

*Original Article*

## *Hevea* rubber physiological status and relationships under different rubber-based intercropping systems\*

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### Abstract

The study aimed to preliminarily explore the relationship of the rubber tree's physiological status and technological properties with response to different agroecosystem components, especially leaf area index (LAI) and soil moisture contents (SMC) under three types of rubber-based intercropping, notably rubber-bamboo (RB), rubber-coffee (RC) and rubber-melinjo (RM) compared to a rubber monocrop (R). RB and RM significantly showed the highest LAI values of around 1.4, while that of R had the smallest value (about 30% lesser than the highest values). RM, RB dramatically increased the SMC with the soil depths. The rubber-based intercropping farms indicated better biochemical composition in the latex, showing efficient metabolism of the latex biosynthesis. Technological properties of the raw rubber from the rubber-based intercropping farms expressed the premium results in both non-rubber components and rheological properties with higher molecular weights. The correlation analysis revealed some significant relations among the LAI, SMC, biochemical composition and technological properties.

**Keywords:** rubber-based intercropping, leaf area index, soil moisture content, physiological status, technological properties

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### 1. Introduction

*Hevea* rubber, cis-1, 4-polyisoprene, is a secondary metabolite of *Hevea brasiliensis* biosynthesized from sucrose in the laticiferous system for physiological defense mechanism (Jacob *et al.*, 1989). Since the sucrose is the resultant of photosynthesis by consuming natural resources such as sunlight, nutrients and water from the environment, the technological properties of natural rubber are strongly linked to the rubber tree's physiological responses to its environment (d'Auzac *et al.*, 1997; Roux *et al.*, 2000; Van Gils, 1951;).

Conventionally, natural rubber has been sourced from *Hevea* rubber monocrop cultivation, which generates major incomes of the rubber smallholders, for the requirement

of the world rubber consumption. However, commercial rubber monocropping has degraded the environment and natural ecosystem with adverse consequences such as deforestation, agricultural pollutions, changing local climate, and losses of natural resources (Zhang, Yang, & Du, 2007; Ziegler, Fox, & Jianchu, 2009). Moreover, due to the extensive involvement of smallholders as the major rubber producers, socio-economic issues like low income, high production cost, and shortage of workers have been raised associated with instability of rubber price (Fox & Castella, 2013).

Many researches highlighted that the agroecosystem of rubber growing area and socio-economic factors were improved under rubber-based intercropping compared to the rubber monocropping (Chen, Liu, Wu, Jiang, & Zhu, 2019; Elmholt, Schjonning, Munkholm, & Debosz, 2008; Guardiola-Claramonte *et al.*, 2008; Werner *et al.*, 2006; Zhang *et al.*, 2007;). Thus, rubber-based intercropping became a recommended practice for smallholders to reduce the impacts and develop sustainable natural rubber production of the smallholders (Langerberger, Cadish, Martin, Min, & Waibel, 2017; Polthanee, Promkhambut, & Khamla, 2016; Rodrigo, Stirling, Naranpanawa, & Herath, 2001). However, studies

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related to the technological properties of raw rubber sourced from the rubber-based intercropping systems are limited.

In this context, it needs to extend the realization on variation in biochemical composition of latex under the rubber-based intercropping and its relations to the technological properties of raw natural rubber. Therefore, a preliminary study was conducted to investigate changes in the rubber tree's physiological status and the technological properties of raw rubber related to agroecosystem components, especially leaf area index (LAI) and soil moisture contents (SMC), in the rubber-based intercropping systems.

## 2. Materials and Methods

Three farms of rubber-based intercropping: rubber-bamboo (*Gigantochloa nigrociliata*) (RB); rubber-coffee (*Coffee canephora*) (RC) and rubber-melinjo (*Gnetum gnemon*) (RM), and one monocrop rubber farm (R) were selected for the study structured in complete randomized design at Khao Phra village located at Khao Phra village located at 6° 59' N, 100° 08' E, in Rattaphum district, Songkhla province in Southern Thailand. The soil in the area is under the Tha Sae Series which is suitable for *Hevea* rubber, horticulture, and upland crops and vegetables, and generally it is well-drained with moderate permeability (Land Development Department, 2003). Specifically, it is found that the soil in the RC, R and RB are medium to coarse textured with sandy loam, loamy sand and sandy clay loam, while that of RM is fine to medium textured consists of silt loam, clay and silty clay loam.

Rubber trees in the farms were RRIM 600, aged around ten years and planted in 6 m x 3 m spacing on flat land. The trees were tapped in S/3 2d3 tapping system (one-third spiral cut of tapping length with two-day tappings in three days) (Vijayakumar *et al.*, 2009) and their tapping had been implemented for four years. Farms were converted into intercropping in the first year of tapping, and the associated crop was planted between the rubber rows.

Weather data, such as temperature, relative humidity, rainfall, and raining days in the area were monitored by a mini weather station installed in the area. Leaf area index (LAI) of the farms were measured using the hemispherical photography method (Chen, Rich, Gower, Norman, & Plummer, 1997). Fisheye photos were captured by Nikon Coolpix 8400 camera from five different points in each farm. The captured images were analyzed using the GAP Light Analyzer software version 2.0. Soil moisture content (SMC) of the experimental farms were measured by PR2/6 profile probe (Delta-T Devices, Cambridge, UK) at the soil depths of 0-10 cm, 11-20 cm, 21-30 cm and 31-40 cm, respectively from two locations in each farm through three access tubes installed at each location.

Latex samples were collected from ten trees of each plot with three replications from each farm for measuring biochemical composition: sucrose content (Suc), inorganic phosphorus content (Pi), reduced thiols (R-SH) and dry rubber content (DRC). The samples were analyzed using the latex diagnosis method of CIRAD (Gohet & Chantuma, 1999).

From each farm, field latex from ten trees was collected with three replications to investigate the technological properties: ash content, nitrogen (N) content,

initial plasticity ( $P_0$ ), plasticity retention index (PRI), and Mooney viscosity (MV). The test methods followed the RRIM test methods (Tong, 1992). Molecular weights (Mw) of the rubber from the samples were tested using the Gel Permeation Chromatography method with Agilent GPC/SEC software (Agilent Technologies, 2015). Field latex of each farm was also sampled from ten trees by three replications to analyse the average yields (gram per tapping per tree – g/t/t) (Malaysian Rubber Board [MRB], 2009).

All data collections and measurements were taken between May and August of 2020. The data were analyzed in ANOVA and compared by Duncan's multiple range test (DMRT) at  $P \leq 0.05$ . Multiple correlation analysis was applied to investigate relationships among the studied data.

## 3. Results and Discussion

### 3.1 Weather condition

Generally, there are two seasons comprised of the rainy season and the dry season in the area. The average maximum and minimum temperatures are typically 30 °C and 37 °C, respectively. The monsoon rainy season starts from May to January with 2,200 mm of average annual rainfall. The dry season lasts three to four months from January to April with around 6 to 7 hours of average sunshine period per day while the other months have around 4 to 5 hours per day (Thai Meteorological Department, 2019). Table 1 describes monthly weather conditions (from May to August 2020) analyzed from the records of the mini weather station where the experiments were conducted. The total rainfall in the farms' area during the study period was over 900 mm which was accounted for around 40% of the average total annual rainfall. The mean temperatures were relatively lower in June, July and August, compared to that of May. The maximum and minimum relative humidity increased over 95% and 50%, respectively in those three months also.

### 3.2 Soil moisture content

In the soil depth of 0-10 cm, SMCs of R and RC were 21.6% and 23.1%, respectively, and higher than that of RB and RM (Table 2). However, it was noticed that RM showed the highest SOM significantly in the deeper soil layers. It was also observed that SMC of RM and RB increased dramatically with the soil depths. RC could show the highest SMC only in the soil depth of 0-10 cm, but in the other soil depths, its SMCs were at the lowest level compared to that of the other experimental farms. Chen, Liu, Wu, Jiang, and Zhu (2019) reported that rubber-intercropping significantly improved SMCs because of its significant moisture-holding capacity of capillary porosity in the average soil depth due to its better root distribution. The higher SMC in the average deep soil could be the complementary result of a rubber-based intercropping system as the crops shared nutrients, soil water and also the space in the soil (Langenberger *et al.*, 2017; Wu, Liu, & Chen, 2016) that enhanced the root proliferation which improved soil porosity, hydraulic conductivity, water infiltration and soil water holding capacity (Chen, Liu, Jiang, & Wu, 2017; Elmholt *et al.*, 2008).

Table 1. Monthly weather condition in the study area (May to August 2020)

Month	Mean temperature (°C)	Maximum relative humidity (%)	Minimum relative humidity (%)	Rainfall (mm)	Raining days
May	29.4	80	42	304.7	21
June	28.5	96	53	208.9	18
July	28.2	97	51	319.9	22
August	28.3	96	51	83.7	13

Table 2. Soil moisture content (SMC) among the farms

Studied farms	Soil moisture content (%)			
	0-10 cm	11-20 cm	21-30 cm	31-40 cm
Monocrop rubber	21.62 ab	24.09 b	27.88 b	26.12 b
Rubber-coffee	23.06 a	20.73 c	21.58 c	23.66 c
Rubber-bamboo	15.27 b	21.27 c	26.57 b	26.62 b
Rubber-melinjo	12.59 c	30.95 a	34.53 a	40.26 a

Means with different letters in each column are significantly different at  $p \leq 0.05$  and ranked by the DMRT.

Table 3. Leaf area index (LAI) among the farms

Studied farms	Leaf area index (LAI)
Monocrop rubber	1.02 c
Rubber-coffee	1.26 b
Rubber-bamboo	1.44 a
Rubber-melinjo	1.39 ab

Means with different letters in the column are significantly different at  $p \leq 0.05$  and ranked by the DMRT.

### 3.3 Leaf area index

Table 3 represents the LAIs among the farms. RB and RM significantly showed the highest LAI values of around 1.4, followed by RC with 1.26, while that of R was the smallest value, which was about 30% lesser than the highest value. The results of LAIs confirmed that the above-ground vegetative growth of RB, RM and RC were greater than that of the monocrop rubber farm. High LAI values in a multi-story rubber intercropping allowed efficient light distribution through the canopies of the crops in the system, thus a greater light energy capture, ensuring in improved photosynthesis function (Chow, Qian, Goodchild, & Anderson, 1988; Vandermeer, 1992).

### 3.4 Latex biochemical composition and yield

Table 4 depicts the physiological status expressed in biochemical composition, namely Suc, Pi and R-SH contents, DRC of the latex and yield sourced from the farms. It was found that Suc content of R was the highest at 4.87 mM, followed by that of RC with 3.70 mM, while RB and RM showed around 2 mM as the lowest content. RM significantly showed the highest values of Pi with 31.49 mM that was around 96% higher than that of RB. Pi's lowest values were observed in R and RC with about 12 mM, which was 162 and 34% lesser than that of RM and RB, respectively. R-SH contents of RM, RB and RC were 0.80 mM, 0.76 mM, and 0.67 mM, respectively, and higher than that of R, which was the minimum level at 0.62 mM. DRC of RC and R were the highest at 40.02 and 39.44%, respectively, followed by RB

with 36.14%, while RM showed the lowest DRC of 28.93%. Average yields of a tree from the farms were ranged between 30 and 40 g/t. Although yield analysis data did not prove statistical differences among the farms significantly, it was found that R delivered the least yield among the others.

Sucrose is the raw material of rubber synthesis and initiates the generation of energy for synthesis. High content of Suc indicates either the efficient supply of lactiferous rings or low metabolic utilization of sucrose for the rubber synthesis (d'Auzac *et al.*, 1997). Since Pi represents the level of laticifer biosynthetic activity or metabolic utilization of sucrose (Jacob *et al.*, 1989), rubber trees in RM and RB with higher values of Pi and lower values of Suc were under the healthy physiological status with efficient utilization of sucrose for latex production. R-SH are antioxidants that reduce the oxidative stresses resulted from latex harvesting. Thus, the high content of R-SH in RM and RB signaled better physiological status of rubber trees. In contrast, the lowest R-SH value in R depicted the lactiferous system's poor physiological condition or overexploitation of latex from the rubber tree (d'Auzac *et al.*, 1997). High DRC with less yield resulted in the farm R also reflected the less efficient metabolism of latex biosynthesis. DRC represents the laticifers' synthesis activity and regeneration capacity, and the standard DRC from fresh latex ranges between 30 and 35% by weight of latex (Sarah Kumara, 2003). A distinct low level of DRC signals a deficient metabolism or incomplete regeneration process. Likewise, a significant high DRC increases latex viscosity and limits the latex flow, resulting in low latex production (Van Gils, 1951). When the water availability is limited for the lactiferous cells, latex production typically reaches the lowest associated with the high DRC of the latex (Pakianathan, Boatman, & Taysum, 1966). Vijayakumar, Chandrashekhar, and Philip (2000) reported that a high turgor pressure increased the latex flow rate leading to a higher latex volume with a slight decrease in DRC.

### 3.5 Technological properties

In comparing the non-rubber components, namely nitrogen (N) content and ash content, of the raw rubbers from

Table 4. Latex biochemical composition (Suc, Pi, R-SH, DRC) and yield among the farms

Studied farms	Suc (mM)	Pi (mM)	R-SH (mM)	DRC (%)	Yield (g/t)
Monocrop rubber	4.87 a	12.16 c	0.62 d	39.44 a	30.69
Rubber-coffee	3.70 b	12.22 c	0.67 c	40.02 a	38.06
Rubber-bamboo	1.93 c	16.09 b	0.76 b	36.14 b	39.97
Rubber-melinjo	2.08 c	31.49 a	0.80 a	28.93 c	35.85

Means with different letters in each column are significantly different at  $p \leq 0.05$  and ranked by the DMRT.

the farms, R and RC had the lowest contents in N around 0.3%, but the highest ash contents with 0.42 and 0.43%, respectively, compared to the others (Table 5). RM showed the highest content of N at 0.39%. The lowest levels of the ash content were found in the raw rubbers of RM and RB with 0.32 and 0.31%, respectively. N content in the raw natural rubber represents a residue of protein content that affects the curing properties and efficiency in vulcanization processes (Sadeesh Babu, Gopalakrishnan, & Jacob, 2000). Results of the nitrogen content ranged between 0.3 and 0.4%, which did not exceed the maximum level of 0.6% recommended for all grades of the technically specified rubber (Sadeesh *et al.*, 2000; MRB, 2009). The ash content represents the presence of nonvolatile mineral oxide (Giraldo-Vasquez & Velasquez-Restrepo, 2017), and the results' values were less than the standard permissible amount 0.6% (MRB, 2009).

Regarding the rheological properties, RM delivered better results in  $P_0$  of 38, PRI of 98, and MV of 69, compared to the others. RB showed high values of  $P_0$  and PRI with 36 and 96, respectively. However, its Mooney viscosity was the lowest at 60 among that of the farms. RC had good results in  $P_0$  and PRI and averaged in MV, whereas R resulted in low values of  $P_0$ , PRI, and MV at 36, 97, and 61, respectively. All results of the  $P_0$  and PRI showed significantly higher levels as they were higher than the standard minimum level recommended for most technically specified rubber (MRB, 2009). The MV of the raw rubber from RB and R had the lowest values showing around 60. These values are in the range of the optimal feature of the MV required by most rubber manufacturer which is around 60 to 65 ML (1+4) 100 °C because harder rubber (over 70 in MV) required larger power consumption for mastication (Sadeesh *et al.*, 2000).

In comparing the average molecular weights (Mw) of the raw rubbers from the farms, RM and RB delivered higher Mw of  $19.45 \times 10^5$  and  $20.99 \times 10^5$  g/mol, respectively, compared to the others, R and RC, which had  $18.08 \times 10^5$  g/mol and  $18.48 \times 10^5$  g/mol, respectively. Mw of raw natural rubber is a major determinant of the raw rubber's processibility. It widely ranges typically between  $10^4$  and  $10^6$  g/mol according to not only cultivars and the tree's age but also the soil condition and seasonal variation of the rubber farm (Kovuttikulrangsie & Sakdapipanich, 2005).

### 3.6 Relationships among parameters

It was found that the LAI was negatively correlated to the Suc content but positively to the RSH content, significantly. Since low content of Suc associated with high RSH content reflects efficient utilization of sucrose reserved by photosynthesis in rubber biosynthesis and less stress of physiological condition ensuring in improved latex production (d'Auzac *et al.*, 1997; Tupy, 1989). Meanwhile, the SMC

expressed a positive relation with the Pi content but a negative association with the DRC. Roux *et al.* (2000) observed that water availability was a significant determinant for latex synthesis and rubber tree metabolism activities. Pi represents a rubber tree's metabolism, and a rubber cultivar that has a high content of Pi is regarded as high metabolism clone that delivered a high volume of latex but a slight decrease in DRC (Jacob *et al.*, 1989).

The LAI also affected the non-rubber components as it increased with a decrease of the ash content and an increase of the N content. These relations replicated the findings of Moreno, Ferreira, Goncalves, and Mattoso (2005) that a minimum level of ash content was observed during the rubber tree's leaf area developed its maximum. They also found that the N content was falling when the leaf defoliation period. High N content in natural rubber was associated with increased protein biosynthesis (Othman, Hepburn, & Hasma, 1993), showing high photosynthesis efficiency which could be improved by leaf area development (Moreno *et al.*, 2005).

SMC did not show a significant correlation to the non-rubber components in the study. Regarding the associations with the rheological properties, however, it expressed a significant association with the MV, whereas the LAI values were directly proportional to the Mw. Although the study did not show an apparent association between the MV and Mw, some studies reported that maximum leaf area and sufficient moisture availability favored low evapotranspiration (Jacob *et al.*, 1989; Roux *et al.*, 2000), leading to more significant isoprene biosynthesis resulting in higher Mw. A study by Kovuttikulrangsie and Sakdapipanich (2005) observed the latex from matured rubber trees delivered high Mw compared to the young rubber trees.

There were some associations among the technological properties as  $P_0$ , PRI and MV had positive correlations themselves. These relations replicated an observation of Roux *et al.* (2000) that seasonal variations of  $P_0$ , PRI and MV followed a similar trend. The Mw showed negative correlations with the ash content and the PRI values, but it was positively related to the N content. Since the Mw was positively associated with the LAI, it showed similarly as the LAI's relations with the ash content and N content. Roux *et al.* (2000) also reported that high production of the macromolecular chain of a high metabolism clone could not be protected completely, leading to high sensitivity to thermo-oxidative degradation resulting in lower PRI value.

There were some associations between the biochemical compositions and the technological properties since the ash content increased with the Suc content and decreased with the N content and the Mw. Ash contents were at a high level associated with high DRC when water availability was limited, causing less translocation of inorganic elements from the absorbed nutrients (Giroldo-

Table 5. Technological properties (Ash, N, P<sub>0</sub>, PRI, MV, Mw) of raw rubber sourced from the farms

Studied farms	Ash (%)	N (%)	P <sub>0</sub>	PRI	MV (ML (1+4) 100 °C)	Mw (g/mol)
Monocrop rubber	0.42 a	0.30 c	36 b	97 b	61 c	18.08 x 10 <sup>5</sup> b
Rubber-coffee	0.43 a	0.32 c	38 a	99 a	67 b	18.48 x 10 <sup>5</sup> b
Rubber-bamboo	0.31 b	0.37 b	36 b	96 b	60 c	20.99 x 10 <sup>5</sup> a
Rubber-melinjo	0.32 b	0.39 a	38 ab	98 a	69 a	19.45 x 10 <sup>5</sup> a

Means with different letters in each column are significantly different at  $p \leq 0.05$  and ranked by the DMRT.

Table 6. Pearson correlation coefficients within the study parameters: LAI, SMC, latex biochemical compositions (Suc, Pi, R-SH) and technological properties (Ash, N, P<sub>0</sub>, PRI, MV, Mw)

	LAI	SMC	DRC	Suc	Pi	R-SH	Ash	N	P <sub>0</sub>	PRI	MV	Mw
LAI	1.00											
SMC	0.17	1.00										
DRC	-0.60	<b>-0.81</b>	1.00									
Suc	<b>-0.97</b>	-0.25	<b>0.73</b>	1.00								
Pi	0.55	<b>0.88</b>	<b>-0.99</b>	-0.66	1.00							
R-SH	<b>0.90</b>	0.52	<b>-0.89</b>	<b>-0.96</b>	<b>0.84</b>	1.00						
Ash	<b>-0.79</b>	-0.23	<b>0.76</b>	<b>0.90</b>	-0.66	<b>-0.89</b>	1.00					
N	<b>0.88</b>	0.52	<b>-0.90</b>	<b>-0.95</b>	<b>0.86</b>	<b>0.99</b>	<b>-0.91</b>	1.00				
P <sub>0</sub>	0.28	0.58	-0.29	-0.16	0.40	0.28	0.20	0.23	1.00			
PRI	-0.21	0.53	-0.02	0.31	0.16	-0.15	0.58	-0.19	<b>0.88</b>	1.00		
MV	0.21	<b>0.81</b>	-0.50	-0.16	0.61	0.35	0.10	0.32	<b>0.94</b>	<b>0.87</b>	1.00	
Mw	<b>0.84</b>	-0.18	-0.42	<b>-0.88</b>	0.30	<b>0.73</b>	<b>-0.89</b>	<b>0.74</b>	-0.28	<b>-0.70</b>	-0.33	1.00

The correlation coefficients in bold are significantly different at  $p \leq 0.05$  (Pearson linear coefficients,  $|r| \geq 0.7$ ).

Vasquez & Velasquez-Restrepo, 2017). These conditions could delay metabolic utilization of sucrose, leading to high sucrose content in the latex so that lower Mw resulted. On the other hand, the N content showed a significant positive association with Pi and R-SH contents. Pi is essential for the formation of nucleic acid, which induces protein biosynthesis (Coupe & Chrestin, 1989). High N content was followed by high Pi and R-SH values that improve the physiological status of laticiferous system and stability of organelles, particularly lutooids in latex (d'Auzac *et al.*, 1997).

#### 4. Conclusions

The study comparing the LAIs confirmed the higher remarkable growth of the above-ground vegetative parts in the rubber-based intercropping system than that of the monocrop rubber farm. The rubber-based intercropping farms conducted in the study expressed a higher content of the soil moisture, one of the below-ground components of the agroecosystem, in the soil depth of 11-40 cm. And it was also worth noting that the intercropping farms, RM and RB, dramatically increased the SMC with the soil depths. Regarding the rubber trees' physiological status in the farms, the rubber-based intercropping farms indicated better biochemical composition in the latex, particularly in Suc, Pi, R-SH, showing efficient metabolism of the latex biosynthesis. Technological properties of the raw rubber sourced from the rubber-based intercropping farms also reflected the premium results in both non-rubber components and rheological properties with higher molecular weights. The correlation analysis found some significant relations notably between the LAI and technological properties of N content, ash content and Mw, and between the SMC and MV. Likewise, the associations of the physiological changes to the technological properties were observed

particularly between the biochemical composition (Suc, Pi and R-SH) and the non-rubber components (N content, ash content) and Mw. In conclusion, this preliminary study could highlight some changes in the rubber-based intercropping system in terms of mainly LAI, SMC and physiological status, and their relations to the technological properties of the raw natural rubber sourced from the system.

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