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Enhanced Efficiency Phosphorus Fertilizer Impact on Physiological Characteristics of Coffee Seedlings

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Abstract

The effects of phosphorus (P) nutrition on various aspects of photosynthetic metabolism have been examined for coffee plants (*Coffea arabica*) grown under greenhouse conditions. The experimental design was completely randomized, and the treatments were carried out in a 2×5 factorial scheme: two P sources [Triple Superphosphate – TSP (46% P_2O_5) and Policote coated TSP – TSP + Policote (43.7% P_2O_5)] and five P rates (0; 5; 10; 15 and 20 g P_2O_5 plot^{-1}), with three replications. The net photosynthetic rate (A), the leaf temperature (LT), the atmospheric CO_2 concentration (Ca), the stomatal conductance (gs), the transpiration rate (E), the substomatal CO_2 concentration (Ci) and carboxylation efficiency (Ce) were measured in the youngest fully expanded leaf, with the use of an infrared gas analyzer. All photosynthesis measurements were affected by P fertilization. P fertilization increased net photosynthetic rate, leaf temperature, substomatal CO_2 concentration, stomatal conductance, transpiration rate, and carboxylation efficiency. Policote coated P fertilizer resulted in a higher net photosynthetic rate, leaf temperature, stomatal conductance, and transpiration rate than conventional P fertilizer. The data indicated that Policote coated P fertilizer, a kind of enhanced efficiency P fertilizer (EEPF), alters the plant's photosynthetic carbon metabolism, explaining higher crop development and yield resulted from this EEPF.

Keywords

Efficiency Use, Policote, Net Photosynthetic Rate

1. Introduction

Phosphorus (P) is essential for all life on earth [1] and a non-replaceable nutrient in biology, with finite global reserves [2]. An insufficient P supply not only limits economically acceptable yields and the inputs of other nutrients but also threatens food security for a growing world population. Several studies have shown that global crop production needs to double by 2050 to meet the projected demands of rising population, diet shifts, and increasing biofuels consumption [3]. Two main strategies exist to meet rising global food and biofuel demand: intensify the existing agricultural land area as much as possible, and/or expand into areas with native vegetation which would be detrimental to global biodiversity [4]. Boosting crop yields to meet these rising demands, rather than clearing more land for agriculture has been highlighted as a preferred solution to meet this goal [3].

Coffee is the second most traded commodity in the world after oil, generating approximately U$ 90 billion per year and involves about 500 million people in its management from cultivation to final product for consumption [5] [6]. With the rise in popularity of coffee among Europeans, Brazil became the world's largest producer in the 1840s and has been ever since. Until the 60s, Brazilian coffee plantations were sited in high fertility areas that were originally forested, but with the tightening of environmental legislation and the increased cost of the most fertile areas, the coffee crop has expanded into areas that were marginal in terms of fertility, where constant soil correction and fertilization are needed [7]. Brazilian coffee producing areas are concentrated in the tropical regions, whose soils are highly weathered with low plant-available phosphate [8]. This is not just a Brazilian soil problem. Phosphorus is known to be one of the most recognized limiting factors for coffee production in most soils of southwestern Ethiopia, the primary diversity center of this crop [9]. P is one of the important nutrients for coffee because it causes an increase in root development and plant vigor to ensure the formation of crops with high productivity and low rates of replanting [10]. High phosphate fertilizer rates are necessary to coffee planting, but relatively small amounts of P are extracted by the plants, indicating that a large part of the added phosphates would be unavailable to the growing coffee tree [11]. Amelioration attempts by the addition of phosphatic fertilizers are economically and ecologically unsound as the efficiency of added phosphatic fertilizers is very low [12]. This scenario increases the pressure to improve P use efficiency. The low availability of P is because it readily forms insoluble complexes with cation such as aluminum and iron under acidic soil condition and with calcium and magnesium under alkaline soil conditions whereas the poor P fertilizer recovery is because the P applied in the form of fertilizers is mainly adsorbed by the soil and is not available for plants lacking specific adaptations [13]. Currently, most commercial P fertilizers are water-soluble and P sorbs rapidly onto soil minerals, causing low P use efficiency and low residual values of these fertilizations [14]. The low efficiency of P fertilization has been reported in several papers [15] [16] [17] [18]. Phosphorus is commonly bound to iron and aluminum oxides and

hydroxides through chemical precipitation or physical adsorption [19]. As a result of adsorption, precipitation, and conversion to organic forms, only 10% - 30% of the applied phosphate mineral fertilizer can be recovered by the crop grown after the fertilization [20] [21] [22] [23] [24]. The rest stays in the soil and may be used by crops in the following years. Because of low P solubility and desorption, only a small proportion of phosphate ions exist in the soil solution for plant uptake even under optimum P fertilization making P fertilizer recovery to be lower compared to other nutrient containing fertilizers [20]. On weathered and tropical soils, it can be necessary to apply up to five-fold more P as fertilizer than is exported in products [25]. For this reason, much of the input P fertilizer is not used by crops. This suggests that traditional chemical fertilizer application alone is not a cost-effective way of increasing crop production in many P-limiting soils.

Due to the importance of food, economy, and environmental safety, it is necessary to carry out studies aimed at increasing P fertilizers use efficiency in agriculture [24]. Several strategies have been used to increase P fertilization efficiency. Lately, the most frequently used strategy has been the application of increased efficiency fertilizers. One of the strategies used in enhanced efficiency nitrogen fertilizers is the use of inhibitors such as NBPT [N-(n-butyl) thiophosphoric triamide] to control the hydrolysis of urea to ammonia gas in the soil [26]. A similar strategy could be applied with additives of Fe and Al affinity (responsible for the fixation of phosphorus in tropical soils) in P fertilizers, increasing its agronomic efficiency. New P fertilizer additives have been recently developed to combat P-limited crop productivity by reducing phosphate fixation in soil [27]. Polymer additives with a higher affinity for Fe and Al than P could be used to produce EEFs. Some reports point out the advantages of polymer-coated P fertilizer [24] [28]-[34], while others indicate its inefficiency, compared with common fertilizer [27] [35]-[40]. The need to increase P fertilization efficiency and the lack of information with enhanced efficiency P fertilizers justify the performance of studies evaluating the performance of this type of fertilizer.

The present study aimed to evaluate physiological characteristics in response to P sources (conventional and enhanced efficiency) and rates on coffee seedlings in different soils.

2. Material and Methods

2.1. Experimental Site

The experiment was carried out at the Department of Soil Science/Lavras Federal University, in Minas Gerais/Brazil, under greenhouse conditions, from February to November.

The experiment was laid out at a Red Oxisol, characterized following the methodology described by [41]. The Red Oxisol presented the following chemical characteristics: pH (H_2O) = 5.0; P (Mehlich-1) = 0.90 mg·dm^{-3}; K (Mehlich-1) = 10 mg·dm^{-3}; Ca = 9.0 mmol$_c$·dm^{-3}; Mg = 1.0 mmol$_c$·dm^{-3}; Al = 1.0 mmol$_c$·dm^{-3};

clay = 670 g·kg⁻¹; silt = 170 g·kg⁻¹ and sand = 160 g·kg⁻¹.

2.2. Experimental Design

The experimental design was completely randomized, and the treatments were carried out in a 2 × 5 factorial scheme: two P sources [Triple Superphosphate – TSP (46% P_2O_5) and Policote coated TSP – TSP + Policote (43.7% P_2O_5)] and five P rates (0; 5; 10; 15 and 20 g P_2O_5·plot⁻¹), with three replications. Each experimental plot consisted of one pot filled with 14 kg of soil in which two plants were grown. The Policote additive, a biodegradable and soluble anionic polymer, was used to coat TSP and to reduce the contact of P fertilizer with Al and Fe [28] [29], decreasing Fe^{2+} and Al^{3+} activity near P fertilizer granules and the precipitation reactions of P with these cations [31].

2.3. Crop Management

Nitrogen (5.33 g N plot⁻¹, as ammonium sulfate) and potassium (6.72 g K_2O plot⁻¹, as KCl) fertilization and all treatments were homogenized with the plot's soil on February 15th. Then, five-month-old coffee (*Coffea arabica* L., cultivar Acaiá IAC 474-19) seedlings produced with Plantmax substrate [N (4.9 g·kg⁻¹), P_2O_5 (4.1 g·kg⁻¹), K_2O (3.8 g·kg⁻¹), Ca (9.0 g·kg⁻¹), Mg (17.8 g·kg⁻¹), Fe (20 g·kg⁻¹), Cu (41.3 mg·kg⁻¹), Mn (312 mg·kg⁻¹) and B (8.2 mg·kg⁻¹)] were transplanted. The seedlings presented five pairs of true leaves and were produced from seeds that had been sown in washed and sieved sand. During the entire experimental period, the soil moisture was maintained at 70% of the total pore volume by weighing the pots and adding deionized water. At 60 days after transplanting, foliar fertilization, with boric acid (0.3% boron) and zinc sulfate (0.3% zinc), was carried out. Coffee plant characteristics such as height, dry weight of plants, leaf area, content, and P accumulation in coffee leaves were evaluated as reported by [29].

2.4. Photosynthesis Measurements

On November 10th, the net photosynthetic rate (A), the leaf temperature (LT), the atmospheric CO_2 concentration (Ca), the stomatal conductance (gs), the transpiration rate (E), the substomatal CO_2 concentration (Ci) and carboxylation efficiency (Ce) [42] [43] were measured in the youngest fully expanded leaf, with the use of an infrared gas analyzer (LI-6400XT Portable Photosynthesis System, Licor, Lincoln, USA), equipped with an artificial light and automatic CO_2 injection system. The carboxylation efficiency (Ce) was obtained by the Ca/Ci ratio. The leaves were illuminated at 1500 µmol photon m⁻²·s⁻¹ for 30 min in a growth chamber, before the measurement. The assimilation rate of CO_2 in the chamber was measured with the ambient concentration of CO_2 equal to 400 ± 9 µmol CO_2 mol⁻¹.

2.5. Statistical Analysis

All data were analyzed by analysis of variance, and the F-test was used to deter-

mine treatment significance. Appropriate regression equations were also used to further analyze relations between evaluated parameters and P rates. All the statistical procedures were performed with Assistat software [44].

3. Results

All photosynthesis measurements were affected by P fertilization (Table 1). P fertilization resulted in increasing net photosynthetic rate (A), leaf temperature (LT), and carboxylation efficiency (Ce) while increasing P rates resulted in lower substomatal CO_2 concentration (Ci). Higher A, LT, transpiration rate (E), and stomatal conductance (gs) were observed with Policote coated P fertilizer use. For gs, E and Ce, the interactions between rates and sources were not significant.

The net photosynthetic rate raised to 2.08 and 2.30 µmol CO_2 m^{-2}·s^{-1}, with 20 g P plot^{-1} and TSP and with 17.1 g P plot^{-1} and PTSP, respectively (Figure 1). PTSP resulted in higher average A (2.05 µmol CO_2 m^{-2}·s^{-1}) than TSP (1.84 µmol CO_2 m^{-2}·s^{-1}). P excess reducing A was only observed with PTSP. P fertilization increased leaf temperature (LT) up to 30.4°C and 31.7°C, with 17.7 g P plot^{-1} and TSP and with 18.3 g P plot^{-1} and PTSP, respectively (Figure 1). PTSP resulted in higher average LT (30.7°C) than TSP (29.8°C). Increasing P rates reduced substomatal CO_2 concentration (Ci) from 254.72 µmol CO_2 mol air^{-1} to 138.28 µmol CO_2 mol air^{-1} (Figure 1). The Ci differences between sources were not significant. The gs and E did not differ among P rates but were different between P sources. PTSP increased gs and E in 47.4% and 60.3%, respectively, when compared to TSP. The carboxylation efficiency (Ce) increased up to 1.75 × 10^{-3} with 20 g P plot^{-1} (Figure 1). The Ce differences between sources were not significant.

4. Discussion

Increasing net photosynthetic rate, transpiration rate, and stomatal conductance with P supply were also reported by [45] because low-P decreases the initial activities of Calvin-cycle enzymes [46].

PTSP resulted in a higher net photosynthetic rate with a lower P rate than TSP. The PTSP use resulted in a 10.5% increase in the net photosynthetic rate. Inorganic phosphate has a major role in the regulation of photosynthetic carbon metabolism [47]. Reduced nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) is an essential electron donor in plants and provides the reducing power that drives the photosynthesis process. Adenosine triphosphate (ATP) is an important source of energy for the photosynthesis process. As NADPH and ATP have P in their constitution, the increasing P fertilization increase cytoplasmatic inorganic P concentration and NADPH and ATP production, resulting in higher oxidative phosphorylation and, consequently, higher net photosynthetic rate. Reference [47] concluded that the limitation of Pi supply to isolated chloroplasts reduced stromal Pi to the point where it limits ATP synthesis. Reduced photosynthetic rate with low phosphate nutrition was also reported [48]
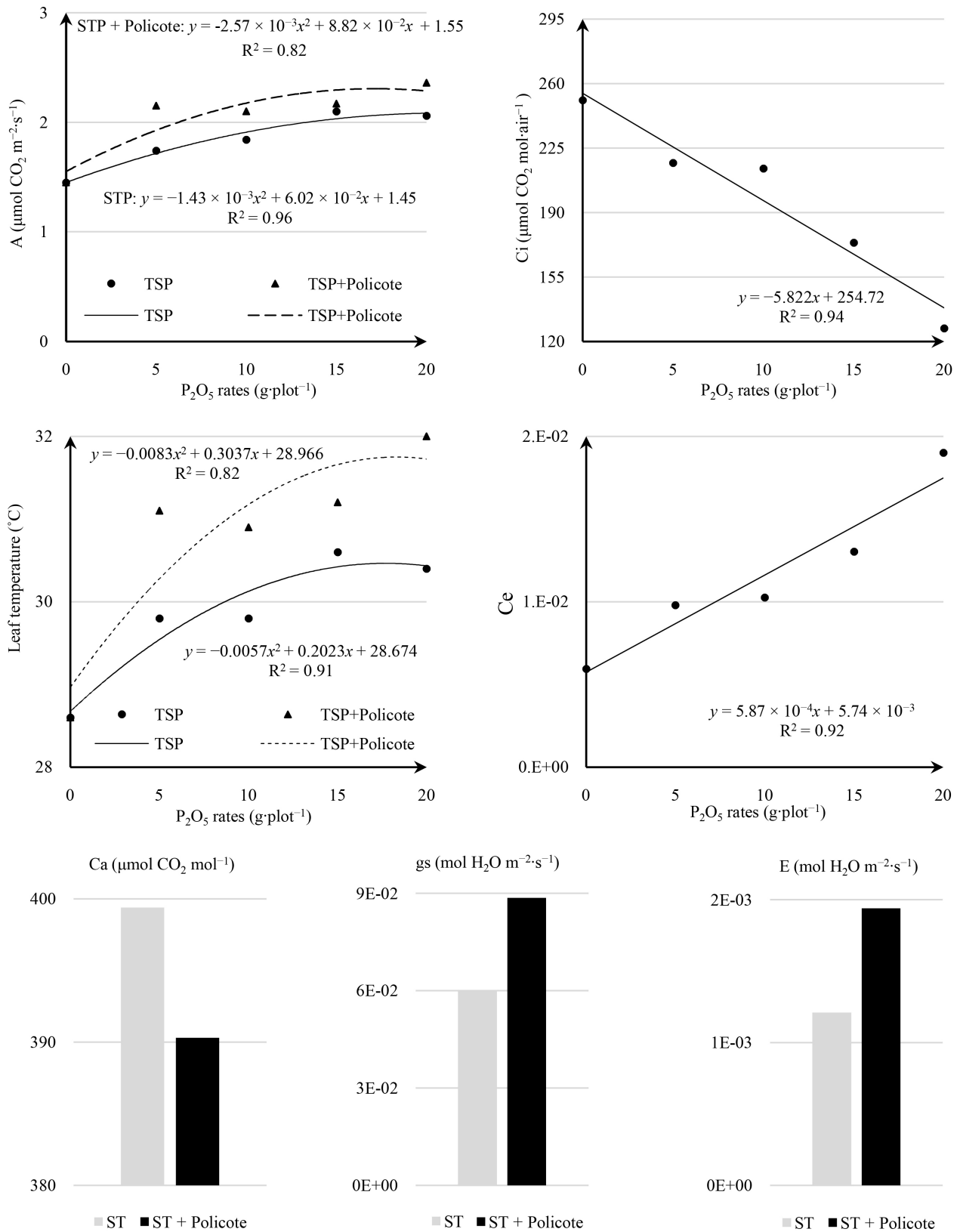
Figure 1. Relationship between net photosynthetic rate (A), leaf temperature (LT), the substomatal CO_2 concentration (Ci), the stomatal conductance (gs), transpiration rate (E) and carboxylation efficiency (Ce) and P sources (Triple Superphosphate – TSP and Policote coated TSP – TSP + Policote) and rates.

Table 1. The net photosynthetic rate (A), the leaf temperature (LT), the substomatal CO_2 concentration (Ci), the stomatal conductance (gs), the transpiration rate (E) and carboxylation efficiency (Ce).

		A ($\mu mol\ CO_2\ m^{-2} \cdot s^{-1}$)	LT (°C)	Ci ($\mu mol\ CO_2\ mol\ air^{-1}$)	gs ($mol\ H_2O\ m^{-2} \cdot s^{-1}$)	E ($mol\ H_2O\ m^{-2} \cdot s^{-1}$)	Ce
Average	TSP (00 g P_2O_5 plot^{-1})	1.45	28.6	251.0	0.0936	1.57×10^{-3}	5.95×10^{-3}
	TSP (05 g P_2O_5 plot^{-1})	1.74	29.8	229.3	0.0475	1.01×10^{-3}	8.14×10^{-3}
	TSP (10 g P_2O_5 plot^{-1})	1.84	29.8	202.0	0.0507	1.14×10^{-3}	1.11×10^{-2}
	TSP (15 g P_2O_5 plot^{-1})	2.10	30.6	152.6	0.0560	1.28×10^{-3}	1.47×10^{-2}
	TSP (20 g P_2O_5 vaso^{-1})	2.06	30.4	199.3	0.0525	1.30×10^{-3}	1.26×10^{-2}
	TSP + Policote (00 g P_2O_5 plot^{-1})	1.45	28.6	251.0	0.0936	1.57×10^{-3}	5.95×10^{-3}
	TSP + Policote (05 g P_2O_5 plot^{-1})	2.15	31.1	204.4	0.0902	2.02×10^{-3}	$1,15 \times 10^{-2}$
	TSP + Policote (10 g P_2O_5 plot^{-1})	2.10	30.9	225.7	0.0637	1.49×10^{-3}	9.40×10^{-3}
	TSP + Policote (15 g P_2O_5 plot^{-1})	2.17	31.2	194.6	0.1187	2.70×10^{-3}	1.14×10^{-2}
	TSP + Policote (20 g P_2O_5 plot^{-1})	2.36	32.0	54.9	0.0765	1.93×10^{-3}	2.54×10^{-2}
	TSP	1.84b	29.8b	186.1	0.0601b	1.21×10^{-3}b	1.05×10^{-2}
	TSP + Policote	2.05a	30.7a	206.8	0.0886a	1.94×10^{-3}a	1.27×10^{-2}
	00 g P_2O_5 plot^{-1}	1.45	28.6	251.0	0.0936	1.57×10^{-3}	5.95×10^{-3}
	05 g P_2O_5 plot^{-1}	1.95	30.4	216.9	0.0689	1.51×10^{-3}	9.80×10^{-3}
	10 g P_2O_5 plot^{-1}	1.97	30.3	213.9	0.0572	1.31×10^{-3}	1.03×10^{-2}
	15 g P_2O_5 plot^{-1}	2.13	30.9	173.6	0.0874	1.99×10^{-3}	1.30×10^{-2}
	20 g P_2O_5 plot^{-1}	2.21	31.2	127.1	0.0645	1.61×10^{-3}	1.90×10^{-2}
	Average	1.94	30.3	196.5	0.0743	1.60×10^{-3}	1.16×10^{-2}
	CV	6.07	1.40	32.4	31.9	26.6	56.3
F Value (ANOVA)	Source	22.5**	34.8**	0.79ns	10.8**	19.1**	0.87ns
	Rate	37.8**	35.3**	3.34*	2.56ns	1.98ns	3.29*
	Source * Rate	3.05*	3.61*	2.00ns	1.63ns	2.54ns	1.45ns
	Rate/TSP	15.0**	10.7**	-	-	-	-
	Rate/TSP + Policote	25.8**	28.1**	-	-	-	-

(**) significant at 1% probability by the "F" test. (*) significant at 5% probability by the "F" test. Means followed by the same letter lowercase in the column do not differ from each other by the Tukey test at 5%.

[49]. A 40% drop in net CO_2 exchange in low-P soybean plants was reported by [46]. Several studies have demonstrated that using Policote as a coating for phosphate fertilizer leads to increased crop yields [24] [28] [30] [31] [32] [33]

[34]. Higher soil phosphorus diffusion was reported with Policote-coated phosphate fertilizer [34], resulting in higher phosphorus delivery to plants per unit of used fertilizer. This phosphorus-increased delivery, an essential element of photosynthesis, promotes increased photosynthetic activity even when using lower rates of Policote-coated phosphate fertilizer.

During photosynthesis, plants fix CO_2 from the atmosphere onto ribulose-bisphosphate, producing 3-phosphoglycerate, which is reduced to triose phosphates [50]. *In vivo*, triose phosphate is exported from the chloroplast in exchange for the uptake of inorganic phosphate (Pi), via the phosphate transporter, which maintains the chloroplast Pi pool and allows photosynthesis to continue [47]. The triose phosphate is used in the cytosol predominantly for sucrose synthesis, thereby releasing Pi to maintain the cytosolic Pi pool and allow the process to continue [47]. Therefore, adequate P supply (by P fertilization) is important for adequate carbon partitioning. Despite the decreased export from chloroplasts, chloroplasts depleted of Pi had lower levels of triose phosphate while the percentage of total phosphate in 3-phosphoglycerate was increased [47]. Low phosphate nutrition results in increased chlorophyll fluorescence, reduced photosynthetic rate, accumulation of starch and sucrose in leaves, and low crop yields [48]. Plant growth and development depend on the photosynthetic rate and carbon partitioning between roots and shoots. Starch and sugar accumulation in the leaves of P-deficient plants may indirectly also be a result of low export due to ATP limitation for phloem loading. Therefore, increasing phosphorus supply, either by increasing the P rate or using enhanced efficiency P fertilizer, is vital to promote the photosynthetic rate and carbon partitioning.

PTST resulted in higher LT than TSP. Probably it is a reflex of increasing chemical reactions in the leaf tissue. The higher the leaf temperature typically the greater the rate of chemical reactions, such as photosynthesis and respiration. The significant reductions in intracellular Pi and ADP levels that follow extended Pi deprivation will impede respiratory electron flow through the cytochrome pathway at the sites of coupled ATP synthesis [21]. The low-P availability limits important processes (photosynthesis and respiration) to promote plant growth and development.

In principle, increases in stomatal conductance (gs), which regulates gas exchange (CO_2 and water), can allow plants under well-watered growth conditions to increase their CO_2 uptake and subsequently enhance photosynthesis [51]. Reference [52] reported a positive and exponential relation between gs and net photosynthetic rate. PTSP resulted in higher stomatal conductance than TSP (Figure 1), leading to a higher net photosynthetic rate and leaf temperature.

Transpiration allows the diffusion of carbon dioxide gas from the air for photosynthesis, cools plants, and enables mass flow of mineral nutrients and water from roots to shoots. Therefore, increasing transpiration rate will result in a higher net photosynthetic rate and plant growth and development. PTSP resulted in a higher transpiration rate than TSP, leading to a higher net photosynthetic rate (Figure 1).

Relationships between P and photosynthesis can also arise if the P supply affects the resistance to CO_2 movement from the atmosphere to chloroplasts [53]. The Ci reduction described above did not reduce A because it increased with P fertilization. Probably this Ci reduction was a result of CO_2 consumption promoted by higher A. This Ci reduction was not sufficient to limit A.

Increasing Ce results from increasing ribulose biphosphate (Rubisco) activity [54], an enzyme involved in the first major step of carbon fixation. It has been reported that low Pi decreases the Ce [48]. These results show the importance of adequate P nutrition to enhance Rubisco activity, Ce and A, leading to high plant growth and development.

Plant growth, expressed by increasing dry matter yield, is a result of increasing net photosynthetic rate. Photosynthesis in low-P plants appears limited by the rate of Rubisco regeneration, limiting the response of carbon acquisition of plants [55]. For example, legumes in a calcareous grassland do not respond to elevated CO_2 content unless P is added [56]. Therefore, higher coffee height and dry mass reported by [29] are consequences of the higher net photosynthetic rate observed with increasing P rates and Policote coated P fertilizer. Enhanced efficiency fertilizer (EEF) studies can bring advantages to several crops. Policote coated P fertilizer can be used as an EEF and is a more efficient way to deliver required phosphorous to plants.

All photosynthesis measurements were affected by P fertilization. P fertilization increased net photosynthetic rate, leaf temperature, substomatal CO_2 concentration, stomatal conductance, transpiration rate, and carboxylation efficiency. Policote coated P fertilizer resulted in a higher net photosynthetic rate, leaf temperature, stomatal conductance, and transpiration rate than conventional P fertilizer.

The data indicated that Policote coated P fertilizer, a kind of enhanced efficiency P fertilizer (EEPF), alters the plant's photosynthetic carbon metabolism, explaining higher crop development and yield resulted from this EEPF.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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