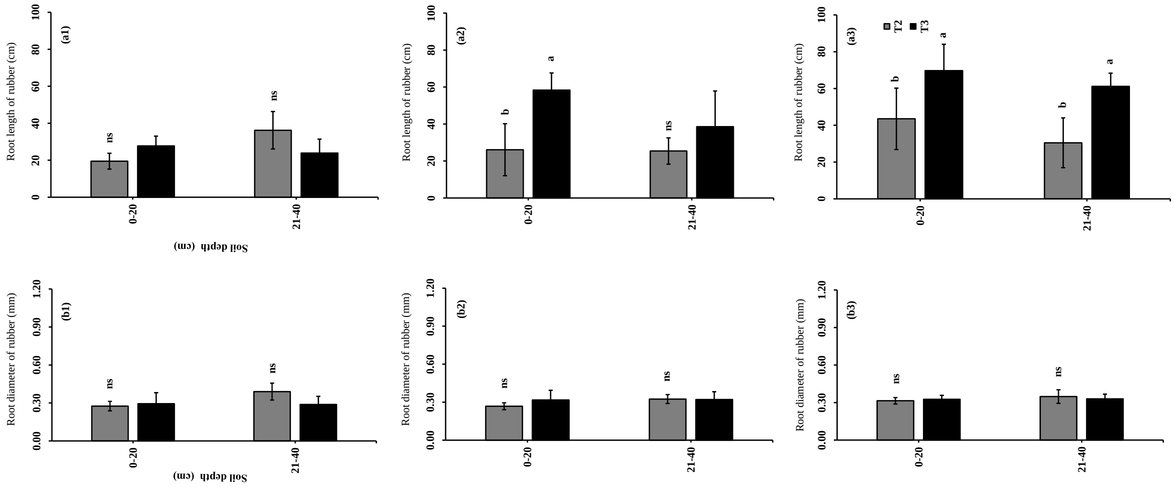

Figure 6. Root lengths and root diameters of rubber trees in 8-year old (T2) and 16-year old (T3) at 0 - 20 cm and 21 - 40 cm soil depth as intercropped with Robusta coffee saplings at 12- (a1 and b1), 15- (a2 and b2), and 18-month old (a3 and b3). Bars represent the mean and standard derivation followed by the same letter have no significant difference by LSD (P≤ 0.05). ns= No significant difference.

between the 15- and 18-month plants (Figure 5; a2 and a3). The root diameters from the observation tubes were similar between the top 20 cm and 40 cm of the soil at the 18-month plants (Figure 5; b2 and b3).

For the fine root distributions of rubber trees, the study also found evidence for the differences in root lengths of rubber trees among the soil layers (Figure 6). There were significant values, especially, for older rubber trees (T3) root increased with the surface area sampled from the 15^{th} to 18^{th} month (Figure 6; a2 and a3). However, the root diameters in the 21-40 cm soil layer were lower than in 0-20 cm. The root diameters of rubber trees were slightly increased with tree ages (in the top 20 cm) but did not change significantly from the 12^{th} to 18^{th} month (Figure 6; b1, b2, and b3).

The root core sampling methods were used to determine fine root biomass in the field. Root dry weight increased between coffee and rubber trees at different horizontal distances from the Robusta coffee saplings intercropped with rubber trees. At each of the growth stages of Robusta coffee saplings, the study found no horizontal root spread in both rubber plantations with an increasing distance of 0-150 cm from the coffee sapling row (Figures 7-a to -d).

DISCUSSION

Effects of Light Intensity on Robusta Coffee Intercropped with Rubber Plantation

There was a negative effect of rubber shade on Robusta coffee potential growth. The growths of coffee saplings were higher under the full sunlight than under shaded rubber plantations during the first and the second year. The shaded Robusta coffee plants showed smaller canopy-size Many reports indicate the higher rate of coffee plant growth under full sunlight conditions (Bote and Struik, 2011). Moreover, sunlight passes through the rubber tree canopies in different LAI. Radiation interception capacity will be altered. There was evidence showing that annual rainfall change influenced phenological shift in both species.

Although Robusta coffee had generally been grown under the shade of an intercropping system, some growth characteristics of Robusta coffee were significantly influenced by the shade of rubber plantation. For example, Robusta coffee saplings showed similar increases in plant heights and stem diameter with increased shading of 8-year-old rubber plantation. However, there was a long drying period from December 2015 to April 2016 when the highest evapotranspiration was 181.80 mm per month, which was higher than the total rainfall by 11.77 mm per month (Figure 1). As a result, drought events caused decreases in canopy size, and new leaves flushing also led to the growth reduction of all coffee saplings during the study from the 15th to 18th month. Furthermore, high light intensity and high temperature occurred during the summer period (January to April). Then, sole coffee saplings might be limited by stomata closure, high leaf temperature, and water stress leading to suppressing the growth and development or the low seedlings survival rate

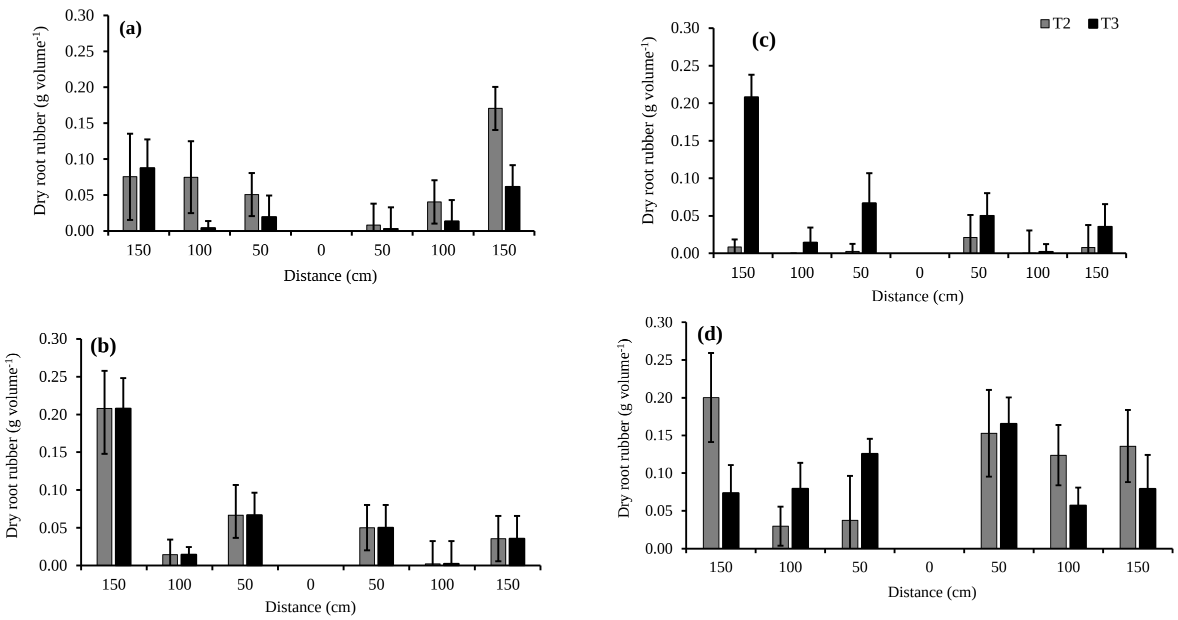

Figure 7. Root dry matter of 8- (T2) and 16-year old (T3) of rubber trees at distances of 50, 100, and 150 cm from Robusta coffee saplings (0 cm) at 9- (a), 12- (b), 15- (c), and 18-month old (d). Bars represent the mean and standard derivation.

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(Franck and Vaast, 2009; Baliza *et al*., 2012). Similarly, growth irradiance levels of coffee plant under approximately 50% shading did not limit the photosynthetic values compared to exposed leaves (Pompelli *et al*., 2010).

Some other studies found higher rates of leaf area under shade than non-shaded coffee plants. For example, leaf area was stimulated under shaded light when adequate amounts of nutrients and water were supplied (Jaramillo-Botero *et al*., 2010). This is consistent with responses observed in coffee intercropped with rubber trees where significant growth gains were achieved by temperature reduction, irradiance level, and increased relative humidity variations (Partelli *et al*., 2014).

This result showed that rubber plantations induced a difference of light transmission rates with the development of canopy. The shaded area of PAR then fell below approximately 80-90% throughout the annual season as down to 7.00 and 21.78% measured in 8- and 16-year old plantations. There was a similarity for rubber plantation that showed a low light transmission between 5-12 years Stür and Shelton, 1990). This may be because Robusta coffee saplings have gradually adapted to grow under low irradiance condition of rubber plantation.

Regarding adapted leaves in the second year (2016), the study found that the lower *LA* of Robusta coffee saplings was obtained from full sunlight condition which was mainly attributed to the higher Nitrogen content found in their leaves (N_a). Furthermore, full sunlight leaves were relative to he ratio of DW (LMA) (Yasumura *et al.*, 2006) and the concentration of total N and TNC (Jaramillo-Botero *et al.*, 2010). Because leaf nitrogen was positively correlated with high irradiance levels, there were changes in morpho-physiological characteristics (Carelli *et al.*, 2006). For photosynthetic pigments (Chl_a, Chl_b, Chl_a/Chl_b, Chl_{total}, and Car), our results are in agreement with Yasumura *et al.* (2006) and Araujo *et al.* (2008) who demonstrated that the increased photosynthetic pigments were lower in high-light leaves than those in low-light leaves. In contrast, Robusta coffee in full sunlight might present Chl_a/Chl_b ratio more than 3.0, whereas the ratio might be lower than 3.0 in some environments (Netto *et al.*, 2005). Although no significant differences in leaf photosynthetic pigments were observed between the shade treatment (8-year-old rubber plantation) compared to full sunlight treatment, this study hypothesized that the variations in photosynthetic pigments were not due to the light conditions as observed in Robusta coffee grown in low nitrogen and water status (Da Matta *et al*., 2002; Pompelli *et al*., 2010). Thus, photosynthetic pigments can be considered as an indicator of plasticity in leaves to different sunlight levels of coffee trees.

Considering the leaf physiological data, the study found that light intensity led to different physiological characteristics of Robusta coffee leaves. Moreover, coffee plants grown in open sun condition could develop leaf burn symptom that would more likely lead to plant stress responses compared with the microclimates under shaded plants, the reason being that shaded condition was often deployed over crops to reduce heat stress (Da Matta *et al*., 1997; Araujo *et al*., 2008). This study showed that light intensity and temperature under rubber plantations was slightly lower than in the open sun, throughout the growing season. Therefore, the success of intercropped productivity for the rubber-based intercropping system depends on the amount of visible light transmission through the canopy. Getting productivity on a long-term basis, effects of light competition on the potential Robusta coffee growth that will contribute to the appropriate design and management of rubber-coffee intercropping systems for more suitability of the existing local agricultural practices.

Effect of Root Competition on Growth of Robusta Coffee as an Intercrop

In the rubber plantation, Robusta coffee saplings under shaded rubber trees were not only influenced by light but also by root competition. Also, the importance of soil depths and widespread root systems of rubber trees could affect Robusta coffee saplings in competing for growth and development.

In both growing seasons, most of the roots observed were in both soil depths at 20 cm and 40 cm with finding that relatively more root growth occurred in the second year. Previous studies indicated that roots of rubber trees could penetrate deeply at least 1.0 m into the ground, and the greatest number of surface feeder roots

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occurred at the inter-row space interfering between soil surface to soil depth at 40 cm (Chairungsee *et al*., 2013). Similarly, fine root length density at soil depths fron 0 to 30 cm was higher than those at other soil depths in mixed coffee and *Erythrina poeppigiana* plantations (Defrenet *et al*., 2016).

There were also more root densities at the between-row area in the older rubber trees (16 years) which had 76.28±5.98 cm of trunk diameter compared with those in the younger rubber trees (61.25±4.76 cm). This study indicated that the variation in root spread could be explained by trunk growth. However, the root extent in the older tree increases very slowly relative to trunk diameter (Day *et al*., 2010). Meanwhile, elongation of the root in the rubber tree may decrease during the tapping period (Chairungsee *et al*., 2013).

Besides, the larger diameter of roots did not change significantly in both soil depths compared to both rubber plantations. Thus, root size distributions observed in minirhizotron tubes were similar to root dry matter distributions obtained by the destructive method as shown in Figure 7.

Under sole Robusta coffee with many sunny day conditions, the saplings were grown individually or at extremely low densities. Saplings often were subjected to a high light level even though the roots were in the lower soil moisture compared to those under the rubber plantation. The response of root length and root diameter to different plantation conditions and soil environmental factors might have effects on the growth and development of the saplings. Similarly, the fine-root dynamics and development fluctuated due to the various environmental conditions in rubber plantation, especially for soil moisture in the rainy season (Saelim *et al*., 2019). Thus, the complex natural soil environments might be more favorable for inducing adventitious root growth and its architecture from the dynamics of root meristem distribution in the soil (Dupuy *et al*., 2010; Hosseini *et al*., 2019).

Meanwhile, the growth of Robusta coffee saplings grown under rubber plantation was virtually affected by competition as the inter-row space received the interference from rubber roots. Rubber trees showed a high density of root spread penetrated to depths of soil from 20 and 40 cm.

However, the considerable variation of root growth competition could be expected due to species and site conditions. For example, root length density of coffee tree intercropped with *Eucalyptus deglupta* had low competition in both horizontal and vertical distributions by increasing distance from the shade tree from 1.5 to 5.5 m, as reported by Schaller *et al*. (2003).

The size of the root system morphology (weight, volume, and surface) usually was reduced while growing at competitive conditions among other crop plants (Padovan *et al*., 2015). Thus, the diameters of the fine roots in Robusta coffee plants were smaller when they were grown in the rubber intercropping system, especially in the 16-year-old plantation that had more intense root spread in the shallow soil layer.

Besides, Robusta coffee roots seemed to have more competition in the soil surface at 20 cm. This evidence of root mechanism indicated that coffee roots were prevented from interfering with the penetration of rubber fine roots. A similar root distribution in soil was observed, which indicated that the competition between Robusta coffee plants and rubber trees could not be avoided. Consequently, the root system of Robusta coffee saplings was restricted by overlapping with the root of rubber trees. This study hypothesized that most of the root growth differences observed in the Robusta coffee saplings under different rubber trees ecological conditions were due to response in the root restriction. The independence of the root growth of Robusta coffee saplings is probably associated with the factors of root competition to acquire water and nutrients in different ages of rubber trees, which was strongly depressed in both shoot and root growth of intercrops (Defrenet *et al*., 2016).

For sustainable reasons, the coffee-rubber intercropping system provides better opportunities to rubber smallholders for long-term production until reaching the log harvesting period of the rubber tree. Although Robusta coffee is often suggested as rubber intercropping to the rubber smallholders, growth limiting factors should be emphasized when Robusta coffee plants are grown in rubber plantations with different ages. Previous studies

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demonstrated that rubber and coffee association in double spaces (16 m) was more profitable than the traditional planting system (Snoeck *et al*., 2013). Hence, widening of rubber row spacing is still needed for further study on the long-term rubber-Robusta coffee intercropping system.

The results suggest that the plasticity in the root distribution of Robusta coffee can be used as an indicator of growth adaptation in rubber plantation conditions. The mechanisms of root dynamics and changing environments are recommended for further intercropping study. Thus, this study provides insights into the potential ecophysiological responses of Robusta coffee plants to manipulate the production under rubber intercropping systems. Also, results of this study will be useful to apply for improving the current agricultural practices for the rubber trees smallholders.

CONCLUSIONS

Under the rubber-based intercropping, variations in the leaf characteristics of Robusta coffee saplings growing in various light transmissions reflect ecophysiological adaptations to changes in the light environments. Based on the light transmission, decreased light intensity caused by the rubber tree canopy could decrease the shoot and root growth of Robusta coffee sapling. This result indicates that the approximately 10-20% of full sunlight under rubber plantations should not be considered as the optimal condition for long-term rubber-based intercropping systems. Concomitantly, the results of this study may help assess the growth potential related to adaptive ecophysiological characteristics in Robusta coffee under light conditions grown in different ages of rubber plantations.

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ویژگی های اکوفیزیولوژیکی سازگاری برگ ها و توزیع ریشه نهال های قهوهRobusta تحت تاثیر سن درختان لاستیک در سامانه کشت مخلوط

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چکیده

قهوه Robusta به طور سنتی درجنوب تایلند کشت می شود. هدف این پژوهش ارزیابی سازگاری رشد و نمو نهال های قهوه Robusta (*Coffea canephora*) به تنهایی و نیز در یک سامانه کشت مخلوط "قهوه Robusta- درختان لاستیک با سن ۸ و ۱۶ ساله " بود که در ختان قهوه در شرایط زیر سایه و غیرسایه کشت شده بودند. نتایج نشان داد که در طول دوره اندازه گیری ها، دریافت نور توسط نهال های قهوه Robusta که به تنهایی کشت شده بود(برابر ۱۰۰٪) بیشتر از آنهایی بود که مخلوط با درختان ۸ و ۱۶ ساله لاستیک (به ترتیب برابر ۹۳٪ و۷۸/۲۲٪) کشت شده بود. در هر دو محل درختان لاستیک در مقایسه با شرایط جایی که تابش آفتاب کامل بود، مقدار رطوبت خاک در طول فصل رشد به طور بارزی بیشتر بود (۶/۳۴٪ و ۷/۲۵٪). افزون بر آستانه گیاهی(canopy) درختان لاستیک، شاخص سطح برگ (LAI) برای

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درختان ۸ و ۱۶ ساله در تمامی دوره زمانی به ترتیب بین ۰/۳۵ و ۳/۲۹ و بین ۰/۳۸ تا ۲/۳۳ تغییر می کرد. نتایج ۱۸ ماه بعد از رشد نیز چنین حکایت داشت که نهال های قهوه Robusta رشد کرده در شرایط آفتابی کامل تعداد بیشتری برگ تازه داشتند و عرض آستانه گیاهی آنها نیز بیشتر از نهال هایی بود که در سامانه کشت مخلوط قرار داشتند. همچنین، هیچ تفاوت معنی داری در پیگمنت های فتو سنتزی (Chl_a, Chl_b, Chl_a/Chl_b, Chl_{total} و Car) بین محل درختان لاستیک ۸ ساله در مقایسه با آنهایی که در تابش کامل آفتاب بودند مشاهده نشد. از نظر رقابت بین ریشه ها، تفاوت معناداری بین طول ریشه های نازک در لایه های ۲۰ و ۴۰ سانتی متری بود که در نهال های قهوه ۱۸ ماهه واقع در زیر آفتاب کامل، فراوان یافت میشد. نیز، درختان لاستیک مسن تر (۱۶ ساله) در مقایسه با درختان جوان (۸ ساله) تراکم زیادی از ریشه که به همه اعماق زمین فرو رفته بودند نشان دادند. به این قرار، این نتایج چنین اشاره داشتند که ترکیبی از شرایط سایه و رقابت ریشه، پتانسیل رشد نهال های قهوه Robusta را در زیر درختان لاستیک به شدت محدود کرده بود. تغییرات در صفات ساختار برگ و ریشه نهال های قهوه Robusta در شرایط اکولوژیکی زیر درختان لاستیک می تواند برای مدیریت کارآمد و دراز مدت رشد نهال های قهوه در سامانه کشت مخلوط ارزشمند باشد.

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