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The Dynamics of Immature Rubber Photosynthetic Capacities Under Macronutrients Deficiencies

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Abstract

Pará rubber produces natural latex which is essential for the industries. Rubber plant in immature phase is prone to macronutrient deficiencies due to improper management practices in the field and the nature of immature plants that have sensitive physiological responses under stress conditions. The study aimed to assess the effect of macronutrient limitation on immature rubber trees' photosynthetic capacity and growth. The immature rubber was pot-grown inside the greenhouse with a completely randomized design experiment and nutrient limitations used as the treatments. The treatments consisted of 5 levels, namely, NPK; NP (-K); NK (-P); PK (-N); Control (-NPK). Photosynthetic capacity parameters ($V_{c\ max}$: maximum rate RuBisCO carboxylation, J_{max}: RuBP regeneration rate, and TPU: Triose Phosphate Utilization), tree growth (plant height, flush number, leaf number, stem diameter), and leaf macronutrient (N, P, and K) concentrations were periodically measured. Welsch's test ($\alpha = 0.05$) continued with Games-Howell pairwise comparison, followed by Pearson's correlation test and polynomial regressions were performed to describe the nutrient limitation and photosynthetic capacity relationships. Results showed that the leaf nutrient concentration corresponds with the given treatments, even though it was above the critical level for immature rubber. The limitation of N fertilization slightly reduced plant development and growth such as height, leaf number, flush number, relative growth rate, and photosynthetic capacities. However, the P and K limitation effect could not be observed clearly in the observation periods on growth and photosynthetic capacity parameters. Furthermore, the mobility rate of nutrients from the soil to the plants and its translocation inside plant organs played more essential role in plant growth and photosynthetic capacities. Prolonged observation periods on various rubber clones have to be performed to deeply understand the effects of nutrient deficiencies on immature rubber tree morphophysiological activities.

Keywords: Immature rubber, Macronutrient deficiencies, Photosynthetic capacities, Photosynthesis, RRIM 600

Introduction

Pará rubber (*Hevea brasiliensis* Müll. Arg.) is an essential industrial tree that produces latex components for various products [1]. According to Chambon *et al.* [2], Thailand's excellent performance in natural rubber production resulted from adopting high-yielding varieties and intensive fertilizer inputs. Fertilization is known to influence both plant growth and latex production [3,4]. Furthermore, rubber tree requirements for primary nutrients such as nitrogen (N), phosphorus (P), potassium (K), and secondary nutrients sulfur (S), calcium (Ca) as well as magnesium (Mg) might depend on its growth stage [4]. The immature rubber tree is prone to macronutrient deficiency due to improper management practices in the field and the sensitivity of its physiological response under stress conditions that could lead to abnormal growth [5].

Macronutrients such as N, P, and K have specific roles during plant photosynthesis. Nitrogen is essential for synthesizing important plant molecules involved in plant photosynthesis, especially the Calvin cycle, thylakoid adaptation, and CO_2 assimilation [6-8]. Phosphorus is a component of ADP and ATP, which are essential in energy storage and transfer. Phosphorus is a constituent of polyphosphate and phospholipids in-plant leaves for the Calvin cycle and RuBisCO regeneration [9]. Osmotic regulation, photosynthesis, respiration, carbon allocations, stomatal movement, and protein synthesis is prominent roles of K [10,11].

Hunger for nutrients on immature rubber trees may lead to various growth problems. Nitrogen deficiencies might lead to chlorosis, lower photosynthetic activity, and growth [6,7,12,13]. P deficiency symptoms are stunted height, poor root development, reduced leaf number, reduced leaf area, leaf anthocyanosis, and decreased starch synthesis rate [12,13]. Potassium deficiency reduces photosynthetic capacity, shorter leaf lifespan, lower leaf-area index, and low aboveground gross tree biomass production [5,10,11,14,15].

Photosynthetic capacity is measured as the CO_2 assimilation rate that leaves can achieve under optimal environmental conditions and is determined by several leaf photosynthetic traits [15]. Photosynthetic capacity measurements consist of assessing essential parameters such as the maximum rate of RuBisCO-catalysed carboxylation ($V_{c\ max}$), the maximum electron transport capacity (J_{max}) that controls the ribulose biphosphate (RuBP) regeneration rate, the rate of triose phosphate utilization (TPU), the mesophyll and stomatal conductance (g_m and g_s), and mitochondrial respiration rate in the light (R_d) [16,17]. Those measurements could uncover the plant's physiological activities that could explain the trees' growing ability. The research on the effect of macronutrient limitation on growth of immature rubber plants and their photosynthetic capacity is limited. Thus, this research aimed to assess the effect of macronutrient limitation on immature rubber trees' growth and photosynthetic capacity. Furthermore, our research could also guide photosynthetic capacity, and nutrient omissions experiment in young industrial tree species.

Materials and methods

Materials

Soil samples (Ultisols (Fang Daeng series, Fine-loamy, kaolinitic, isohyperthermic, Rhodic Kandiudults)) were taken from Sitthiporn Kritdakon Research Station, Bang Saphan district, Prachuap Kirikan province, Thailand (10°59'13.35" N, 99°29'22.41" E)**.** Before application of the treatments, properties of the soil used were, soil pH = 5.3 (acidic soil), cation exchange capacity = 20.80 cmol (+) kg^{-1}, organic matter content = 2.11 %, total N = 0.25 ± 0.08%, available P Bray I = 8.950 ± 0.290 $mg.kg^{-1}$ and available K = 0.044 ± 0.001 $mg.kg^{-1}$. The pot experiment was conducted in greenhouse of the Soil Science Department, Kasetsart University, located in Bangkok, Thailand.

Grafted RRIM 600 rubber varieties grown in 5×20 cm polybag filled with approximately 0.5 kg soil (2 months old) were used. The rubber tree seedlings were acclimated for 1 month, then planted in the pots (35 cm diameter and 20 cm height) and placed above their plates (40 cm diameter and 5 cm height). Pre-prepared soil (soil sample was sieved with a 3 mm sieve, and plant debris was removed) was then added to the pots after the rubber seedlings (25 kg of soil for each pot). The first stage of this test was growing 100 young rubber trees until they formed a new flush (2 months), and only uniformly grown trees (**Table 1**. July 15th 2019 observation - similar height, flush number, and leaves number) were chosen for the forthcoming experiment.

Research design

The macronutrient omission experiment on young rubber trees was designed according to Completely Randomized Design (CRD) with 6 replications and 5 levels of treatments: Treatment A: NPK (5.29-8.01-5.34 g $plants^{-1}$ $month^{-1}$); Treatment B: NP (-K) (5.29-8.01-0 g $plants^{-1}$ $month^{-1}$); Treatment C: NK (-P) (5.29-0-5.34 g $plants^{-1}$ $month^{-1}$); Treatment D: PK (-N) (0-8.01-5.34 g $plants^{-1}$ $month^{-1}$); Treatment E: Control (-NPK). Fertilizers as the main source of nutrients used for this research were: Urea, superphosphate 36, and KCl containing 46 % N, 36 % P_2O_5, and 60 % K_2O, respectively, followed by the fertilizer doses in Baulkwill & Webster [18]. Soil water content in the pots was maintained at field capacity and was not a limiting factor for this experiment. Fertilizer was applied when the rubber trees were 5 - 7 months old (July 21st, August 22nd, and September 25th, 2019).

Methodology

The measurement of plant height, leaves number (fully expanded leaves), flush number, and stem diameter was performed once every 2 weeks to measure the growth of the trees. The fully expanded leaves that were used to measure the photosynthetic capacity were 30 - 35 day after emergence (After treatment application). The photosynthetic capacities were measured on August 21st - 24th, 2019, for the second flush, and September 24th - 29th, 2019, for the third flush. A portable photosynthesis system (Li-6,400XT Li-Cor Inc.) was used for leaf photosynthetic capacity measurements, and it was done at the daytime from 9.00 am - 3.00 pm to avoid stomata closure. Leaves used for photosynthetic capacities and leaf area measurement were also analyzed for nutrient contents. These measurements of A-Ci curves were done on 1 leaflet in each flush starting on the second flush, according to Kositsup *et al.* [19]. The leaf chamber was equilibrated for at least 15 min to reach a steady-state before starting A/Ci curves measurements: This was done through an automatic program that controlled CO_2 concentration in the leaf chamber, with stepwise decreases of CO_2 concentrations as follows 250, 200, 150, 100, 50 and 0 µmol. mol^{-1}, followed by a stepwise increase from 400 to 600, 800, 1,000, 1,100, 1,200, 1,400 and 1,600 $\mu mol.mol^{-1}$ to obtain A_c and A_j (RuBisCO carboxylation and RuBP regeneration). CO_2 response curves were fitted according to the Farquhar model [17] to explain the limitation of net assimilation by Rubisco activity at saturating point or by RuBP concentration (A_j).

Photosynthetic capacities V_{cmax} (RuBisCO carboxylation Rate), J_{max} (RuBP regeneration rate), and TPU (Triose Phosphate Utilization) were estimated from the response of assimilation result (A) to intercellular leaf CO_2 concentration (Ci) with leaf temperature adjusted into 25 °C. A-Ci best-fitted data only was used for further statistical analysis. Leaf area measurement of fully emerged leaves was measured using a scanner (LI-Cor LI 3,100 C) on August 21st - 24th, 2019, for the second flush; and September 24th - 29th, 2019, for the third flush (only leaves that were used for photosynthesis and nutrient measurement). Nutrient status in sample leaves (N, P, and K) was analyzed through the acid digestion method using Morgan extractant [20]. Nitrogen content in leaves was analyzed with the micro Kjeldahl method [21], Phosphorus content was analyzed using the colorimetry method [22], and Potassium content was analyzed with the atomic absorption method [23].

Statistical analysis

Plant height, leaves number, flush number, and stem diameter data were analyzed using Analysis of Variance (ANOVA) with a 95 % confidence level, followed by the Duncan Multiple Range Test (DMRT) for mean comparisons. Welsch's test unequal ANOVA test ($\alpha = 0.05$) continued with Games-Howell pairwise comparison used on measured leaf nutrient concentrations and photosynthetic capacities because not all the A-Ci curves data could be fitted normally; therefore, we only used best fitted A-Ci data and leaf nutrient content accordingly to increase the accuracy. A polynomial regression graph plotting the leaf nutrient concentrations and photosynthetic capacities was used to determine a simple perspective of nutrient concentration and photosynthetic capacity dynamic.

Results and discussion

Growth of immature rubber trees

The growth parameters of immature rubber measured on 15 July 2019 (7 days after transplanting) until 04 October 2019 (88 days after transplanting) (**Table 1**) showed that the immature rubber trees that were selected before treatment application had homogenous properties while at the end of the experiment the growth varied with the treatments. As shown by the analysis results presented on **Table 1** and **Figure 1**, rubber tree height at the end of the experiment was the highest on N fertilized treatments (NPK, NP (-K), and NK (-P)), reaching 115.34 ± 23.64, 115.86 ± 29.72, 115.52 ± 21 cm^3 consecutively. The shortest tree was observed in the Control (-NPK) treatment at 86.25 ± 10.64 cm which is not significantly different with PK (-N) treatment with 91.06 ± 19.89 cm height. The plant height's relative growth rate (**Figure 2**) also indicates a slight increment over time on treatments with N fertilizer application, even though its relative growth rate was not significantly different compared to the other treatments. These results highlight the role of N for tree height growth, as already reported by several studies [5,24]. However, a prolonged observation period is needed due to the slow growth rate of the perennial trees.

The number of fully expanded leaves still intact with the main tree at the end of the experiment exhibited significant differences between treatments. The highest number of fully expanded leaves was observed in NP (-K) treatment (27.60 ± 8.36 leaves per tree), followed by Control (-NPK) (25.90 ± 6.15 per tree); however, it needs to be noted that fully expanded leaves tend to falls when the new flush with young leaves grew [18,25]. Leaf number was significantly lower in NK (-P), NPK and PK (-N), with

18.70 ± 8.77, 17.00 ± 5.31 and 10.40 ± 13.9 leaves per tree, respectively, compared with those in NP(-K) and control. Furthermore, as shown in **Figures 1** and **2**, there were 2 periods of leaf falls and flush formation (formation of young leaves), as well as slightly different RGR of leaves and flush. Therefore, the treatment with the least newly developed flush had most of the fully expanded leaves still intact. Flush formation on the immature rubber was reported to be affected by the availability of nutrients, especially N [26]. On average, NPK treatment showed the highest flush formation with 3.70 ± 0.48 formed flush, followed by NP (-K) and NK (-P) consecutively, and it was significantly different with PK (-N) and Control (-NPK) which showed 3.10 ± 0.32 and 3.00 ± 0.00 formed flushes. N, P, K availability had various effects on plants, especially on its growth and development of rubber, which is related to the nutrient mobilization as well as sink and source relationship, in which the younger parts of the tree became the sink and the older parts became the nutrient source [5,18,27-33].

The widest stem diameter of rubber trees was recorded in Control (-NPK) treatment (1.29 ± 0.11 cm). Significantly narrower stem diameter was observed in the NPK treatment (1.16 ± 0.15 cm). The low growth and development activity of the upper parts of plants, such as height development and flush formation on control treatments, could be the factors in the wider stem diameter growth. However, there were no significant differences between both of them and NP(-K), NK(-P), and PK(-N) treatments in stem diameter, as well as in RGR of stem diameter (**Figure 2**). Taiz and Zeiger [34] explained that under certain abiotic stress, plants' sink and source roles could be changed as a stress response mechanism. Apart from that, Loescher [35] explained that perennial tree bark could also be used for carbohydrates reserve; with inadequate nutrients to be used for leaves and flush development, the carbohydrate accumulation in the bark is increasing. However, other perspectives related to imbalance nutrient availability, as shown by Mokhatar *et al.* [5], indicate that imbalance N fertilizer on immature rubber could induce leaves scorching, weak stem, and root damage. The reduction of the leaf number affected the total leaf area and produced biomass [14]. Furthermore, according to Ahmad *et al.* [1] and Vrignon-Brenas *et al.* [13], P and K fertilizer application on immature rubber trees showed limited effects.

Leaf nutrients concentration of immature rubber trees

Leaf nutrient contents in each treatment were consistent with the treatment applied, as shown on **Table 2**. For the second flush area-based leaf N content was the highest (1.319 ± 0.109 $g.m^{-2}$) on NP (-K) treatments, followed by NPK and NK (-P) treatments, while the lowest N content was observed on control (-NPK) treatment (0.552 ± 0.309 $g.m^{-2}$). The highest area-based phosphorus content in second flush leaves was observed on PK (-N) treatment with 0.367 ± 0.084 $g.m^{-2}$, but it was not significantly different with NPK and NP (-K) treatments. NK (-P) treatments exhibited the lowest area-based leaf P content (0.042 ± 0.017 $g.m^{-2}$). Leaf potassium contents on PK (-N), and NPK were 0.998 ± 0.207 $g.m^{-2}$ and 0.749 ± 0.251 $g.m^{-2}$, respectively, but without significant difference between NPK treatment and NK (-P) treatment (0.613 ± 0.104 $g.m^{-2}$). The lowest area-based leaf K content was observed on the control (-NPK) treatment (0.229 ± 0.098 $g.m^{-2}$).

In the third flush stage, the NK (-P) treatment exhibited the highest area-based leaf N content (2.097 ± 0.313 $g.m^{-2}$), that was significantly higher than in PK (-N) treatments (1.151 ± 0.165 $g.m^{-2}$) and Control (-NPK) (0.893 ± 0.096 $g.m^{-2}$), but not significantly different from the treatments NPK (1.970 ± 0.257 $g.m^{-2}$) and NP (-K) (2.071 ± 0.405 $g.m^{-2}$). Highest area-based leaf P content was observed on PK (-N) treatment (0.374 ± 0.092 $g.m^{-2}$) followed by NPK treatment (0.288 ± 0.091 $g.m^{-2}$), and NP (-K) (0.221 ± 0.084 $g.m^{-2}$). These values were significantly higher than those in NK (-P) treatment (0.077 ± 0.084 $g.m^{-2}$) and Control (-NPK) (0.102 ± 0.012 $g.m^{-2}$). Highest area-based leaf K content was observed in NK (-P) (1.051 ± 0.174 $g.m^{-2}$) but without significant difference with PK (-N) treatment (1.002 ± 0.144 $g.m^{-2}$). Lowest leaf K contents were observed on NP(-K) and (-NPK) treatments, (0.571 ± 0.322) and (0.698 ± 0.133 $g.m^{-2}$) consecutively.

The nutrient absorption rate (%) in single leaf on the second and third flush, as listed in **Table 2**, showed that nutrient limitation affects the absorption rate of N and the NK (-P) treatment showed a significantly higher absorption rate of N and P than the other treatments. The P absorption (%) indicated that NPK, NP(-K), and PK (-N) had low P uptake in the third flush leaves compared to the second flush because the leaf phosphorus concentration of the third flush was translocated for the height, leaves, and new flush development, as indicated in **Figure 1**. However, in PK (-N) treatment, the low absorption rate of P is probably caused by the limited availability of N because of synergistic N and P co-limitation [29,36].

NK(-P) and control treatments showed a higher absorption rate with 81.4 and 21.57 %. However, the uptake nutrient ($g.m^{-2}$) on the second and third flush for both treatments remained significantly lower than in other treatments. The inconsistency in P absorption due to the P limitation caused the plants to strive to absorb more available P through various mechanisms to reach a critical uptake level to sustain the plant's survival [29,30,37]. Moreover, the K absorption rate in Control (-NPK) treatment showed the highest absorption rate compared to the other treatments. The variations of nutrient absorption rate most likely were caused by the nutrient translocation inside plant tissue, as the lack of plant growth and development of the flush on control treatment causes the accumulation of K on the third flush, while on the NPK, NP (-K), and NK (-P), the nutrient in the third flush already being used for further development of height, leaves, and new flush. The capability of the plants to absorb nutrients depends on the plant growth activity, internal nutrient concentration, nutrient mobility or translocation rate, and interaction among nutrients inside plants [9,10,29-31,34,37].

Table 1 Statistical analysis of growth parameters before and after treatment.

Treatment	**Plant height (cm)**		**Leaves number**		**Flush number**		**Stem diameter (cm)**	
	July 15th 2019	**October 4th 2019**	**July 15th 2019**	**October 4th 2019**	**July 15th 2019**	**October 4th 2019**	**July 15th 2019**	**October 4th 2019**
NPK	68.89 ± 12.28^{a}	115.34 ± 23.64^{a}	18.1 ± 2.99^{a}	17.00 ± 5.31^{cd}	2 ± 0.82^{a}	3.70 ± 0.48^{a}	0.798 ± 0.1^{a}	1.16 ± 0.15^{b}
NP(-K)	75.07 ± 13.41^{a}	115.86 ± 29.72^{a}	19.9 ± 2.73^{a}	27.60 ± 8.36^{a}	2.4 ± 0.69^{a}	3.70 ± 0.48^{a}	0.832 ± 0.08^{a}	1.24 ± 0.14^{ab}
NK(-P)	69.06 ± 9.95^{a}	115.52 ± 21^{a}	19.4 ± 2.59^{a}	18.70 ± 8.77^{bc}	2.4 ± 0.52^{a}	3.70 ± 0.48^{a}	0.804 ± 0.06^{a}	1.18 ± 0.11^{ab}
PK(-N)	76.73 ± 16.15^{a}	91.06 ± 19.89^{b}	20.4 ± 3.2^{a}	10.40 ± 13.9d	2.8 ± 0.42^{a}	3.10 ± 0.32^{b}	0.856 ± 0.11^{a}	1.24 ± 0.12^{ab}
Control (-NPK)	68.96 ± 13.15^{a}	86.25 ± 10.64^{b}	18.8 ± 2.44^{a}	25.90 ± 6.15^{ab}	2.6 ± 0.52^{a}	3.00 ± 0.00^{b}	0.861 ± 0.07^{a}	1.29 ± 0.11^{a}
CV %	18.21	23.742	14.54	53.70	26.39	14.58	10.46	10.58
F-test	ns	**	ns	**	ns	**	ns	*

The Mean ± SD followed by the same letter in a row and column shows not significantly different (α = 5 %).

Table 2 Leaf nutrient content in the second flush and third flush (g m^{-2}).

Observation date	**2nd Flush (22 August 2019)**			**3rd Flush (24 September 2019)**			**Nutrient absorption (%)**		
Treatment	**Leaf nutrient (g m−2)**			**(24 September 2019)**					
	N	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**
NPK	1.186 ± 0.248^{abc}	0.289 ± 0.075^{a}	0.749 ± 0.251^{ab}	1.97 ± 0.257^{a}	0.288 ± 0.091^{a}	0.971 ± 0.209^{ab}	66.06 ± 25.2^{ab}	−0.21 ± 8.29^{a}	29.56 ± 22.98^{ab}
NP(-K)	1.319 ± 0.109^{a}	0.302 ± 0.048^{a}	0.380 ± 0.051^{bc}	2.071 ± 0.405^{a}	0.221 ± 0.084^{ab}	0.571 ± 0.322^{ab}	57.03 ± 5.69 b	−26.80 ± 6.6^{b}	50.09 ± 18.67^{bc}
NK(-P)	1.018 ± 0.023^{bd}	0.042 ± 0.017^{b}	0.613 ± 0.104^{b}	2.097 ± 0.313^{a}	0.077 ± 0.018^{c}	1.051 ± 0.174^{a}	105.90 ± 16.83^{a}	81.40 ± 1.75^{e}	71.56 ± 13.90^{c}
PK(-N)	0.722 ± 0.084^{cd}	0.367 ± 0.084^{a}	0.998 ± 0.207^{a}	1.151 ± 0.165^{b}	0.374 ± 0.092^{a}	1.002 ± 0.144^{a}	59.32 ± 12.44^{b}	1.98 ± 8.81^{c}	0.37 ± 17.59^{a}
Control (-NPK)	0.552 ± 0.309^{de}	0.084 ± 0.029^{b}	0.229 ± 0.098^{c}	0.893 ± 0.096^{b}	0.102 ± 0.012^{bc}	0.698 ± 0.133^{b}	61.92 ± 20.23^{b}	21.57 ± 2.04^{d}	204.13 ± 11.51^{d}
CV %	16.1	23.4	23.9	15.1	28	22.9	25.95	233.02	99.13
Welch's test	**	**	**	**	**	**	**	**	**

The Mean ± SD followed by the same letter or no letter in a row shows not significantly different in Games-Howell pairwise comparison.
Welsch's test means the ANOVA for the given treatment with unequal sample size (ns = $p > 0.05$, * = $p < 0.05$, ** = $p < 0.01$

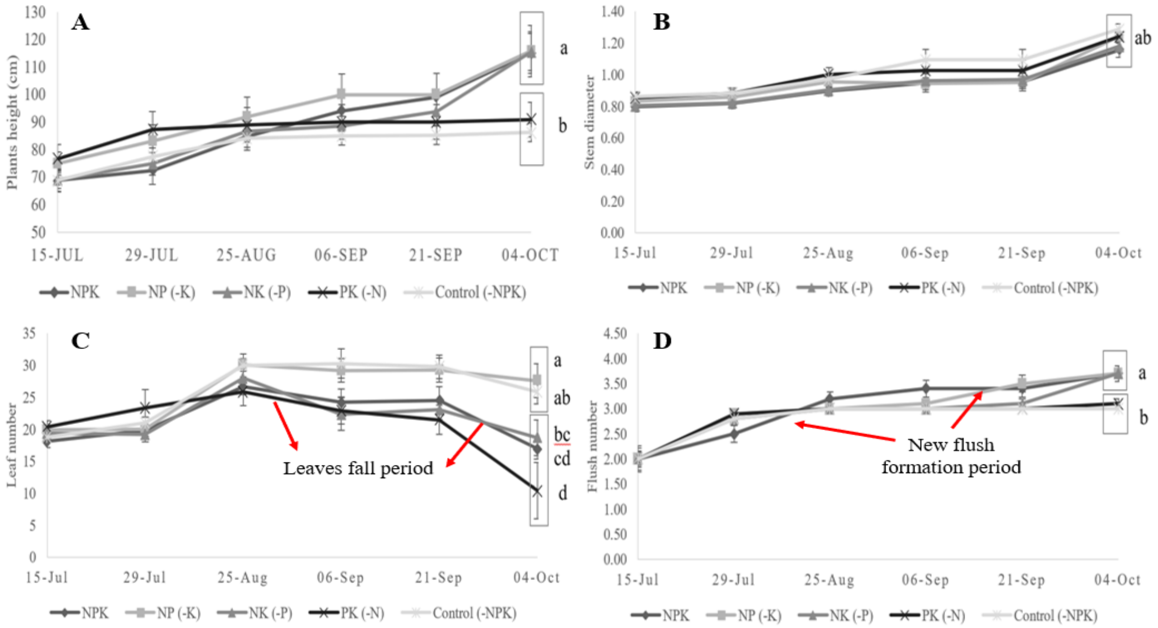

Figure 1 Immature rubber trees growth (A) plant height, (B) stem diameter, (C) leaves number, and (D) flush number.

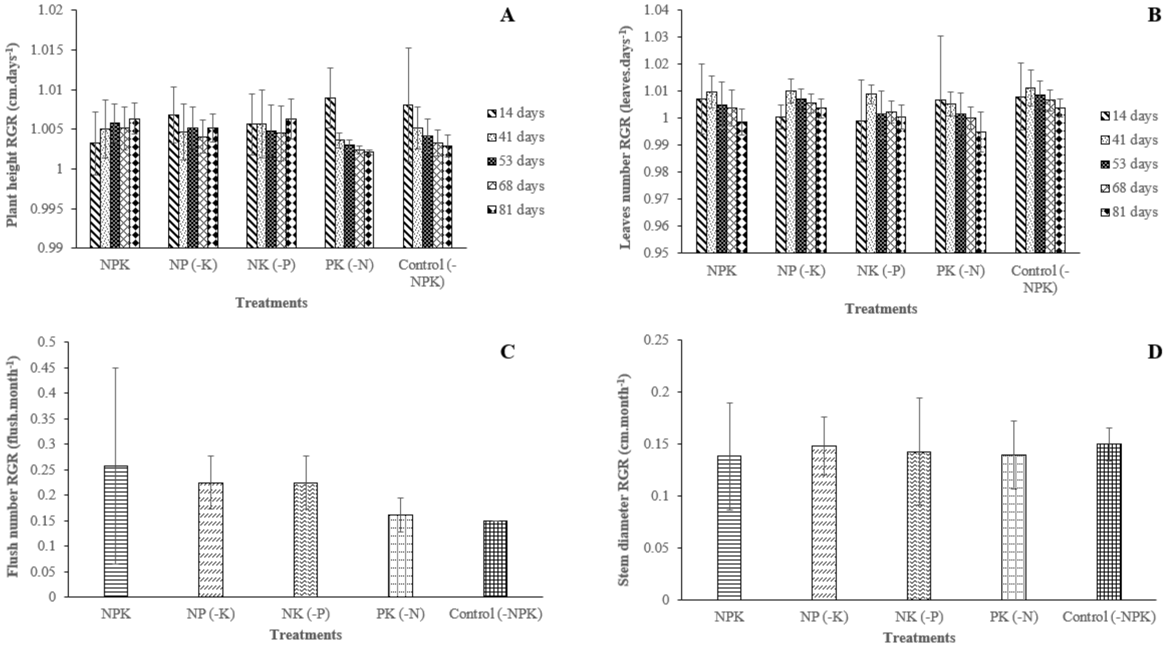

Figure 2 Immature rubber trees growth rate (A) plant height, (B) leaves number, (C) flush number, and (D) stem diameter.

Critical percentages of N, P, and K content in young mature leaves of immature rubber trees, according to Reuter and Robinson [38], were N (< 1.7 %), P (< 0.15 %), and K (0.85 %). Thus, the data showed that the leaf N, P, and K concentrations in the second and third flush were above the critical level. The plant mechanism to absorb and uptake nutrients could depend on the interaction of nutrient concentration inside plants [37]. However, soil properties and various environmental factors might affect

N, P, and K fertilizer application in the field [9,29,30,36,37]. Moreover, there were possibilities that observable deficiency symptoms in rubber leaves were limited during the early flush (30 - 35 days after leaves emergence and after 81 days of treatment application) except for the PK (-N) and control treatment that reflects on its height and flush growth.

Immature rubber trees leave photosynthetic capacities

The highest leaf photosynthetic capacities on the second flush were observed in the complete fertilizer application treatment (NPK), with values of $V_{c\ max}$, J_{max}, and TPU of 103 ± 37.3, 89.2 ± 17.46 and 7.025 ± 1.29 µmol.m^{-2}.s^{-1}, respectively (**Table 3**), while the lowest leaf photosynthetic capacities were found in NK (-P) treatment, with $V_{c\ max}$, J_{max}, and TPU values of 38.18 ± 6.64, 60.14 ± 4.91 and 4.722 ± 0.48 µmol.m^{-2}.s^{-1}, respectively due to the limitation of P input, even though the absorption was high, the nutrient uptake was still significantly lower than other treatments. The third flush photosynthetic capacity data showed that omission of N, P, or K did not substantially affect the leaf photosynthetic capacities, possibly due to the adaptation of the rubber tree to its growing medium. The adaptation could come into the limitation of growth when the nutrient was scarce and swift growth when there was vast nutrient available while maintaining the photosynthetic activity homeostasis [29-33,39]. The NP (-K) treatment exhibited the highest $V_{c\ max}$, J_{max}, and TPU values (113.70 ± 33, 82.15 ± 16.09 and 6.543 ± 1.333 µmol.m^{-2}.s^{-1}, respectively. Furthermore, despite an insignificant difference in photosynthetic capacities value on the third flush, treatment with N and P omissions showed relatively lower $V_{c\ max}$, J_{max}, and TPU. The correlation of leaf nutrient and photosynthetic capacities on **Table 4** showed significant R values ranging from 0.348 to 0.501.

Table 3 Leaf photosynthetic capacity (µmol.m^{-2}.s^{-1}) on second and third flush.

Leaf Photosynthetic capacities (µmol.m^{-2}.s^{-1})

Treatment	2nd Flush (22 August 2019)			3rd Flush (24 September 2019)		
	$V_{c\ max}$	J_{max}	TPU	$V_{c\ max}$	J_{max}	TPU
NPK	103 ± 37.3^{a}	89.20 ± 17.46^{ab}	7.025 ± 1.297^{ab}	81.70 ± 34.3^{a}	71.52 ± 19.74^{a}	5.534 ± 1.144^{a}
NP(-K)	88.47 ± 18.76^{a}	83.06 ± 13.47^{a}	6.556 ± 0.795^{a}	113.70 ± 33^{a}	82.15 ± 16.09^{a}	6.543 ± 1.333^{a}
NK(-P)	38.18 ± 6.64^{bc}	60.14 ± 4.91^{b}	4.722 ± 0.484^{b}	73.80 ± 29.1^{a}	64.99 ± 13.44^{a}	5.147 ± 0.947^{a}
PK(-N)	67.88 ± 20.81^{abc}	73.29 ± 11.08^{ab}	5.625 ± 0.863^{ab}	66.96 ± 16.9^{a}	74.09 ± 13.52^{a}	5.779 ± 0.908^{a}
Control (-NPK)	57.82 ± 8.05^{c}	83.11 ± 8.58^{a}	6.493 ± 0.608^{a}	75.77 ± 19.7^{a}	64.03 ± 8.43^{a}	5.187 ± 0.747^{a}
CV %	25.77	14.27	13.30	32.29	19.96	18.02
Welch's test	**	**	**	ns	ns	ns

The Mean ± SD followed by the same letter in a row and column shows not significantly different in Games-Howell pairwise comparison with (*p*-value: 0.05).

Table 4 Correlation between leaf nutrient concentration and photosynthetic capacity parameters.

Nutrient concentration	2nd Flush			3rd Flush		
	$V_{c\ max}$	J_{max}	TPU	$V_{c\ max}$	J_{max}	TPU
N (%)	0.477*	0.081	0.082	0.448**	0.274	0.216
P (%)	0.501**	0.135	0.096	0.075	0.321	0.322
K (%)	0.177	−0.168	−0.192	−0.203	−0.193	−0.255
N (g.m^{-2})	0.318	0.141	0.134	0.486**	0.371*	0.32
P (g.m^{-2})	0.451*	0.19	0.147	0.06	0.348*	0.357*
K (g.m^{-2})	0.079	−0.123	−0.156	−0.251	−0.13	−0.172

The number inside the table represent the Pearson correlation value (R) and * or ** symbol showed that there was strong correlation ($p < 0.05$ or $p < 0.01$).

The leaf photosynthetic capacities highly depend on leaf N contents. Low leaf N concentrations reduce chlorophyll-a concentration and the maximum rate of photosynthesis [6,40]. Leaves N content and photosynthesis rate also could be affected by phosphorus availability in the soil since phosphorus is crucial for a plant's ability to absorb nitrogen in the soil, which leads to higher biomass production [40]. Potassium also plays an important role in regulating stomata [24], In addition, leaf N, P & K contents were above the critical contents was shown in this research. Therefore, macronutrient concentration in leaves would greatly impact photosynthesis-related activities [6-9,24,39-41]. However, our research results could indicate that higher N and P concentration in leaves potentially increase RuBisCO carboxylation, RuBP regeneration, and triose phosphate utilization.

Conclusions

The assessment of the macronutrient omission effect in immature rubber trees showed that the limitation of N fertilization slightly reduced plant development and growth such as height, leaves number, flush number, relative growth rate, and photosynthetic capacities. However, the P and K limitation effect could not be observed clearly in the observation periods on growth and photosynthetic capacity parameters. Furthermore, the mobility of nutrients from the soil to the plants and its translocation inside plant organs played an essential role in plant growth and photosynthetic capacities, and further studies regarding those topics were essential. However, our study was limited to a short period on very young trees grown in pots. Prolonged observations should be performed under field conditions to clearly understand the effects of nutrients on the growth of immature rubber trees.

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