



RESEARCH ARTICLE

Carbohydrate utilization by belowground organs defines the capacity of faba bean to exploit organic phosphorus sources

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Abstract

Background: Limited phosphate (P_i) reserves and the economic and adverse environmental impacts of excessive application of mineral fertilizers in soils are alarming. Therefore, the use of organic phosphorus (P_{org}) is suggested to reduce reliance on chemical fertilizers.

Aims: There is currently little information regarding the accessibility of P_{org} to N_2 fixation plants. Therefore, the present study aims to address the question of to what extent P_{org} substrates can serve as reliable sources of phosphorus (P) for faba bean in its nodulated phase. Specifically, we intended to explore whether N_2 -fixing plants employ intrinsic mechanisms to facilitate P_{org} availability.

Methods: A pot experiment was conducted to assess the inherent capacity of two faba bean varieties (Tiffany vs. Mallory) to utilize P_{org} supplied as phytic acid and Ca-glycerophosphate. Plants were inoculated with *Rhizobium leguminosarum* bv. *viciae* 3841 strain and cultivated on sterilized loamy sand under greenhouse conditions.

Results: Phytic acid was relatively less efficient than Ca-glycerophosphate in sustaining plant growth. Compared to Mallory, the variety Tiffany displayed a higher capability to use P_{org} , as evidenced by the relatively higher biomass production, nodulation, phosphorus use efficiency, and P- and N-accumulations in plant tissues. P_{org} exploitation was intimately linked to the efficient utilization of the host's carbohydrate budget into nutrient-acquiring structures (roots and nodules). In addition, the preferential allocation of P_i to prolific nodules is essential for plants to thrive best under these circumstances.

Conclusions: Together with N profitability, these findings suggested that N_2 -fixing plants (e.g., faba bean) can promote the sustainable exploitation of P_{org} , thereby diminishing the sizeable environmental footprint.

KEYWORDS

available phosphate, Ca-glycerophosphate, inorganic phosphate, legumes, phytic acid, symbiosis, *Vicia faba*

1 | INTRODUCTION

Phosphorus (P) is an essential macronutrient for plant growth and development (Liu, 2021; Plaxton & Tran, 2011). It is actively involved

in a broad range of structural and metabolic functions in plants. However, P is frequently unavailable in most agricultural soils (Bechtaoui et al., 2021). Poor P availability is likely because plants do not recover much of the applied P fertilizer due to precipitation,

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adsorption, or conversion to organic complexation inaccessible by plant roots (Menezes-Blackburn et al., 2018; Richardson et al., 2005). Nevertheless, global food production is highly dependent on phosphatic fertilizers produced from mined rock phosphate (Bechtaoui et al., 2021). As rock phosphate is a finite, nonrenewable resource, several studies have warned about its maximum production potential reaching within the next few decades (Cordell & White, 2015). Traditionally, excessive and nonjudicious application of mineral fertilizers has posed a severe threat to watercourses and associated water quality and eutrophication pressing challenges (George et al., 2018).

Soil P pools consist of two significant fractions: inorganic P (P_i) and organic P (P_{org}) (Hayes et al., 2000). Compared to the P_i fraction, organically bound P is enormously abundant in soil and may reach up to 80% of the total P (Islam et al., 1979). Despite this, much less attention has been given to soil P_{org} forms than P_i forms, presumably due to the more complex structures in soil (George et al., 2018; Richardson et al., 2005). It is now widely known that P_{org} is classified into phosphate esters (monoesters, diesters), anhydrides, and phosphonates (Condon et al., 2005). Consistently, inositol phosphates are the dominant monoester phosphates, while myo-inositol hexakisphosphates, commonly known as phytates, are the most abundant form among inositol phosphates (Condon et al., 2005). Before utilization by plant roots, the P_{org} fraction must first be mineralized to the P_i form, and extracellular enzymes produced by plants and microbes can carry out this biochemical reaction at the soil-root interface (Tarafdar & Claassen, 1988).

Numerous studies on the utilization of P_{org} have shown interspecific variability among different crops, including cereals, pasture, and legumes (e.g., Belinque et al., 2015; Hayes et al., 2000; Steffens et al., 2010). Tarafdar and Claassen (1988) reported that many plant species (wheat, oat, barley, clover) could utilize phytin as effectively as P_i substrates. Other investigations, in sharp contrast, revealed that P_{org} could not fulfil P requirements for plant growth and development (Hübel & Beck, 1993; Richardson et al., 2005). This is in agreement with the findings by Hayes et al. (2000), who attributed the limited acquisition of phytate by six pasture species to the low capacity of plant roots to hydrolyze P_{org} and to phytate fixing reactions with the soil matrix. Based on this, we can infer that contrasting reports regarding P_{org} utilization by plants might be related to P_{org} substrate, growing media, plant intraspecies, genotypic differences, soil type, and the analytical approach.

A recent Game Changer has drawn attention to the premise that symbiotic legumes can efficiently mobilize the P_{org} pool in soil (Sulie-man & Mühling, 2021). This assumption is likely because significant amounts of P_i are required to sustain nodule organogenesis and functioning, especially under P_i scarcity (Lambert et al., 2020; Lazali et al., 2017). However, there is not much direct information regarding the potential mechanisms (external and internal) underlying the differential P_{org} uptake and utilization by nodulating plants. Many researchers revealed that N_2 -legumes could acidify the rhizosphere, exude organic anions, and increase their extracellular enzymatic activities, probably contributing to P_i release from poorly available P_{org} resources

(Hinsinger et al., 2003; Sulieman et al., 2022). To meet these goals, we need to focus not only on the dynamics of soil organically bound P but also on exploring the various regulatory pathways of the symbiotic nodules at the whole-plant level. These advances can significantly assist in preserving the limited P_i resources and minimizing the wasteful applications of mineral fertilizers, as also endorsed by VDLUFA (2018) and Organic Phosphorus Workshop (2016).

Faba bean (*Vicia faba* L.) is a globally important leguminous crop widely cultivated in large parts of the world (Etemadi et al., 2019). It has a relatively high N_2 -fixing potential that can dramatically improve soil fertility. In addition to the superior N_2 fixation capacity, it was discovered that faba bean possesses higher P_i mobilization potential than other crops when grown in the field (Maltais-Landry et al., 2014). For instance, the faba bean showed a strong acidification potential compared to soybean and maize (Zhou et al., 2009). Thus, we hypothesized that faba bean could promote P_{org} solubility in soil and then uptake by numerous root nodule-related strategies (Sulieman & Mühling, 2021). Therefore, the underlying purpose of the present study was to evaluate the potential capacity of two faba bean varieties to P_{org} sources when exclusively relying on nodule symbiosis for nitrogen (N).

2 | MATERIALS AND METHODS

2.1 | Plant materials and growth conditions

Two varieties of faba bean (Tiffany and Mallory) were grown in a semicontrolled greenhouse with approximately 20/15°C day/night temperature, photosynthetic active radiation of 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and 60% relative humidity. Seeds were surface-sterilized with 2% sodium hypochlorite for 5 min, thoroughly rinsed 10 times with deionized water, and subsequently soaked in 0.5 mM CaCl_2 with aeration (12 h) to facilitate germination. Plants of each variety were cultivated individually in Mitscherlich pots with 6 kg soil with the following properties: 86.7% sand, 3.2% silt, 10.1% clay, pH 6.7 (CaCl_2), and 55 mg kg^{-1} soil CAL-P. Before seed sowing, the soil samples were sterilized three times at 121°C temperature (200 kPa pressure) and then replenished with the following composition per 6 kg of soil: 4.50 g K, 0.36 g Mg, 0.75 g Ca, 15 mg Zn, 15 mg Mn, 15 mg Fe, 7.5 mg Cu, 7.5 mg Mo, 3 mg B, and 0.75 mg Co (Kadiata et al., 2002).

2.2 | Inoculum preparation and experimental treatments

Rhizobium leguminosarum bv. *viciae* 3841 was cultured for 5 days at 28°C in a tryptic yeast broth recipe (glucose 4 g L^{-1} ; tryptone 5 g L^{-1} ; yeast extract 3 g L^{-1} ; CaCl_2 0.7 g L^{-1} ; pH 6.8). As *R. leguminosarum* bv. *viciae* 3841 is a spontaneous antibiotic-resistant strain; streptomycin was added to the broth solution at 10 mL L^{-1} . Freshly prepared inoculum (3 mL per plant) reaching the optical density value of 0.5 was used for inoculation after the seeds were sown in the soil. Subsequently, four P treatments were added to the soil alongside the basal

fertilization: no P (P_0 ; negative control), KH_2PO_4 (P_i ; positive control), and two P_{org} sources (phytic acid [PA; $C_6H_{18}O_{24}P_6$] and Ca-glycerophosphate [Ca-gly-P; $C_3H_7CaO_6P$]). P was added to each pot at a level equivalent to 1.8 g P pot^{-1} (Kadiata et al., 2002). The experiment was arranged in a completely randomized design with four replicates for each P treatment.

2.3 | Gas exchange measurements

Photosynthetic-related traits were estimated using an open-flow portable photosynthesis system (LI-6400XT; Li-COR Biosciences Inc., Lincoln, NE, USA) on an intact, youngest, fully expanded leaf placed on a 6 cm^2 leaf chamber between 10:00 AM and 2:00 PM. The incoming photosynthetic photon flux density provided by a red/blue LED light source amounted to $600 \mu\text{mol m}^{-2} \text{ s}^{-1}$, and the ambient CO_2 concentration was adjusted to $400 \mu\text{mol mol}^{-1}$ by CO_2 injection. Gas exchange measurements, including the net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), stomatal conductance (g_s), and transpiration rate (Tr), were then recorded. All photosynthetic measurements were repeated six times, and the average values were recorded.

2.4 | Determination of P and N contents

Plants were harvested 45 days after sowing, and the harvested plant material was separated into nodules, roots, and shoots for chemical analysis. Next, oven-dried plant materials (65°C , 72 h) were milled to a fine powder, and approximately 200 mg was digested with 10 mL of 69% HNO_3 (ROTIPURAN Supra for ICP) in an 1800 W microwave oven (MARS 6 Xpress; CEM, Matthews, MC, USA). After that, the samples were diluted to 100 mL with distilled water ($18.2 \text{ M}\Omega \text{ cm}$ conductivity) and kept at 4°C until analysis. Finally, the total P concentration was quantified using inductively coupled plasma-mass spectrometry (ICP-MS; Agilent Technologies 7700 Series, Böblingen, Germany). For N determination, approximately 1 mg of finely ground plant tissue was weighed into aluminium capsules and fed onto a Thermo 2000 Elemental Analyzer coupled via a Thermo Conflo IV to a Thermo Delta V Plus stable light isotope mass spectrometer (Thermo Corporation, Bremen, Germany).

2.5 | Phosphorus use efficiency

Indices of phosphorus use efficiency (PUE) were estimated to characterize the relationship between P_{org} supply and plant growth and nodulation for both faba bean varieties. P uptake efficiency (PUPE), P utilization efficiency (PUtE), P absorption efficiency of roots (RPAE), and physiological PUE (PPUE) were determined following the procedures described elsewhere (Deng et al., 2018; Hammond et al., 2009):

$$\begin{aligned} \text{Total P uptake (mg plant}^{-1}\text{)} &= \text{tissue P concentration (mg g}^{-1}\text{)} \\ &\times \text{plant dry matter (g plant}^{-1}\text{)}, \end{aligned} \quad (1)$$

$$\begin{aligned} \text{PUPE (mg P g}^{-1}\text{ P applied)} &= \text{total P uptake (mg plant}^{-1}\text{)} / \\ &\text{quantity of P applied (g plant}^{-1}\text{)}, \end{aligned} \quad (2)$$

$$\begin{aligned} \text{PUtE (g DM mg}^{-1}\text{ P)} &= \text{plant dry matter accumulation (g plant}^{-1}\text{)} / \\ &\text{total P uptake (mg plant}^{-1}\text{)}, \end{aligned} \quad (3)$$

$$\begin{aligned} \text{RPAE (mg P g root}^{-1}\text{)} &= \text{total P uptake (mg plant}^{-1}\text{)} / \\ &\text{root dry matter (g plant}^{-1}\text{)}, \end{aligned} \quad (4)$$

$$\begin{aligned} \text{PPUE (g}^2\text{ DMmg}^{-1}\text{ P)} &= \text{plant dry matter (g plant}^{-1}\text{)} / \\ &\text{tissue P concentration (mg g}^{-1}\text{)}. \end{aligned} \quad (5)$$

2.6 | Sugars and organic acid analyses

Finely ground plant tissue samples ($\approx 20 \text{ mg}$) were treated with 1 mL double deionized water and preheated to 100°C for 5 min to extract soluble sugars and free inorganic anions following a modified protocol (Cataldi et al., 2000). Afterwards, the samples were immediately placed on ice for 30 min and then centrifuged at 12,000 rpm for 10 min. Subsequently, the supernatants were collected and diluted 10 times with double deionized water and vortexed for protein precipitation. Finally, chloroform was added, and samples were centrifuged at 12,000 rpm (4°C) for 5 min. Supernatants were purified using C18 columns (Strata® 8B-S001-DAK, Phenomenex, Torrance, CA, USA). Sugar (glucose, fructose) and organic acid (citrate, malate, and oxalate) concentrations were determined by isocratic ion chromatography (IC-5000 Capillary Reagent-Free IC System, Thermo Scientific).

2.7 | Statistical analysis

The data were analyzed by two-way analysis of variance using Statistix 10 analytical software (Tallahassee, Florida, USA). In addition, mean separation was carried out using Tukey's test at 95%.

3 | RESULTS

3.1 | Plant biomass production

The present results indicated that the growth of faba bean is susceptible to P deprivation, with the variety Mallory exhibiting a higher level of sensitivity (Figure 1, Table 1). Under an adequate P_i supply, Tiffany produced significantly higher shoot dry matter (SDM), root dry matter (RDM), and total dry matter (TDM) than Mallory. Both varieties were differentially responsive to PA and Ca-gly-P treatments, as evidenced by the SDM, RDM, and TDM measurements (Table 1). Although both varieties produced similar TDM under P_{org} supply, plants under

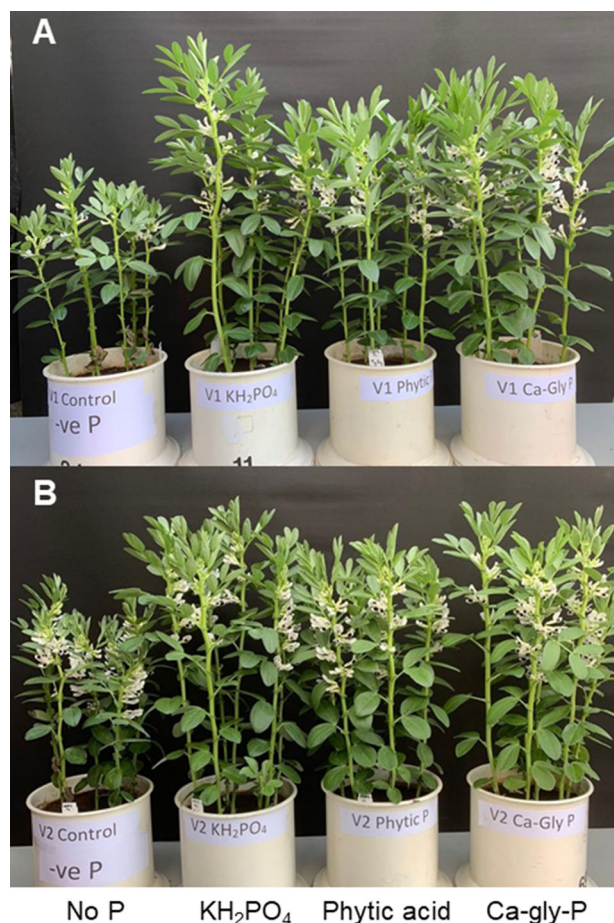


FIGURE 1 Growth response of (A) Tiffany and (B) Mallory varieties inoculated with *Rhizobium leguminosarum* bv. *viciae* 3841 and grown in a pot (6 kg) experiment for 45 days under different phosphorus (P) treatments (no P, KH_2PO_4 , phytic acid, and Ca-glycophosphate). Abbreviation: Ca-gly-P, Ca-glycerophosphate.

TABLE 1 Dry matter production and nodulation levels in two varieties of faba bean (Tiffany and Mallory) inoculated with *Rhizobium leguminosarum* bv. *viciae* 3841 and grown in a pot (6 kg) experiment for 45 days under different phosphorus (P) treatments (no P, KH_2PO_4 , phytic acid, and Ca-glycerophosphate).

	Tiffany				Mallory			
	No P	KH_2PO_4	Phytic acid	Ca-gly-P	No P	KH_2PO_4	Phytic acid	Ca-gly-P
Shoot DM (g plant^{-1})	$1.88 \pm 0.05\text{d}$	$3.25 \pm 0.08\text{a}$	$2.61 \pm 0.12\text{bc}$	$3.18 \pm 0.06\text{a}$	$1.59 \pm 0.05\text{e}$	$2.85 \pm 0.08\text{b}$	$2.56 \pm 0.10\text{c}$	$2.81 \pm 0.08\text{b}$
Root DM (g plant^{-1})	$0.87 \pm 0.05\text{d}$	$1.05 \pm 0.09\text{abc}$	$1.16 \pm 0.04\text{a}$	$0.96 \pm 0.05\text{cd}$	$0.74 \pm 0.03\text{e}$	$0.89 \pm 0.08\text{d}$	$1.14 \pm 0.04\text{ab}$	$1.03 \pm 0.06\text{bc}$
Whole plant DM (g plant^{-1})	$2.88 \pm 0.06\text{d}$	$4.61 \pm 0.12\text{a}$	$4.00 \pm 0.15\text{c}$	$4.40 \pm 0.06\text{ab}$	$2.46 \pm 0.07\text{e}$	$4.03 \pm 0.12\text{c}$	$3.96 \pm 0.06\text{c}$	$4.15 \pm 0.13\text{bc}$
Root/shoot ratio (g/g)	$0.46 \pm 0.04\text{a}$	$0.32 \pm 0.03\text{bc}$	$0.44 \pm 0.02\text{a}$	$0.30 \pm 0.02\text{c}$	$0.47 \pm 0.02\text{a}$	$0.31 \pm 0.03\text{bc}$	$0.45 \pm 0.03\text{a}$	$0.37 \pm 0.02\text{b}$
Nodule/root ratio (g/g)	$0.16 \pm 0.02\text{e}$	$0.30 \pm 0.03\text{ab}$	$0.20 \pm 0.01\text{de}$	$0.27 \pm 0.02\text{bc}$	$0.17 \pm 0.01\text{de}$	$0.34 \pm 0.03\text{a}$	$0.23 \pm 0.01\text{cd}$	$0.31 \pm 0.02\text{ab}$
Nodule number plant^{-1}	$345.3 \pm 17.1\text{cd}$	$432.3 \pm 18.5\text{ab}$	$476.5 \pm 25.6\text{a}$	$399.3 \pm 33.8\text{bc}$	$305.8 \pm 12.6\text{d}$	$406.3 \pm 19.2\text{bc}$	$430.8 \pm 16.5\text{ab}$	$387.3 \pm 18.4\text{bc}$
Nodule DM (g plant^{-1})	$0.14 \pm 0.011\text{c}$	$0.31 \pm 0.011\text{a}$	$0.24 \pm 0.006\text{b}$	$0.26 \pm 0.009\text{b}$	$0.13 \pm 0.006\text{c}$	$0.30 \pm 0.014\text{a}$	$0.26 \pm 0.011\text{b}$	$0.32 \pm 0.018\text{a}$
Individual nodule DM (mg)	$0.41 \pm 0.03\text{d}$	$0.72 \pm 0.05\text{ab}$	$0.50 \pm 0.03\text{cd}$	$0.66 \pm 0.08\text{b}$	$0.41 \pm 0.02\text{d}$	$0.73 \pm 0.06\text{ab}$	$0.60 \pm 0.04\text{bc}$	$0.82 \pm 0.06\text{a}$

Note: Data presented are the means \pm SE of four replicates. Different letters within a row indicate a significant difference from each other in all combinations (Tukey's test, $p \leq 0.05$).

Abbreviations: Ca-gly-P, Ca-glycerophosphate; DM, dry matter.

Ca-gly-P displayed the highest records. Concerning the PA condition, we observed no significant differences between both varieties regarding SDM, RDM, and TDM (Table 1). Our findings suggested that Tiffany was relatively more acclimated under low- P_i regimes and highly efficient in P_{org} utilization, especially under Ca-gly-P. These results imply that Ca-gly-P can be used as a reliable P source, whereas PA is less efficient for biomass production (Table 1).

Faba bean varieties exhibited remarkable variations in biomass partitioning under different P treatments (Table 1). Both varieties responded similarly by producing a remarkably higher root/shoot ratio (RSR) under P_0 supply (Table 1). A similar response was noted for plants under PA application (Table 1). These results further support the lower efficiency of PA as a sole source for the P_i nutrition of plants (Hayes et al., 2000). In contrast, the RSR was significantly reduced in plants under P_i or Ca-gly-P (Table 1). No significant differences were noted between the varieties under different P treatments, except for Ca-gly-P, in which Mallory exhibited a higher RSR than Tiffany (Table 1). Apart from the RSR, both faba bean varieties exhibited significant variations regarding the nodule/root ratio (NRR) (Table 1). Under P_i and Ca-gly-P conditions, a higher proportion of the biomass was allocated to support nodulation, leading to a higher NRR. In stark contrast, a lower NRR was noted for plants under PA or P_0 supply. On the other hand, no significant effects on the NRR were noted between Tiffany and Mallory when both varieties were likely treated (Table 1).

3.2 | Nodulation

P_i starvation negatively affected the nodulation of faba bean varieties (Table 1). However, compared to nodule number (NN), total nodule dry matter (NDM) and individual nodule dry matter (INDM) were relatively

more susceptible to suboptimal P_i availability (Table 1). Concerning the NDM, both varieties responded similarly to all P treatments, except Ca-gly-P, in which Mallory displayed higher values (Table 1). Furthermore, no significant variation was noted between both varieties regarding PA in all quantified parameters (Table 1). However, when each variety was incubated with different P_{org} sources, it became evident that Mallory produced significantly higher NDM and INDM under Ca-gly-P. In contrast, Tiffany exhibited significantly higher NN and INDM under PA and Ca-gly-P, respectively (Table 1). Thus, Ca-gly-P may be more readily available than PA, with the variety Mallory exhibiting better performance than Tiffany.

3.3 | Tissue-specific P allocation

P concentrations (mg g^{-1} DM) were significantly reduced in plants under the P_0 treatment (Table 2). P_{org} applications significantly increased the P concentrations in different plant organs compared to the negative controls (Table 2). Notably, the P concentration was relatively higher in nodules than in other plant organs (Table 2). Tiffany exhibited a higher shoot P concentration than Mallory under PA supply. However, the P concentration was significantly decreased compared with that under Ca-gly-P (Table 2). Mallory's markedly higher RSR (Table 1) was constantly reflected in the higher root P concentration under Ca-gly-P (Table 2). However, Tiffany was more efficient in maintaining the nodule P concentration under the same experimental conditions (Table 2). For total P uptake (mg plant^{-1}), Tiffany significantly accumulated higher P contents in the shoot and whole plant across all P treatments (P_i and P_{org}) (Table 2). Coincident with the root P measurements, the root P uptake was significantly higher for Mallory under Ca-gly-P (Table 2). These data support the premise that Tiffany was more efficient than Mallory in P_{org} utilization and P_i distribution among plant organs.

3.4 | Total N accumulation

The shoot and total plant N content (mg plant^{-1}) was significantly higher in Tiffany plants under P_i and Ca-gly-P applications (Table 2). Compared to Ca-gly-P, plants under PA accumulated minor N amounts in the shoots, nodules, and whole plants in both varieties (Table 2). No significant differences were revealed between the varieties regarding the PA treatment (Table 2). For the N concentration (mg g^{-1}), no significant difference was noted except for nodules when both varieties were treated similarly (Table 2). Tiffany under P_i and Ca-gly-P showed significantly higher N concentrations in nodules (Table 2). The C/N ratios were markedly affected by the Ca-gly-P supply, in which Tiffany showed significantly lower C/N ratios for the roots and nodules (Table 2). In addition, the N/P ratios in the shoots and roots were significantly higher for plants under P_0 , followed by PA, whereas P_i and Ca-gly-P displayed the lowest values. No significant differences were shown for the nodule N/P ratio when both varieties were likely treated (Table 2).

3.5 | Phosphorus use efficiency

Indices of PUE were significantly affected by P treatments (Table 3). Plants under sufficient P_i supply increased PUE while decreasing PUE (Table 3). In this instance, a contrasting response was noted between both varieties, with the variety Tiffany displaying a higher PUE (Table 3). Under PA incubation, the faba bean varieties displayed the least PUE than under other P treatments, with the variety Tiffany exhibiting better performance than Mallory (Table 3). Generally, an opposite trend was noted between both varieties, with Tiffany displaying higher PUE, while Mallory exhibited higher PUE across all P treatments, except under Ca-gly-P supply (Table 3). RPAE was the highest for plants treated with $P_i > \text{Ca-gly-P} > \text{PA}$, whereas plants exposed to P limitation displayed the lowest values (Table 3). Compared with the P_i and Ca-gly-P treatments, the PPUE was significantly higher in plants under PA, with the variety Mallory exhibiting the highest value (Table 3). Together, these results provided further support for the relatively lower efficiency of PA and suggested that the variety Tiffany might be potentially more efficient in accessing P_{org} sources.

3.6 | Gas exchange measurements

Plant biomass production relies on photosynthetic efficiency, which largely depends on P_i availability interns (Nguyen et al., 2021). P deprivation significantly reduced the P_N of faba bean plants, with the variety Tiffany exhibiting the lowest value (Figure 2A). Both varieties did not significantly differ in response to other P treatments, except Mallory, which showed lower P_N than Tiffany under PA supply (Figure 2A). In addition, no more considerable variation was found between both varieties regarding the g_s (Figure 2B), Tr (Figure 2C), and C_i (Figure 2D) across all P treatments. The present results suggested that P treatment did not stringently affect the photosynthetic efficiency among different plants.

3.7 | Major carbon (C) metabolites

The data presented in Table 4 indicated no remarkable differences between both varieties regarding the shoot concentrations of sugars. Mallory experiencing P limitation showed significantly higher nodule concentrations of hexoses than Tiffany. A similar response was noticed under PA treatment regarding nodule glucose concentration (Table 4). For organic acids, P limitation significantly increased citrate formation in the roots of both varieties, whereas the concentration of this organic acid remained unaffected across the other P treatments (Table 4). A similar trend was noted for this metabolite in nodular fractions, except for PA, which significantly increased over P_i and Ca-gly-P treatments (Table 4). On the other hand, no significant difference was indicated between both varieties regarding the malate concentration in plant fractions (Table 4). Similar to citrate, higher significant oxalate concentrations were measured in the roots under the P_0 treatment (Table 4). The statistical analysis revealed no significant differences between

TABLE 2 Phosphorus (P) and nitrogen (N) assimilation and N/P and C/N ratios in two varieties of faba bean (Tiffany and Mallory) inoculated with *Rhizobium leguminosarum* bv. *viciae* 3841 and grown in a pot (6 kg) experiment for 45 days under different P treatments (no P, KH_2PO_4 , phytic acid, and Ca-glycerophosphate).

	Tiffany				Mallory			
	No P	KH_2PO_4	Phytic acid	Ca-gly-P	No P	KH_2PO_4	Phytic acid	Ca-gly-P
Shoot P conc. (mg P g^{-1} DM)	$1.63 \pm 0.09\text{e}$	$5.57 \pm 0.08\text{a}$	$3.05 \pm 0.10\text{c}$	$4.19 \pm 0.10\text{b}$	$1.64 \pm 0.07\text{e}$	$4.19 \pm 0.05\text{b}$	$2.33 \pm 0.06\text{d}$	$3.98 \pm 0.06\text{b}$
Root P conc. (mg P g^{-1} DM)	$1.38 \pm 0.05\text{e}$	$3.29 \pm 0.09\text{a}$	$1.90 \pm 0.05\text{d}$	$2.43 \pm 0.07\text{c}$	$1.22 \pm 0.04\text{e}$	$2.93 \pm 0.03\text{b}$	$1.80 \pm 0.10\text{d}$	$2.76 \pm 0.09\text{b}$
Nodules P conc. (mg P g^{-1} DM)	$2.82 \pm 0.18\text{d}$	$6.02 \pm 0.17\text{a}$	$4.35 \pm 0.07\text{c}$	$5.38 \pm 0.12\text{b}$	$2.67 \pm 0.11\text{d}$	$4.38 \pm 0.10\text{c}$	$4.01 \pm 0.07\text{c}$	$4.12 \pm 0.10\text{c}$
Shoot P content (mg plant^{-1})	$3.06 \pm 0.20\text{f}$	$18.10 \pm 0.60\text{a}$	$7.94 \pm 0.25\text{d}$	$13.31 \pm 0.36\text{b}$	$2.61 \pm 0.10\text{f}$	$11.92 \pm 0.31\text{c}$	$5.95 \pm 0.09\text{e}$	$11.18 \pm 0.37\text{c}$
Root P content (mg plant^{-1})	$1.20 \pm 0.09\text{e}$	$3.43 \pm 0.20\text{a}$	$2.19 \pm 0.05\text{d}$	$2.32 \pm 0.07\text{cd}$	$0.90 \pm 0.05\text{e}$	$2.59 \pm 0.20\text{bc}$	$2.05 \pm 0.16\text{d}$	$2.82 \pm 0.13\text{b}$
Nodules P content (mg plant^{-1})	$0.40 \pm 0.05\text{d}$	$1.87 \pm 0.07\text{a}$	$1.02 \pm 0.04\text{c}$	$1.39 \pm 0.07\text{b}$	$0.33 \pm 0.01\text{d}$	$1.29 \pm 0.05\text{b}$	$1.03 \pm 0.05\text{c}$	$1.31 \pm 0.09\text{b}$
Total P content (mg plant^{-1})	$4.66 \pm 0.29\text{f}$	$23.39 \pm 0.60\text{a}$	$11.15 \pm 0.32\text{d}$	$17.02 \pm 0.35\text{b}$	$3.85 \pm 0.12\text{f}$	$15.80 \pm 0.35\text{c}$	$9.03 \pm 0.12\text{e}$	$15.31 \pm 0.45\text{c}$
Shoot %N in DM	$3.12 \pm 0.06\text{bc}$	$3.63 \pm 0.05\text{a}$	$3.33 \pm 0.11\text{ab}$	$3.68 \pm 0.08\text{a}$	$2.95 \pm 0.14\text{c}$	$3.56 \pm 0.13\text{a}$	$3.17 \pm 0.14\text{bc}$	$3.46 \pm 0.15\text{ab}$
Root %N in DM	$2.24 \pm 0.24\text{ab}$	$2.48 \pm 0.06\text{a}$	$1.97 \pm 0.19\text{b}$	$2.20 \pm 0.16\text{ab}$	$2.24 \pm 0.10\text{ab}$	$2.37 \pm 0.08\text{ab}$	$2.14 \pm 0.10\text{ab}$	$2.35 \pm 0.15\text{ab}$
Nodules %N in DM	$3.99 \pm 0.44\text{cd}$	$5.88 \pm 0.29\text{a}$	$4.78 \pm 0.25\text{b}$	$5.70 \pm 0.27\text{a}$	$3.66 \pm 0.16\text{d}$	$4.66 \pm 0.19\text{bc}$	$4.43 \pm 0.12\text{bc}$	$4.84 \pm 0.18\text{b}$
Shoot N content (mg plant^{-1})	$58.51 \pm 2.47\text{d}$	$117.94 \pm 2.98\text{a}$	$86.42 \pm 1.45\text{c}$	$116.93 \pm 3.86\text{a}$	$46.81 \pm 1.66\text{e}$	$101.24 \pm 4.98\text{b}$	$81.02 \pm 4.01\text{c}$	$96.97 \pm 3.16\text{b}$
Root N content (mg plant^{-1})	$19.72 \pm 3.02\text{bc}$	$26.04 \pm 2.26\text{a}$	$22.66 \pm 1.92\text{ab}$	$21.07 \pm 1.55\text{abc}$	$16.62 \pm 1.38\text{c}$	$20.92 \pm 1.57\text{abc}$	$24.47 \pm 1.83\text{ab}$	$24.20 \pm 2.34\text{ab}$
Nodules N content (mg plant^{-1})	$5.73 \pm 0.99\text{e}$	$18.22 \pm 1.04\text{a}$	$11.20 \pm 0.52\text{d}$	$14.64 \pm 0.72\text{b}$	$4.56 \pm 0.23\text{e}$	$13.69 \pm 0.59\text{bc}$	$11.40 \pm 0.46\text{cd}$	$15.47 \pm 1.40\text{b}$
Total N content (mg plant^{-1})	$83.96 \pm 2.16\text{d}$	$162.19 \pm 4.04\text{a}$	$120.28 \pm 2.92\text{c}$	$152.63 \pm 4.88\text{a}$	$67.98 \pm 0.27\text{e}$	$135.85 \pm 3.59\text{b}$	$116.89 \pm 5.23\text{c}$	$136.63 \pm 5.50\text{b}$
Shoot C/N ratio (%/%)	$11.63 \pm 0.23\text{ab}$	$10.36 \pm 0.16\text{d}$	$11.00 \pm 0.34\text{bcd}$	$10.28 \pm 0.22\text{d}$	$12.39 \pm 0.49\text{a}$	$10.57 \pm 0.26\text{cd}$	$11.60 \pm 0.46\text{abc}$	$10.83 \pm 0.37\text{bcd}$
Root C/N ratio (%/%)	$12.87 \pm 0.19\text{ab}$	$11.94 \pm 0.27\text{bc}$	$12.66 \pm 0.16\text{abc}$	$11.78 \pm 0.46\text{c}$	$13.25 \pm 0.24\text{a}$	$12.50 \pm 0.30\text{abc}$	$12.74 \pm 0.54\text{abc}$	$13.02 \pm 0.55\text{a}$
Nodules C/N ratio (%/%)	$8.84 \pm 0.55\text{ab}$	$6.72 \pm 0.30\text{cd}$	$7.96 \pm 0.21\text{ab}$	$6.26 \pm 0.85\text{d}$	$9.16 \pm 0.29\text{a}$	$8.15 \pm 0.36\text{ab}$	$8.25 \pm 0.09\text{ab}$	$7.76 \pm 0.22\text{bc}$
Shoot N/P ratio (%/%)	$19.27 \pm 0.91\text{a}$	$6.52 \pm 0.13\text{e}$	$10.90 \pm 0.16\text{c}$	$8.80 \pm 0.37\text{d}$	$18.03 \pm 1.07\text{a}$	$8.48 \pm 0.25\text{d}$	$13.61 \pm 0.58\text{b}$	$8.71 \pm 0.45\text{d}$
Root N/P ratio (%/%)	$16.26 \pm 1.81\text{a}$	$7.56 \pm 0.25\text{d}$	$10.34 \pm 0.75\text{bc}$	$9.06 \pm 0.55\text{cd}$	$18.30 \pm 0.64\text{a}$	$8.10 \pm 0.24\text{cd}$	$12.05 \pm 1.07\text{b}$	$8.55 \pm 0.62\text{cd}$
Nodules N/P ratio (%/%)	$14.05 \pm 0.99\text{a}$	$9.81 \pm 0.67\text{c}$	$11.00 \pm 0.57\text{bc}$	$10.61 \pm 0.53\text{bc}$	$13.79 \pm 0.89\text{a}$	$10.62 \pm 0.21\text{bc}$	$11.06 \pm 0.31\text{c}$	$12.29 \pm 0.54\text{ab}$

Note: Data presented are the means \pm SE of four replicates. Different letters within a row indicate a significant difference from each other in all combinations (Tukey's test, $p \leq 0.05$).

Abbreviations: Ca-gly-P, Ca-glycerophosphate; DM, dry matter.

P_i and P_{org} treatments regarding the nodule oxalate concentration (Table 4).

4 | DISCUSSION

The present study demonstrated that N_2 -fixing faba bean plants were differentially able to access P_i from P_{org} substrates when grown

under sterile conditions (Figure 1, Table 1). However, PA was relatively less efficient than Ca-gly-P in sustaining plant growth and nodulation (Table 1). Despite its surplus abundance in soil, there is a broad consensus that PA is markedly resistant to rapid mineralization and is highly difficult to access by a broad range of plants (Giles et al., 2018; Hayes et al., 2000; Hübel & Beck, 1993). The precipitation or strong association of PA with the solid phase of the soil is presumably related to its multiple P_i groups and high anionic charge

TABLE 3 Phosphorus (P) uptake efficiency (PUpE), P utilization efficiency (PUtE), P absorption efficiency of roots (RPAE), and physiological PUE (PPUE) in two varieties of faba bean (Tiffany and Mallory) inoculated with *Rhizobium leguminosarum* bv. *viciae* 3841 and grown in a pot (6 kg) experiment for 45 days under different P treatments (no P, KH_2PO_4 , phytic acid, and Ca-glycerophosphate).

Variety	P treatment	PUpE (mg P g ⁻¹ P applied)	PUtE (g DM mg ⁻¹ P)	RPAE (mg P g root ⁻¹)	PPUE (g ² DM g ⁻¹ P)
Tiffany	No P	—	1.71 ± 0.07b	5.41 ± 0.35d	1.84 ± 0.05a
	KH_2PO_4	78.51 ± 2.01a	0.65 ± 0.01f	22.80 ± 1.99a	0.95 ± 0.04e
	Phytic acid	37.42 ± 1.06d	1.09 ± 0.02d	9.68 ± 0.32c	1.53 ± 0.08c
	Ca-glycerophosphate	57.13 ± 1.16b	0.84 ± 0.02e	17.88 ± 1.23b	1.21 ± 0.04d
Mallory	No P	—	1.81 ± 0.03a	5.22 ± 0.20d	1.63 ± 0.08bc
	KH_2PO_4	53.03 ± 1.19c	0.81 ± 0.00e	18.25 ± 1.61b	1.05 ± 0.05de
	Phytic acid	30.32 ± 0.41e	1.24 ± 0.04c	7.95 ± 0.25cd	1.81 ± 0.08ab
	Ca-glycerophosphate	51.37 ± 1.52c	0.86 ± 0.01e	15.06 ± 0.78b	1.16 ± 0.05d

Note: Data presented are the means ± SE of four replicates. Different letters within a column indicate a significant difference from each other in all combinations (Tukey's test, $p \leq 0.05$).

Abbreviation: DM, dry matter.

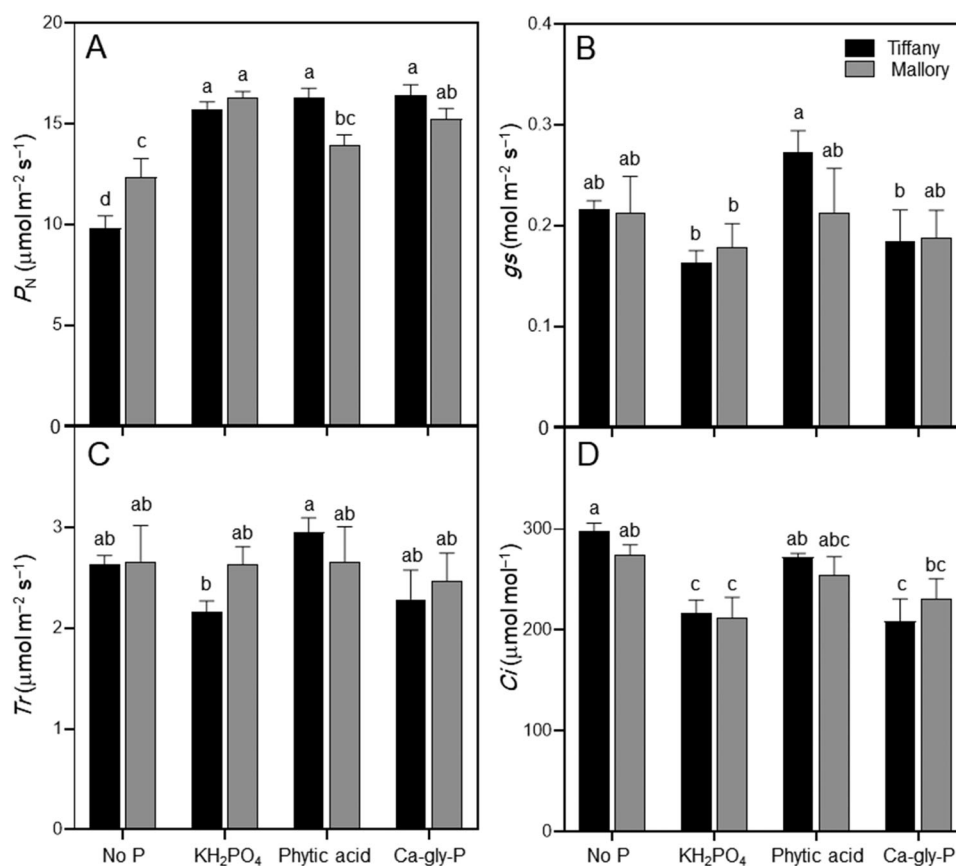


FIGURE 2 (A) Net photosynthetic rate (P_n), (B) stomatal conductance (g_s), (C) transpiration rate (Tr), and (D) intercellular CO_2 concentration (C_i) in two varieties of faba bean (Tiffany and Mallory) inoculated with *Rhizobium leguminosarum* bv. *viciae* 3841 and grown in a pot (6 kg) experiment for 45 days under different phosphorus (P) treatments (no P, KH_2PO_4 , phytic acid, and Ca-glycerophosphate). Data are the means of four replicates. Error bars represent standard errors. Data with different letters are significantly different as measured by Tukey's test ($p \leq 0.05$). Abbreviation: Ca-gly-P, Ca-glycerophosphate.

TABLE 4 Contents of sugars (glucose and fructose; mg g⁻¹ dry matter [DM]) and organic acids (citrate, malate and oxalate: mg g⁻¹ DM) in two varieties of faba bean (Tiffany and Mallory) inoculated with *Rhizobium leguminosarum* bv. *viciae* 3841 and grown in a pot (6 kg) experiment for 45 days under different phosphorus (P) treatments (no P, KH₂PO₄, phytic acid, and Ca-glycerophosphate).

	Tiffany				Mallory			
	No P	KH ₂ PO ₄	Phytic acid	Ca-gly-P	No P	KH ₂ PO ₄	Phytic acid	Ca-gly-P
Glucose in shoot	5.37 ± 1.41abc	1.60 ± 0.47c	3.13 ± 0.89bc	3.86 ± 0.26bc	7.86 ± 0.50a	3.62 ± 0.15bc	4.51 ± 0.79abc	6.44 ± 0.79ab
Glucose in Nodules	5.57 ± 0.92bc	2.27 ± 0.29c	1.75 ± 0.91c	0.13 ± 0.05c	14.69 ± 1.74a	4.01 ± 1.49bc	9.69 ± 2.22ab	3.67 ± 1.28bc
Fructose in shoots	9.33 ± 0.84ab	3.42 ± 1.43b	3.45 ± 1.26b	6.25 ± 0.55ab	10.65 ± 0.46a	6.72 ± 0.79ab	9.23 ± 2.60ab	11.45 ± 1.29a
Fructose in nodules	10.40 ± 1.26bc	5.20 ± 1.58c	12.00 ± 2.42bc	3.98 ± 2.57c	24.29 ± 0.82a	11.02 ± 2.01bc	18.70 ± 3.87ab	9.72 ± 0.96bc
Citrate in roots	11.18 ± 0.26a	1.85 ± 0.83b	2.16 ± 0.41b	0.53 ± 0.09b	9.35 ± 1.28a	1.67 ± 0.15b	3.61 ± 0.18b	1.86 ± 0.66b
Citrate in nodules	3.30 ± 0.09a	0.68 ± 0.01c	1.90 ± 0.22b	0.43 ± 0.06c	2.68 ± 0.20a	0.83 ± 0.10c	1.90 ± 0.12b	0.46 ± 0.13c
Malate in roots	6.54 ± 0.67a	2.09 ± 0.31ab	1.97 ± 0.44ab	0.45 ± 0.10b	4.70 ± 1.99ab	2.17 ± 0.41ab	2.22 ± 0.47ab	2.94 ± 0.91ab
Malate in nodules	3.30 ± 0.77a	3.46 ± 0.78a	1.94 ± 0.97a	1.24 ± 1.17a	2.17 ± 0.81a	1.89 ± 0.56a	3.31 ± 0.54a	0.95 ± 0.46a
Oxalate in roots	1.66 ± 0.21ab	0.73 ± 0.03c	0.65 ± 0.02c	0.68 ± 0.04c	1.89 ± 0.39a	0.66 ± 0.01c	0.75 ± 0.04c	0.92 ± 0.06bc
Oxalate in nodules	0.69 ± 0.05ab	0.82 ± 0.02ab	1.17 ± 0.09a	1.06 ± 0.11ab	0.65 ± 0.05b	0.79 ± 0.06ab	0.76 ± 0.18ab	0.72 ± 0.11ab

Note: Data presented are the means ± SE of three replicates. Different letters within a row indicate a significant difference from each other in all combinations (Tukey's test, $p \leq 0.05$).

Abbreviation: Ca-gly-P, Ca-glycerophosphate.

densities (Findenegg & Nelemans, 1993; Furlani et al., 1987; Menezes-Blackburn et al., 2018). Contrary to PA, Ca-gly-P appeared to be a preferable source to the P_i nutrition of plants that can solubilize more readily under similar conditions. The simplicity of the molecular structure and the surplus P_i produced during hydrolysis may help plants use Ca-gly-P more effectively (Adams & Pate, 1992; Islam et al., 1979). The scarcity of P_i in the sterilized soil substrates was probably the main driver for P_{org} utilization by plants (Islam et al., 1979). Accordingly, as recently suggested, these P_{org} sources can play a prominent role in the P nutrition of N₂-fixing plants (Suliman & Mühling, 2021).

4.1 | Possible mechanisms for P_{org} utilization by faba bean varieties

Limited P_i bioavailability is a significant constraint for faba bean growth and development (Etemadi et al., 2019). The present observations suggested that Tiffany was more responsive to P_i supply and better adapted to cope with P_i starvation (Table 1). Importantly, Tiffany was more efficient than Mallory in P_{org} exploitation (Table 1). Therefore, the following question arises: how can both varieties manage to obtain P_i from P_{org} sources? Based on the results, multifaceted intrinsic mechanisms (morphological and physiological) were employed by both varieties when incubated for 45 days in sterilized soil. Some of these mechanisms aimed to promote P_i acquisition, whereas others were exploited to conserve the internal use of P_i utilization. The following sections discuss the possible mechanisms adopted by both varieties at the whole-plant level.

4.2 | Biomass partitioning at the whole-plant level

Although both varieties produced approximately equivalent TDM under Ca-gly-P, the variety Mallory allocated a more significant frac-

tion of their photosynthetically fixed C to the root systems (Table 1). This response was underpinned by a striking increase in the RSR, which can aid plants in obtaining P_i from Ca-gly-P substrate (Suliman & Mühling, 2021). In this instance, the variety Tiffany showed an opposite trend by diverting C resources predominantly to the shoot (i.e., higher SDM) at the expense of the root (i.e., lower RSR; Table 1), thereby strengthening the utilization efficiency of photosynthate on plant growth. The shoot-root system is intimately linked, and a dynamic adjustment among its constituents is constantly coordinated to ensure minimal C costs while satisfying the plant's nutritional needs (trade-off of resources) (Suliman et al., 2019, 2022). Compared with the positive control, the photosynthetic-related traits (P_N, Ci, gs, and Tr) were insignificantly affected when plants were incubated under Ca-gly-P (Figure 2), indicating no sign of marked Pi deprivation. Numerous published reports have noted that P availability determines the photosynthetic capacity of plants and their instantaneous carboxylation efficiency in organic C and biomass production (Nguyen et al., 2021; Warren, 2011). Thus, it is tempting to suggest that P_{org}-treated plants accessed sufficient amounts of carbohydrates during the nodulation phase.

On the other hand, the SDM of plants under PA showed a marked decrease compared with those under P_i and Ca-gly-P (Table 1). The low efficiency of PA for P nutrition of plants was mirrored by significantly higher RSR at a comparable level to the negative control (Table 1). The remarkably higher RSR may be an active morphological strategy of faba bean to access P supplied as PA (Nuruzzaman et al., 2005). An increase in the RSR has been considered a distinctive hallmark of Pi limitation (Pérez-Fernández et al., 2019). Under such low P_i bioavailability, Mallory's P_N was significantly reduced compared with Tiffany's P_N (Figure 3). Consistent with this notion, it was previously found that the formation of extensive roots represents an important morphological modification for faba bean plants under P_{org}-rich conditions (Nuruzzaman et al., 2005). Accordingly, the faba bean has

been considered a plant with a strong soil P_i mobilization capacity among other plant species. Despite the beneficial effect of the foraging strategy, the proportionally larger root biomass is energetically excessive in C expenditure that can drain the host C reserve, leading to growth retardation (Pérez-Fernández et al., 2019; Sulieman et al., 2019).

A further aspect determining the efficiency of P_{org} exploitation by plants is the biomass partitioning between the underground organs (Sulieman et al., 2019, 2022). Given that nodules incur enormous sink demands for both C and P_i , it is plausible that intense competition between the roots (source of P_i) and the nodular fraction (source of N) may occur, especially under low P_i availability (Sulieman et al., 2019). The host plant might face a tradeoff between root growth and nodulation in this instance. Notably, both varieties increased the NRR under Ca-gly-P supply, whereas a reversible response occurred under PA application (Table 1). Thus, more considerable investment was dedicated to supporting the nodulation of plants under Ca-gly-P. Considerable evidence indicates that high nodule biomass is a good indicator of high symbiotic efficiency (Allito et al., 2021). In support of this finding, the higher nodulation fraction for plants under Ca-gly-P supply coincided with significantly higher SDM and TDM (Table 1). Mallory under Ca-gly-P displayed higher RSR than Tiffany, accompanied by higher TNM and INDM (Table 1). However, Tiffany was relatively more efficient in upgrading the SDM under similar conditions, presumably indicating higher symbiotic efficiency. Indeed, a close association was found between nodulation and shoot biomass production (Allito et al., 2021; Pérez-Fernández et al., 2019). Together, the present findings support the higher efficiency of Ca-gly-P and suggest that the variety Tiffany was relatively more efficient than Mallory in biomass partitioning at the whole-plant level.

4.3 | Exudation of organic anions

Apart from biomass petitioning, other biochemical mechanisms might also be involved in P_{org} utilization by faba bean varieties. Among these, the excretion of organic substances into the rhizosphere is likely to occur, especially under limited P_i availability (Nuruzzaman et al., 2005). In particular, low-molecular-weight organic anions could significantly mobilize sparingly soluble soil P (Touhami et al., 2020). Nevertheless, the exudation of organic substances can severely drain the C budget, thereby reducing the C available for other processes (Sulieman et al., 2022). Our results indicated that substantial amounts of citrate and oxalate were accumulated under P deprivation and more likely to be used for exudation into the rhizosphere (rhizodeposition). Citrate appears to be specifically effective in P_i mobilization among the organic anions released by P_i -stressed plants (Nuruzzaman et al., 2005; Sulieman et al., 2019). Similarly, the nodules of plants under PA significantly increased citrate concentrations compared with P_i and Ca-gly-P (Table 4). Indeed, it has been reported that nodules could behave similarly to roots and exudate organic acids that can promote nodule P_i homeostasis (Sulieman & Tran, 2015; Xue et al., 2018). On the other hand, it cannot be ruled out that nodulating roots

additionally released other organic substances (e.g., extracellular phosphatase, phenolics) to enhance the solubility of organically bound P (Tarafdar & Claassen, 1988; Richardson et al., 2005).

4.4 | PUE and kinetics of P_i distribution

Plants under P_i supply consistently increased PUE while decreasing PUE (Table 3). A similar trend was also employed under Ca-gly-P, with the variety Tiffany exhibiting the highest PUE (Table 3). The capacity of plants to access P_i from P_{org} and invest it in nodulation is a critical factor (Sulieman & Mühling, 2021). The higher RPAE under Ca-gly-P can strongly support the hypothesis that plants were able to use P_{org} substrate more efficiently. On the other hand, the marked increase in PPUE for plants under PA was in agreement with the lower efficiency of P supplied as PA, with the variety Mallory exhibiting lower performance than Tiffany (Table 3). In support of this hypothesis, it has previously been found that PPUE was negatively correlated with P_i availability in plant tissue (Neto et al., 2016). The higher PUE for plants under P_{org} supply was mirrored by the higher P_i uptake (mg plant^{-1}) in the shoots, roots, and nodules over the negative control (Table 2). Consistently, under P_{org} supply, Tiffany accumulated higher P_i contents in the shoot and whole plant (Table 2). The higher P_i availability can substantially reduce the energetic costs when plants exclusively rely on nodule symbiosis as a sole source for N (Pérez-Fernández et al., 2019; Sulieman et al., 2019).

A critical aspect that defines the utilization of the internal P_i pool is the efficient partitioning of P_i within the entire plant (Lazali et al., 2017; Sulieman et al., 2022; Vadez & Drevon, 2001). Under Ca-gly-P, both varieties displayed contrasting behaviour regarding P_i distribution among different organs (Table 2). Mallory increased the root P_i concentration at the expense of the nodular fraction, whereas Tiffany showed the opposite trend (Table 2). It is well established that nodules are preferential P_i sinks with relatively higher P_i concentrations than other plant organs (Míguez-Montero et al., 2020; Sulieman & Tran, 2015; Vadez & Drevon, 2001). This is likely because nodule functioning is highly excessive in C and energy (ATP and NADH) requirements (Lambert et al., 2020; Pérez-Fernández et al., 2019). Indeed, the P_i concentration in nodules was markedly higher than that in other plant organs (Table 2). Therefore, it is tempting to suggest that Tiffany possesses a higher capacity for internal P_i reallocation to nodular tissues (Lazali et al., 2017; Sulieman et al., 2022; Xue et al., 2018). The limited P_i availability under P_0 supply might be the reason for the accumulation of hexoses in nodules (Table 4). Under such conditions, higher P_i -scavenging from small phosphorylated metabolites is more likely to occur, which, as a consequence, can result in a marked accumulation of sugars and likely other low-molecular-weight metabolites (Sulieman et al., 2022; Warren, 2011). This acclimation response can impair cellular metabolic processes (e.g., glycolysis), leading to lower respiratory activity (Nguyen et al., 2021). However, these sugar conjugates might help plants alleviate low- P_i -induced oxidative damage or contribute to cellular osmoregulation (Müller et al., 2015).

4.5 | N assimilation in plant tissue

The higher P_i concentration in nodules under Ca-gly-P resulted in higher N accumulation in plants (Table 2). This is likely due to the higher symbiotic activity of plants incubated under this P_{org} treatment. Although nitrogenase activity was not measured in the present study, all plants were incubated in sterilized N-free substrates. Therefore, we can infer that N accumulated in plants was generated from nodule symbiosis (Míguez-Montero et al., 2020; Pérez-Fernández et al., 2019). In support, the nodules produced under Ca-gly-P appeared normal in size and reddish in colour, indicating functionality (Míguez-Montero et al., 2020). Some documented reports have already found a strong association between nodule function and shoot P_i concentration (Cabeza et al., 2014). The lower N contents in plants under PA supply could further support its poor accessibility by plants (Table 2). Notably, the higher N concentration of Tiffany plants under Ca-gly-P was underpinned by a striking decrease in the C/N ratio in root nodules (Table 2). Shoots of plants incubated under Ca-gly-P displayed a relatively lower N/P ratio (8.7–8.8) than PA (10.9–13.6) or P_0 (18.0–19.3), indicating cellular homeostasis under Ca-gly-P (Table 2). These observations agree with the finding that the optimum N/P ratio in plant cells is approximately 7.0 (Marschner, 1995).

5 | CONCLUSIONS

The present study provides strong lines of evidence that N_2 -fixing legumes (e.g., faba bean) possess strong capacities to utilize P_{org} effectively, thereby reducing reliance on mineral fertilizers. Compared to the P_i supply, it is plausible to suggest that P_{org} could be used as a primary or complementary source for the P nutrition of plants. It is evident that faba bean plants, contrasting genetically in PUE, have a range of differential morphological and physiological strategies to optimize their capacity to access P_i from P_{org} sources. Despite this, our understanding of the potential mechanisms that govern P_{org} exploitation by nodulating plants remains limited. Extending the investigations to other genotypes, leguminous species, and perhaps microsymbionts would be essential to achieve the necessary impact.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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