



RESEARCH ARTICLE

Faba bean (*Vicia faba* L.) varieties reveal substantial and contrasting organic phosphorus use efficiencies (PoUE) under symbiotic conditions

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Abstract

Background: The excessive use of inorganic P (Pi) in soils is alarming as it is causing numerous environmental problems and may lead to the depletion of rock phosphate reserves earlier than expected. Hence, to limit the over-dependence on Pi, there is the need to investigate organic phosphorus (Po), which is the dominant P form of soil P pool, as an alternate P source for plant growth.

Aim: The present study seeks to investigate organic P use efficiency of eight varieties of faba bean grown symbiotically.

Methods: The plants were grown in pots (6 kg soil) under greenhouse condition with three P source, namely, phytic acid (organic P, Po), KH_2PO_4 (inorganic P, Pi), and no-P. The P was applied at the rate of 1.79 g kg^{-1} soil.

Results: The plants grown with Po and Pi produced similar amounts of root, shoot, and total dry matters. Despite producing statistically similar dry matters, P uptake by Pi-fertilized plants was twofold higher than by Po-fertilized plants. Meanwhile, Pi differed significantly from Po in terms of nodulation characteristics such as nodule dry biomass and individual nodule dry biomass. However, Po varied significantly from Pi in P utilization and acquisition efficiencies. Principal component analysis of Pi and Po revealed no significant variation and close association, confirming the nonsignificant differences between the two P treatments. Among the varieties tested, Tiffany tended to accumulate more dry matter, coupled with highest organic P utilization efficiency (0.48 g mg^{-1}) as well as the highest organic P beneficiary factor (80%).

Conclusion: These results provide a solid basis for further comparisons at physiological, biochemical, and molecular levels between Tiffany (Po-efficient) and Fuego (Po-inefficient) varieties, offering deep insights into and making it easier to understand the mechanisms that allow soil Po to be utilized under symbiotic conditions.

KEYWORDS

nodule functioning, organic phosphorus, phosphorus use efficiency, phytic acid, *Rhizobia*, symbiosis

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1 | INTRODUCTION

Contemporary agriculture worldwide has been over-reliant on chemical phosphorus (P) fertilizers (George et al., 2018; Steffens et al., 2010). An estimation of more than 90% of rock phosphate produced is used to manufacture synthetic fertilizers (Li et al., 2016). Unfortunately, inorganic P (Pi) application is sparingly available to plants because it is immobilized and gets precipitated with other soil constituents at different pH regimes (Jamal et al., 2023; Johan et al., 2021; Richardson et al., 2009). Consequently, unsystematic applications of chemical fertilizers pose numerous environmental and ecological problems such as eutrophication (Akhtar et al., 2016; Daneshgar et al., 2018; Mihoub et al., 2022; Xia et al., 2020). These losses make Pi application a poor strategic approach for P fertilization, especially in the tropics and subtropics (Bindrabhan et al., 2020). It is now also known that rock phosphate is a finite resource and is projected to get exhausted shortly (Cordell & White, 2014; Daneshgar et al., 2018). The most worrying and elusive aspect is the uncertainties and inconsistencies in estimating peak production and decline timeframe. Given this scenario, it is quite desirable to find other alternative P resources that can support the growth of plants in a more sustainable and renewable direction.

Organic P (Po) has been suggested as a viable alternative to Pi within the current plans of agricultural sustainability (Steffens et al., 2010; Sulieman & Mühling, 2021). This assumption is very likely because Po constitutes approximately 30%–80% of the total P pool in the soil (Richardson et al., 2001). An estimated 50% of the soil Po fraction exists as inositol-P, 7% as lipids and nucleic acids, with phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate) and its derivatives being the most abundant forms (~40%) (Steffens et al., 2010). It has long been recognized that Po is involved primarily in soil P dynamics and cycling processes (Stewart & Tiessen, 1987; George et al., 2018). Several authors have drawn attention to the fact that numerous plants have the potential to acquire and utilize Po effectively (e.g., Adams & Pate, 1992; Gerke, 2015; Richardson et al., 2001; Tarafdar & Claassen, 2003; Sulieman & Mühling, 2021). This concurs with the finding by Tarafdar and Claassen (1988) who demonstrated that glycerophosphate, lecithin, and phytin exhibited similar results compared to that of Pi sources when exogenously supplied to clover, barley, oats, and wheat plants. These assertions have recently been reported by Jillani et al. (2022) who concluded that *Vicia faba* plants respond effectively to Po supply and produced similar biomass as the Pi counterpart. Consequently, soil Po can help mitigate the inauspicious effects of chemical Pi fertilizers on water bodies and the natural components of the ecosystem (George et al., 2018; Sulieman & Mühling, 2021). Despite this, soil Po bioavailability is questioned, as it is inaccessible for direct plant uptake (Richardson et al., 2001). Thus, soil Po substrates must first be hydrolyzed to render free Pi by unique enzymes of microbial (phytase) and/or plant (acid phosphatase) origins (Richardson et al., 2001; Richardson et al., 2011; Singh & Satyanarayana, 2011). Therefore, it was surprising that Po is neglected and not considered in soil P routine analysis (Steffens et al., 2010) and requires acknowledgment from the scientific communities.

Grain legumes are vital dietary sources for human and animal consumption around the globe (Ferreira et al., 2021; Tabe-Ojong

et al., 2020). In addition, they play a central role in soil conservation and restoring its physical and chemical constituents (Pérez-Fernández et al., 2019; Vanlauwe et al., 2019). This contribution appears likely because of the potential capacity of legumes to establish an intimate symbiotic relationship with N₂-fixing bacteria (*Rhizobia*), which makes them prominent among other crop species (Sulieman & Tran, 2015). Unfortunately, the legume–*Rhizobia* symbiotic relationship is markedly sensitive to many edaphic factors, especially low-Pi availability (Ma & Chen, 2021; Olivera et al., 2004). Not surprisingly, N₂-fixing plants require high amounts of Pi to sustain the high energetic costs of nodules during symbiosis (Olivera et al., 2004; Sulieman et al., 2022). Thus, it is tempting to suggest that N₂-fixing plants might have a robust potential capacity to mobilize Po when soil Pi is limiting. In particular, the strong acidification and massive release of organic exudates induced by Pi scarcity are conceivable to promote Po solubility, thus Pi availability and uptake by the nodulating root. Using soil Po by N₂-fixing plants could be a strategic approach to ensure the sustainable utilization of nitrogen (N) and P in agriculture.

Plants have developed a wide array of morphological, physiological, and biochemical strategies to promote Po mobilization (Sulieman & Mühling, 2021, and references therein). At present, two principal mechanisms are chronicled to improve P use efficiency (PUE) by plants: P acquisition efficiency (PAE) or the capacity of the plant to acquire P from the external environment, and P utilization efficiency (PUtE), which describes the plant's ability to conserve P use internally (Deng et al., 2018; Irfan et al., 2020; Zebrowska et al., 2018). The plant traits related to these strategies could be exploited to create P-efficient genotypes that can grow effectively under low Pi conditions (van de Wiel et al., 2016; Yaseen & Malhi, 2009). This condition is especially true for faba bean (*V. faba* L.), a primary dietary source of protein for the population living in the tropical and subtropical regions lacking Pi availability (Fekadu et al., 2018). As Po use efficiency (PoUE) is not exploited under symbiotic conditions yet, it is plausible that such advances can extend knowledge and pave the way to some elite genotypes/varieties with high PoUE in faba bean and similar legumes.

The present study aimed to screen a collection of faba bean varieties (eight) for PoUE and select the most efficient and less efficient varieties grown under N₂-fixing conditions. In the process of achieving this, we hypothesized that (1) Po in the form of phytic acid could support the growth and nodulation of faba bean plants grown in association with rhizobia and (2) faba bean varieties exhibit differential Po utilization efficiency (PoUE) and Po acquisition efficiency (PoAE) under symbiotic conditions.

2 | MATERIALS AND METHODS

2.1 | Growth conditions and experimental procedures

Seeds of eight commercial Faba beans varieties (Tiffany, Mallory, Victus, Lynx, Trumpet, Fuego, Espresso, and Hiverna) were provided

by the NPZ Innovation GmbH (Hohenlieth) and screened in a pot trial experiment under different P treatments. The seeds were first surface sterilized in NaClO₄ solution (5%–7% v/v) for 4 min, rinsed with sterile deionized water (four times), and subsequently soaked in 1 mM CaSO₄ solution for 48 h to enhance germination. A loamy-sand soil (with properties: 93.4% sand, 1.6% clay, 5.0% silt, pH-CaCl₂ of 6.20, and 53 mg kg⁻¹ soil CAL-P) was sterilized three times (121°C, 200 kPa), mixed thoroughly with basal nutrients and P treatments, and placed in Mitscherlich pots (6 kg soil). Each pot received a modified Kadiata's basal nutrient with the following composition (per kg soil): 10.03 g K₂SO₄, 2.75 g CaCl₂·2H₂O, 3.65 g MgSO₄·7H₂O, 65.96 mg ZnSO₄, 29.47 mg CuSO₄, 18.90 mg Na₂MoO₄·2H₂O, 17.15 mg H₃BO₃, 1.65 mg CoCl₂, 46.14 mg MnSO₄·H₂O, and 113.12 mg Fe-EDTA (Kadiata et al., 2002). Additionally, all plants were supplied with a starter-N (0.29 g N). P was applied by uniformly mixing the Po (phytic acid), Pi (KH₂PO₄; positive control), and without P (–P; negative control) sources with the sterilized soil at a corresponding P application rate equal to 1.79 g P pot⁻¹ (Kadiata et al., 2002). In the control and organic P treatments, K was applied as K₂SO₄ equivalent to the K amount in the inorganic-P treatment. Ten days after germination, seedlings were inoculated with *Rhizobium leguminosarum* bv. *viciae* 3841 (courtesy from the Institute of Plant Genetics, Leibniz University Hannover) and grown in a greenhouse with a 16/8 h day/night cycle, approximately 25/19°C day/night temperature, 55%–75% relative humidity, and 450 μmol m⁻² s⁻¹ photosynthetic active radiation for 8 weeks. The inoculum was prepared under standard conditions in yeast mannitol broth for 48 h at 28°C as previously described by Sulieman and Tran (2015). The experiment was arranged in a completely randomized design with four replicates, with six plants per pot for each treatment. The plants were irrigated with deionized water, and the soil was kept at 70% of the soil water holding capacity.

2.2 | Growth measurements

Stem diameter (SD) was measured with a digital Vernier caliper (CD-20CPX, Mitutoyo Co. Ltd.), whereas chlorophyll content was estimated with a Chlorophyll Meter SPAD-502Plus (Konica Minolta Inc.). Plant height (PH) (cm) and weekly growth were measured with a standard measuring tape. The rest of the growth parameters, including the number of branches (NB), were determined immediately before harvest. Plants were harvested 8 weeks after planting, and the harvested materials were fractioned into the nodules, roots, and shoots. Fresh weights were determined immediately after harvest, and plant samples were oven-dried at 65°C (2–3 days), and the dry matters of shoot (SDM), root (RDM), nodule (NDM), and total (TDM) were recorded. However, results on fresh weights were not considered in our formal statistics and results. TDM was the summation of nodule, root, and shoot dry matter. Fresh roots containing nodules were submerged in liquid N₂, detached, and frozen at –20°C for further analysis.

2.3 | Determination of P and N in plant tissues

Dry plant materials (shoot, root, and nodule) were milled to fine powder and used for P and N chemical analyses. The finely ground subsamples (≈200 mg) were digested with 10 mL 69% HNO₃ at 190°C (45 min) in a microwave oven (MARS 6, Xpress, CEM). P concentration was measured using an inductively coupled plasma mass spectrometry (Agilent Technologies 7700 Series). N concentration was quantified using approximately 1 mg of the finely ground sample in a Thermo Delta V Plus isotope ratio mass spectrometer (Thermo Scientific) following combustion on a Thermo Flash EA 1112 elemental analyzer (Thermo Scientific).

2.4 | P use efficiency (PUE)

Different P-efficiency relations were determined to ascertain the relationship among various P treatments and growth of faba bean varieties. Shortly, P content (mg plant⁻¹) was estimated by multiplying the P concentration (mg g⁻¹) with DM (g plant⁻¹). PUE was calculated as the ratio of DM (g plant⁻¹) to P content (mg plant⁻¹), whereas PAE was estimated as the ratio of P content (mg plant⁻¹) in no P and high P expressed as a percentage as described by Wacker-Fester et al. (2019). Po-beneficiary factor (PoBF) was calculated according to the modified procedure described by Akhtar et al. (2016):

$$PoBF = (SDM_{Po} - SDM_{no-P} / SDM_{Po}) \times 100, \quad (1)$$

where SDM is the shoot dry matter under Po.

2.5 | Statistical analysis

Data were analyzed using a two-way analysis of variance (ANOVA). In addition, the significance of observed differences between P treatments and varieties for each trait was determined by Tukey's honestly significant difference test. The *p*-values below 0.05 were considered statistically significant and those of *p* < 0.01 and *p* < 0.001 as highly significant. All statistical analyses were carried out using R Statistical Software (Version 4.2.2.; R Core Team, 2022).

3 | RESULTS

3.1 | Biomass accumulation in response to Po supply

The data displayed in Table 1 demonstrate that the growth of faba bean varieties is responsive to Po treatment when being exclusively reliant on nodule symbiosis. Nevertheless, significant variations were identified for the studied traits between treatments and varieties. The

TABLE 1 Dry matter accumulation (shoot, root, and total; g plant⁻¹) and root/shoot ratio (g g⁻¹) of eight faba bean varieties inoculated with *Rhizobium leguminosarum* bv. *viciae* 3841 under different phosphorus (P) treatments (no P, KH₂PO₄, and phytic acid) over a period of 8 weeks.

Variety	Shoot dry matter			Root dry matter			Total dry matter			Root/shoot ratio		
	–P	KH ₂ PO ₄	Phytic acid	–P	KH ₂ PO ₄	Phytic acid	–P	KH ₂ PO ₄	Phytic acid	–P	KH ₂ PO ₄	Phytic acid
Espresso	3.67 ± 0.05 ^g	12.28 ± 1.26 ^f	12.50 ± 0.71 ^{ef}	1.93 ± 0.35 ^{fg}	2.66 ± 0.11 ^{c-g}	4.30 ± 0.09 ^a	5.66 ± 0.44 ⁱ	15.79 ± 1.45 ^{gh}	17.23 ± 0.91 ^{d-h}	0.53 ± 0.08 ^{b-d}	0.22 ± 0.02 ^{gh}	0.35 ± 0.02 ^{e-g}
Fuego	5.80 ± 0.97 ^g	16.07 ± 1.45 ^{b-d}	11.72 ± 2.05 ^f	2.62 ± 0.02 ^{c-g}	3.43 ± 0.35 ^{a-d}	2.59 ± 0.33 ^{c-g}	8.81 ± 1.17 ^j	20.33 ± 1.92 ^{bc}	14.79 ± 2.53 ^h	0.46 ± 0.05 ^{c-e}	0.21 ± 0.02 ^{gh}	0.23 ± 0.05 ^{gh}
Hiverna	4.51 ± 0.09 ^g	12.80 ± 0.98 ^{def}	13.96 ± 0.69 ^{c-f}	3.58 ± 0.34 ^{abc}	3.73 ± 0.60 ^{ab}	3.48 ± 0.71 ^{a-d}	8.37 ± 0.45 ^j	17.23 ± 1.71 ^{d-h}	17.96 ± 1.50 ^{c-g}	0.79 ± 0.05 ^a	0.30 ± 0.07 ^{f-h}	0.25 ± 0.06 ^{gh}
Lynx	3.58 ± 0.62 ^g	14.36 ± 2.25 ^{c-f}	14.50 ± 1.67 ^{c-f}	1.98 ± 0.11 ^{fg}	3.55 ± 0.60 ^{a-d}	2.86 ± 0.19 ^{b-f}	5.64 ± 0.74 ⁱ	18.65 ± 2.94 ^{c-g}	17.73 ± 1.89 ^{c-h}	0.56 ± 0.10 ^{bc}	0.25 ± 0.04 ^{gh}	0.20 ± 0.03 ^{gh}
Mallory	4.11 ± 0.43 ^g	13.86 ± 2.09 ^{c-f}	12.44 ± 0.67 ^{ef}	2.56 ± 0.26 ^{d-g}	2.17 ± 0.33 ^{e-g}	2.83 ± 0.30 ^{b-f}	6.97 ± 0.71 ⁱ	16.80 ± 2.61 ^{e-h}	15.65 ± 1.04 ^{f-h}	0.63 ± 0.10 ^b	0.16 ± 0.02 ^h	0.23 ± 0.03 ^{gh}
Tiffany	4.11 ± 0.21 ^g	21.13 ± 1.50 ^a	18.89 ± 2.07 ^{ab}	1.69 ± 0.19 ^g	3.75 ± 0.85 ^{ab}	3.45 ± 0.28 ^{a-d}	5.83 ± 0.42 ⁱ	25.77 ± 2.42 ^a	22.73 ± 2.36 ^b	0.41 ± 0.05 ^{d-f}	0.18 ± 0.05 ^h	0.18 ± 0.01 ^h
Trumpet	4.45 ± 1.05 ^g	15.11 ± 0.38 ^{c-f}	16.22 ± 1.87 ^{bc}	1.95 ± 0.53 ^{fg}	2.84 ± 0.34 ^{b-f}	3.37 ± 0.07 ^{a-d}	6.65 ± 1.61 ⁱ	18.71 ± 0.75 ^{c-g}	20.10 ± 2.00 ^{b-d}	0.44 ± 0.05 ^{cd}	0.19 ± 0.02 ^h	0.21 ± 0.02 ^{gh}
Victus	4.82 ± 0.50 ^g	14.15 ± 1.97 ^{c-f}	15.75 ± 0.40 ^{b-e}	2.58 ± 0.26 ^{c-g}	3.15 ± 0.38 ^{b-e}	3.81 ± 0.19 ^{ab}	7.73 ± 0.79 ^j	17.88 ± 2.53 ^{c-g}	20.27 ± 0.71 ^{b-d}	0.54 ± 0.11 ^{b-d}	0.22 ± 0.02 ^{gh}	0.24 ± 0.01 ^{gh}
Mean	4.38 ± 0.49 ^B	14.97 ± 1.49 ^A	14.50 ± 1.27 ^A	2.36 ± 0.26 ^B	3.16 ± 0.44 ^A	3.34 ± 0.27 ^A	7.4 ± 0.79 ^B	18.90 ± 2.04 ^A	18.32 ± 1.62 ^A	0.55 ± 0.07 ^A	0.22 ± 0.03 ^B	0.24 ± 0.03 ^B
P _V	***	***	***	***	***	***	***	***	***	**	**	**
P _T	***	***	***	***	***	***	***	***	***	***	***	***
P _{V×T}	***	***	***	***	***	***	***	***	***	*	*	*

Note: Data presented means, standard deviations, and ANOVA for varieties (V), treatment (T), and V × T interaction of four replicates. Different capital letters indicate significant differences between the P treatments for all varieties (Tukey's test; $p < 0.05$). Different small letters indicate significant differences among the varieties (Tukey's test; $p < 0.05$). Asterisks indicate significant differences as determined by Tukey's test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Abbreviations: –P, no phosphorus; phytic, phytic acid.

results indicated that the SDM and TDM did not differ significantly between Pi and Po. However, both P treatments (Po vs. Pi) showed highly significant variations ($p < 0.001$) when compared to the negative control (Table 1). Under Po treatment, in relation to $-P$, Tiffany accumulated the highest SDM and TDM (360% and 299%), with Fuego having the lowest (102% and 67.9%), respectively, in these traits. Shoot biomasses of varieties growing under the two sources were threefold higher than $-P$, indicating an effect of P supply on the growth and development of faba bean. Similar trends were observed in the RDM of plants exposed to different P treatments (Table 1). The results showed no significant difference between Po and Pi; however, both P treatments (Po vs. Pi) exhibited a highly significant difference ($p < 0.001$) when compared to $-P$. Under Po, Espresso accounted for 122.8% of the RDM accumulation, with Fuego accounting for the least, and was even reduced by 1.2% compared with $-P$. Conversely, in Hiverna, no significant difference was observed in RDM among $-P$, Pi, and Po ($p < 0.001$) (Table 1).

Nodulation parameters were significantly affected by different P treatments (Table 2). The results indicated Pi varied significantly from Po ($p < 0.05$), but Po was eventually significantly different from $-P$ in terms of NDM. However, no significant variations were observed between both treatments (Pi vs. Po) in terms of nodule numbers (NN), but Po was slightly higher (6.8%) than Pi relative to $-P$ in this regard. Under Po, Victus and Lynx increased by 100% and 362.5%, respectively, in NDM compared with $-P$. However, under the same Po, in terms of NN, Hiverna accounted for the highest NN per plant (240%), with Fuego recording the least when compared to the respective varieties under $-P$ (Table 2). In general, Hiverna was the outstanding variety in terms of NN among the varieties. Individual nodule dry matter (INDM) observed highly significant differences ($p < 0.001$) among treatments and varieties (Table 2). However, plants supplemented with Po saw a general reduction in the INDM relative to the other treatments. Overall, Pi treated plants increased by 9.9%, but Po on the other hand reduced by 34.6% relative to $-P$. Surprisingly, Fuego plants under $-P$ recorded the highest in INDM. Compared with Po, Pi treated varieties increased by 68%. Victus varieties supplied with Po observed the highest INDM but decreased by 13.8% when compared with the corresponding variety under $-P$, with Hiverna obtaining the least and reducing by 38.9% relative to $-P$. Accordingly, Trumpet variety observed the highest INDM under Pi, with the Pi treatment observing 39.5% increment over the $-P$. However, the interactive ($P_{V \times T}$) effect of INDM was not significant across treatment.

The covariation of the various traits was conducted using Pearson's rank correlation (Figure 1). Significant and positive correlations were observed among traits (e.g., SDM, RDM, NDM, NN, and TDM). RDM correlated positively with both NDM and NN ($r = 0.48, 0.61$, respectively). The root-to-shoot ratio (RSR) was also observed to be highly significant between $-P$ and P treatments (Table 1). The $-P$ obtained the highest, indicating the role of high root biomass under P stress (Table 1). Under Po, Espresso recorded the highest RSR, with Tiffany obtaining the least. Interestingly, RSR significantly correlated positively with PUE ($r = 0.80$) but correlated negatively

with SDM, RDM, NDM, NN, and TDM ($r = -0.84, -0.18, -0.64, -0.63, -0.80$, respectively). Generally, variety \times treatment interactions ($P_{V \times T}$) were statistically informative as computed at all significance levels ($p < 0.05, 0.01, 0.001$) and in all traits, respectively (Tables 1 and 2).

3.2 | Growth response of faba bean varieties to Po supply

Several agronomic parameters were measured to quantify the effect of Po on various varieties. The results indicated no significant variation between P treatments but differed significantly ($p < 0.001$) from the no P-treated plants in terms of the NB (Table 3). Hiverna recorded the highest NB compared to all varieties under all three treatments. By contrast, apart from Hiverna under Pi and Po, Hiverna under $-P$ treatment produced similarly and even better than most varieties under the two P sources, respectively, in NB (Table 3). Hiverna supplied with Po recorded the highest NB compared to $-P$ and Pi (103% and 7%), respectively. NB contributed enormously to DM accumulation, and this was confirmed in a positive correlation between TDM, SD, RDM, and NDM ($r = 0.30, 0.32, 0.33, 0.34$), respectively (Figure 1).

The growth of faba bean was highly affected by the absence of P as observed in the $-P$ varieties and treatments (Table 3). Faba bean varieties treated with Po had similar PH as Pi but both differed significantly from $-P$ ($p < 0.001$), as shown (Table 3). Tiffany was the best in terms of PH when compared to the other varieties under the different treatments in the order of $39.25 (-P) < 73.75 (Po) < 82.50 (Pi)$ cm plant $^{-1}$, respectively. Again, Tiffany recorded the greatest PH, which translated into higher DM production under both P treatments. The weekly growth and growth interval between consecutive weeks (growth rate) was noticeably affected by P application (Figures 2 and 3). The results showed no significant differences between both P treatments but differed significantly ($p < 0.001$) above $-P$. Meanwhile, Tiffany plants supplied with Po observed the highest weekly growth compared with $-P$ according to the tagged plants (Figure 2). However, growth rates were found to be slow between weeks 3 and 4 ($W_4 - W_3$), as depicted (Figure 3), with $-P$ severely and Po being slightly affected. Recovery of plants was observed in the subsequent weeks, with Pi and Po growing almost at the same rates compared with $-P$ in weeks 7 and 8 ($W_8 - W_7$), respectively. However, the content of total chlorophyll pigments (SPAD) was not affected by P application (data not shown), with no significant differences among varieties and treatments ($p < 0.05$).

The ANOVA testing the effects of P supply on SD of faba bean varieties revealed no significant differences ($p < 0.05$) among P treatments (Pi vs. Po), with the highest results displayed by the variety Tiffany under both Pi and Po (Table 3). However, both P treatments differed from P deprived plants (Table 3). Significant and positive relationships were observed between PH and traits such as SDM, RDM, NB, and SD (with values $r = 0.92, 0.54, 0.28$, and 0.79 , respectively), indicating the direct effect of these traits on biomass accumulation when plants were treated with the different P sources (Figure 1). However, SD negatively and significantly correlated with RSR and PUE ($r = -0.65$

TABLE 2 Number of nodules (plant^{-1}), nodule dry matter (g plant^{-1}) and individual nodule dry matter (mg nodule^{-1}) of eight faba bean varieties inoculated with *Rhizobium leguminosarum* bv. *viciae* 3841 under different phosphorus (P) treatments (no P, KH_2PO_4 , and phytic acid) over a period of 8 weeks.

Variety	Number of nodules			Nodule dry matter			Individual nodule dry matter		
	–P	KH_2PO_4	Phytic acid	–P	KH_2PO_4	Phytic acid	–P	KH_2PO_4	Phytic acid
Espresso	39.00 ± 7.43^h	222.00 ± 48.39^{cd}	229.00 ± 58.38^{cd}	0.05 ± 0.04^{hi}	0.85 ± 0.08^a	$0.43 \pm 0.11^{c-f}$	1.30 ± 0.73^{ij}	$3.84 \pm 0.71^{b-d}$	$1.88 \pm 0.80^{f-j}$
Fuego	53.00 ± 8.28^h	228.00 ± 5.01^{cd}	207.00 ± 19.77^{cd}	$0.35 \pm 0.18^{e-h}$	0.85 ± 0.12^a	$0.50 \pm 0.15^{b-f}$	6.67 ± 1.79^a	$3.73 \pm 0.58^{b-e}$	$2.41 \pm 0.92^{e-j}$
Hiverna	138.00 ± 16.69^{ef}	314.00 ± 11.67^b	468.00 ± 12.41^a	$0.28 \pm 0.02^{e-i}$	$0.70 \pm 0.13^{a-c}$	$0.58 \pm 0.10^{a-e}$	$2.03 \pm 0.21^{f-j}$	$2.23 \pm 0.38^{e-j}$	1.24 ± 0.19^{ij}
Lynx	$74.00 \pm 18.32^{f-h}$	328.00 ± 8.08^b	232.00 ± 43.20^{cd}	$0.08 \pm 0.01^{g-i}$	0.74 ± 0.09^{ab}	$0.37 \pm 0.03^{d-g}$	1.09 ± 0.30^j	$2.26 \pm 0.22^{e-j}$	$1.59 \pm 0.44^{g-j}$
Mallory	58.00 ± 0.87^h	217.00 ± 3.07^{cd}	233.00 ± 15.89^{cd}	$0.30 \pm 0.02^{e-i}$	0.77 ± 0.19^{ab}	$0.42 \pm 0.07^{c-f}$	5.15 ± 0.19^b	$3.55 \pm 0.74^{c-f}$	$1.80 \pm 0.21^{f-j}$
Tiffany	27.00 ± 5.10^h	273.00 ± 38.50^{bc}	268.00 ± 19.81^{bc}	0.03 ± 0.02^j	0.89 ± 0.07^a	$0.38 \pm 0.01^{d-g}$	1.09 ± 0.54^j	$3.26 \pm 0.63^{c-g}$	$1.42 \pm 0.13^{g-j}$
Trumpet	$81.00 \pm 11.61^{f-h}$	176.00 ± 2.54^{de}	222.00 ± 14.35^{cd}	$0.25 \pm 0.03^{f-i}$	0.76 ± 0.07^{ab}	$0.53 \pm 0.06^{b-f}$	$3.09 \pm 0.33^{c-g}$	4.31 ± 0.42^{bc}	$2.39 \pm 0.37^{e-j}$
Victus	$113.00 \pm 11.19^{e-g}$	231.00 ± 39.44^{cd}	263.00 ± 30.23^{bc}	$0.33 \pm 0.03^{e-i}$	$0.58 \pm 0.18^{a-e}$	$0.66 \pm 0.12^{a-d}$	$2.91 \pm 0.31^{c-h}$	$2.51 \pm 0.55^{d-j}$	$2.51 \pm 0.21^{d-j}$
Mean	73.00 ± 9.94^B	249.00 ± 19.59^A	265.00 ± 26.76^A	0.26 ± 0.04^C	0.77 ± 0.12^A	0.48 ± 0.08^B	2.92 ± 0.55^B	3.21 ± 0.53^A	1.91 ± 0.41^C
P_V	***	***	***	*	*	*	***	***	***
P_T	***	***	***	***	***	***	***	***	***
$P_{V \times T}$	***	***	***	***	***	***	NS	NS	NS

Note: Data presented means, standard deviations, and ANOVA for varieties (V), treatment (T), and $V \times T$ interaction of four replicates. Different capital letters indicate significant differences between the P treatments for all varieties (Tukey's test; $p < 0.05$). Different small letters indicate significant differences among the varieties (Tukey's test; $p < 0.05$). Asterisks indicate significant differences as determined by Tukey's test (* $p < 0.05$; *** $p < 0.001$).

Abbreviations: NS, nonsignificant; –P, no phosphorus.

TABLE 3 Plant height (cm), stem diameter (mm) and number of branches (pot^{-1}) of eight faba bean varieties inoculated with *Rhizobium leguminosarum* bv. *viciae* 3841 under different phosphorus (P) treatments (no P, KH_2PO_4 , and phytic acid) over a period of 8 weeks.

Variety	Plant height			Stem diameter			Number of branches		
	–P		Phytic acid	–P		Phytic acid	–P		Phytic acid
	KH_2PO_4			KH_2PO_4			KH_2PO_4		
Espresso	35.75 ± 0.58^k	53.75 ± 3.51^{hi}	$59.50 \pm 3.12^{e-h}$	$3.39 \pm 0.06^{f-h}$	$4.43 \pm 0.04^{a-e}$	$4.11 \pm 0.19^{b-h}$	7.25 ± 1.26^b	7.50 ± 1.15^b	8.00 ± 0.01^b
Fuego	37.75 ± 6.36^{jk}	$68.75 \pm 3.95^{b-e}$	$60.50 \pm 5.67^{d-h}$	3.26 ± 0.26^{gh}	$4.56 \pm 0.37^{a-c}$	$4.18 \pm 0.51^{b-h}$	6.50 ± 2.08^b	8.50 ± 0.58^b	7.25 ± 0.96^b
Hiverna	44.00 ± 3.61^{jk}	$57.00 \pm 4.76^{f-h}$	$56.00 \pm 3.36^{g-i}$	$3.46 \pm 0.36^{f-h}$	$4.69 \pm 0.29^{a-c}$	$4.49 \pm 0.17^{a-e}$	9.50 ± 1.91^b	18.00 ± 4.62^a	19.25 ± 1.73^a
Lynx	41.75 ± 4.35^{jk}	$64.75 \pm 2.22^{b-g}$	$62.00 \pm 0.82^{d-h}$	3.28 ± 0.68^h	$4.64 \pm 0.25^{a-c}$	$4.22 \pm 0.32^{b-g}$	7.25 ± 2.50^b	8.00 ± 0.82^b	8.25 ± 0.96^b
Mallory	42.00 ± 3.06^{jk}	$65.50 \pm 2.00^{b-g}$	$63.25 \pm 2.22^{c-h}$	3.37 ± 0.02^{gh}	$4.14 \pm 0.21^{b-h}$	$4.07 \pm 0.26^{c-h}$	6.50 ± 0.82^b	7.25 ± 0.58^b	8.00 ± 0.96^b
Tiffany	39.25 ± 7.51^{jk}	82.50 ± 2.65^a	73.75 ± 4.34^{ab}	$3.64 \pm 0.47^{d-h}$	5.01 ± 0.76^{ab}	5.15 ± 0.19^a	6.25 ± 1.26^b	10.33 ± 1.53^b	8.00 ± 1.53^b
Trumpet	41.50 ± 1.79^{jk}	$69.75 \pm 5.50^{b-d}$	$67.25 \pm 3.59^{b-e}$	3.27 ± 0.09^h	$4.43 \pm 0.22^{a-e}$	$4.17 \pm 0.56^{b-h}$	7.00 ± 1.15^b	9.75 ± 1.53^b	6.75 ± 0.96^b
Victus	47.00 ± 1.00^{ij}	$66.25 \pm 7.23^{b-f}$	72.00 ± 1.63^{bc}	$3.58 \pm 0.33^{e-h}$	$4.31 \pm 0.18^{a-f}$	$4.74 \pm 0.36^{a-c}$	6.25 ± 0.58^b	8.50 ± 0.58^b	8.75 ± 1.53^b
Mean	41.13 ± 3.53^B	66.03 ± 3.98^A	64.28 ± 3.09^A	3.41 ± 0.28^B	4.52 ± 0.29^A	4.39 ± 0.32^A	7.06 ± 1.45^B	9.69 ± 1.42^A	9.28 ± 1.08^A
P_V	***	***	***	***	***	***	***	***	***
P_T	***	***	***	***	***	***	***	***	***
$P_{V \times T}$	***	***	***	NS	NS	NS	***	***	***

Note: Data presented means, standard deviations, and ANOVA for varieties (V), treatment (T), and $V \times T$ interaction of four replicates. Different capital letters indicate significant differences between the P treatments for all varieties (Tukey's test; $p < 0.05$). Different small letters indicate significant differences among the varieties at each P treatment (Tukey's test; $p < 0.05$). Asterisks indicate significant differences as determined by Tukey's test (***) $p < 0.001$.

Abbreviations: NS signifies not applicable; –P, no phosphorus.

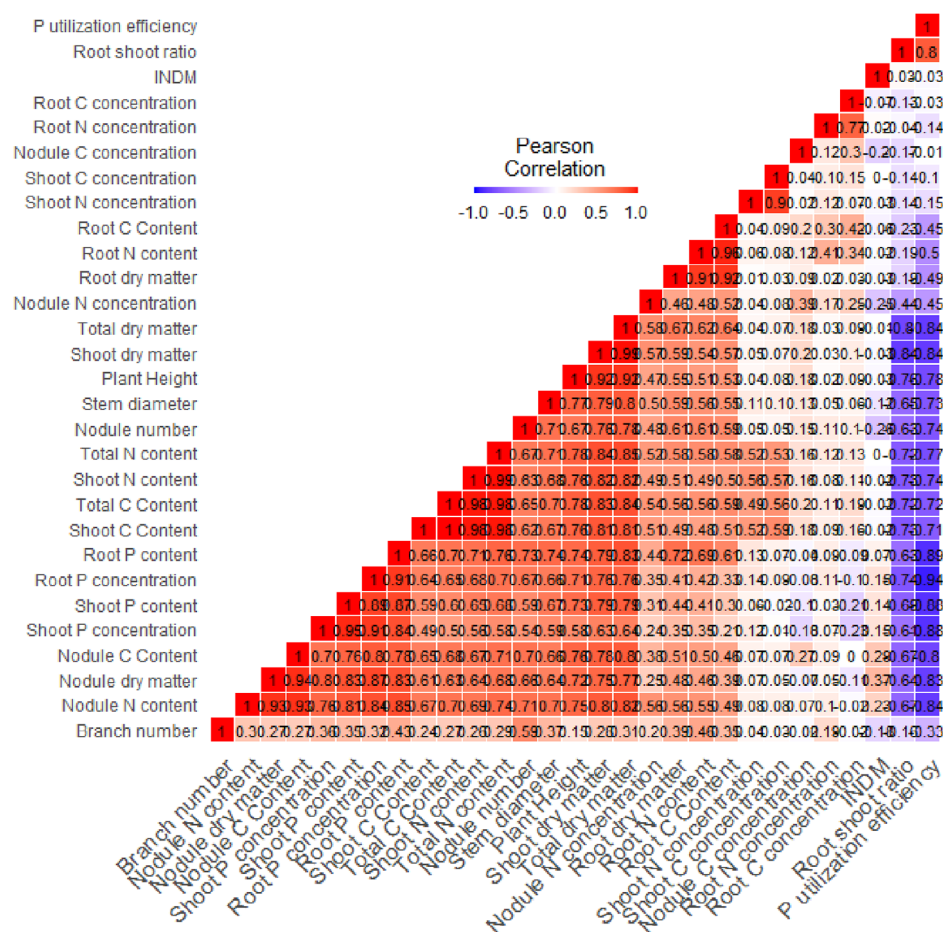


FIGURE 1 Heat map of correlation for 32 morphological, physiological, and biochemical traits of eight faba bean varieties fertilized with no P (–P), KH_2PO_4 (Pi), and phytic acid (Po) and grown under symbiotic conditions for 8 weeks. Created following hierarchical clustering on Pearson's r_s -values. Color, values, and square denote Pearson's ranks correlation r between traits, $n = 4$ with four biological replicates.

and -0.73), respectively. Additionally, INDM correlated positively with NDM ($r = 0.37$), but negatively with NN ($r = -0.26$).

3.3 | P concentration in plant tissues

The P concentration in different plant organs is depicted in Figure 4. Overall, P concentration significantly ($p < 0.05$) increased upon P application in all P-treated varieties compared to varieties without P application (Figure 4). Under Pi supplementation, shoot P concentration (SPC) was significantly ($p < 0.05$) increased when compared with that received by Po (twofold) and –P (fivefold) treatments (Figure 4A). Hiverna produced the highest SPC under Pi treatment and the least by Trumpet. As for the Po application, our ANOVA analysis revealed no significant differences between varieties in terms of SPC (Figure 4A). By contrast, Fuego produced the highest root P concentration (RPC) under Pi, with Lynx obtaining the least under –P. However, Hiverna under Po produced the greatest RPC and Tiffany the least among the Po-treated varieties (Figure 4B). Our analyses revealed statistically significant $P_{\text{V} \times \text{T}}$ interaction under $p < 0.05$, 0.01 , and 0.001 , respectively.

The growths of plant and biomass accumulation were observed to be strongly and positively associated with P concentration, as depicted in Figure 1. For instance, SPC and RPC were positively significant and strongly correlated with the PH ($r = 0.58$ and 0.71 , respectively) and SD ($r = 0.59$ and 0.66 , respectively) across all varieties and treatments (Figure 1). Conversely, RSR was negatively significant and strongly correlated with SPC and RPC ($r = -0.61$ and -0.74 , respectively) in all varieties and treatments.

3.4 | A narrow focus on Po and its related benefits

Information related to PUE could help us select varieties with a contrasting use of Po when plants are solely dependent on N_2 -fixation as a source for N. Factors that could conceivably help to meet this goal include PoUE, PoAE, and PoBF following those proposed (Irfan et al., 2020) with clear modifications. PoBF is defined as the relative increase in biomass accumulation when plants are supplied with Po (e.g., phytic acid). The faba bean varieties revealed significant variations from each other in terms of PoUE under Po supply (Figure 5A–C).

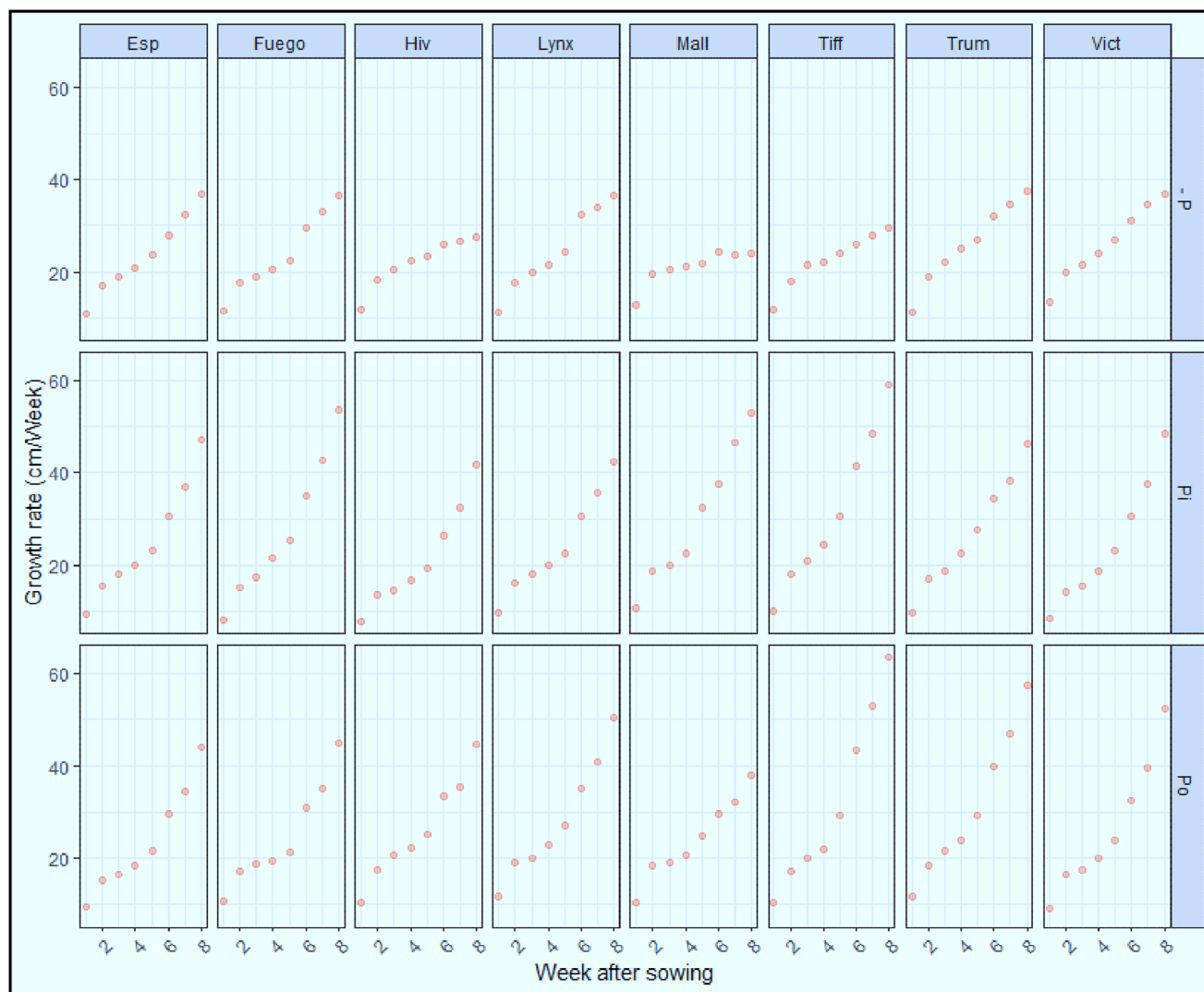


FIGURE 2 Weekly growth of eight faba bean varieties fertilized with no P (–P), KH_2PO_4 (Pi), and phytic acid (Po) under symbiotic conditions for 8 weeks in a soil pot experiment. Two (out of six) plants per pot were tagged, and plant heights were measured at weekly intervals with a measuring tape to monitor the growth of each variety, $n = 4$ with four biological replicates. Esp., Espresso; Fuego; Hiv., Hiverna; Lynx; Mall., Mallory; Tiff., Tiffany; Trum., Trumpet; Vict., Victus.

Under these conditions, Tiffany variety recorded the highest PoUtE and PoBF (0.48 g mg^{-1} and 79.7%), but an intermediary in terms of PoAE (50%) (Figure 5A,C). In contrast, Fuego recorded the second highest PoAE (70.7% ; Figure 5B) but produced one of the least in PoUtE and PoBF (0.34 g mg^{-1} and 51% ; Figure 5A,C), respectively. Hiverna was not only the least variety in terms of PoUtE, but also one of the least in PoBF, with an intermediary in terms of PoAE. Our results indicated again that Espresso had the least acquisition efficiency of (27.5%) and an intermediary in PoUtE when varieties were compared under Po treatment. Tiffany was projected as the best variety because it acquired an average P (50%), but utilized it competently compared with Mallory, that accumulated 93% but could only produce a lesser PoUtE and PoBF than Tiffany. Fuego was the worst-performing variety because it obtained the least in PoUtE and PoBF.

Additionally, Pearson correlation was conducted strictly among Po-treated varieties, and a positive correlation ($r = 0.60$, $p = 0.018$)

was found between PoBF and PoUtE under organic P application, but not significant (Figure 6A–C). However, PoBF correlated negatively ($r = -0.45$, $p = 0.027$) with PoAE and was not statistically informative. Interestingly, a positive correlation was observed between PoUtE and PoAE ($r = 0.07$, $p = 0.75$), but not statistically significant in all Po treated varieties (Figure 6B,C).

3.5 | P contents in plant tissues

The effects of P treatments on faba bean varieties were highly significant ($p < 0.001$) regarding the P content in plant organs (Table S1). Shoot P content (SP_{Cont}) under Pi and Po was (1514.5% and 581.3% , respectively) higher than –P. Pi content, however, increased by 137% relative to Po. Tiffany under Pi, for example, increased by 191% with respect to Po; notwithstanding, Po was also observed

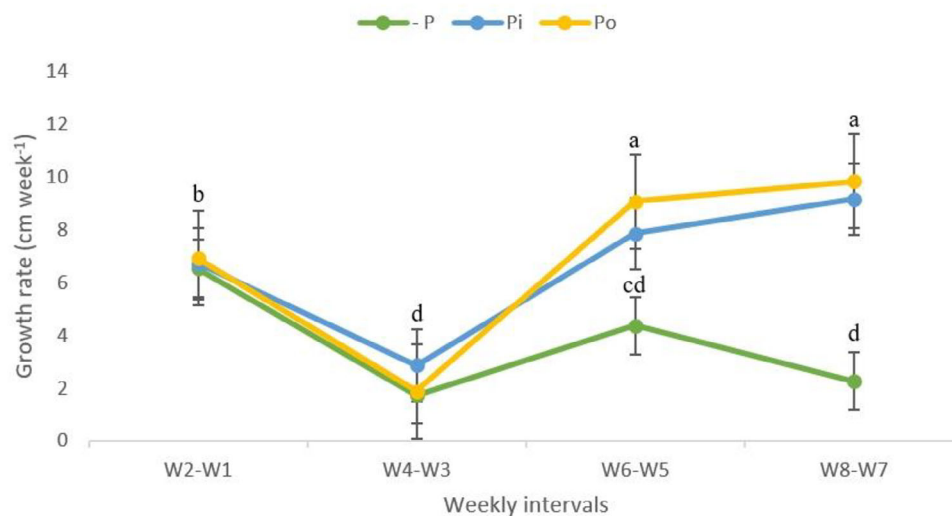


FIGURE 3 The differences in consecutive weekly growth of eight faba bean varieties fertilized with no P (–P), KH_2PO_4 (Pi), and phytic acid (Po) under symbiotic conditions for 8 weeks in a pot experiment.

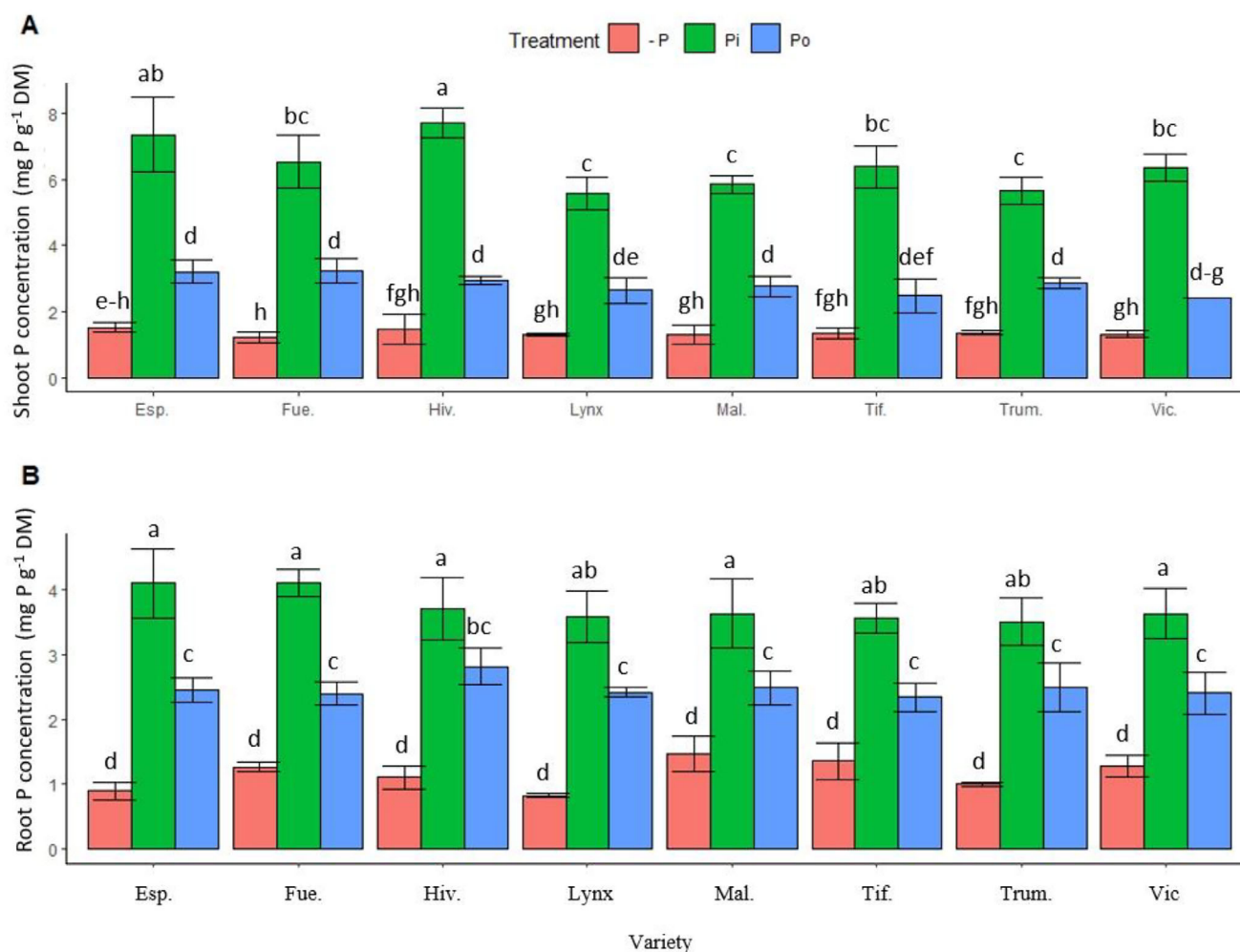


FIGURE 4 Phosphorus concentration in shoots (A) and roots (B) of eight faba bean varieties fertilized with no P (–P), KH_2PO_4 (Pi), and phytic acid (Po) under symbiotic conditions for 8 weeks. Letters signify significant differences in treatments and varieties.

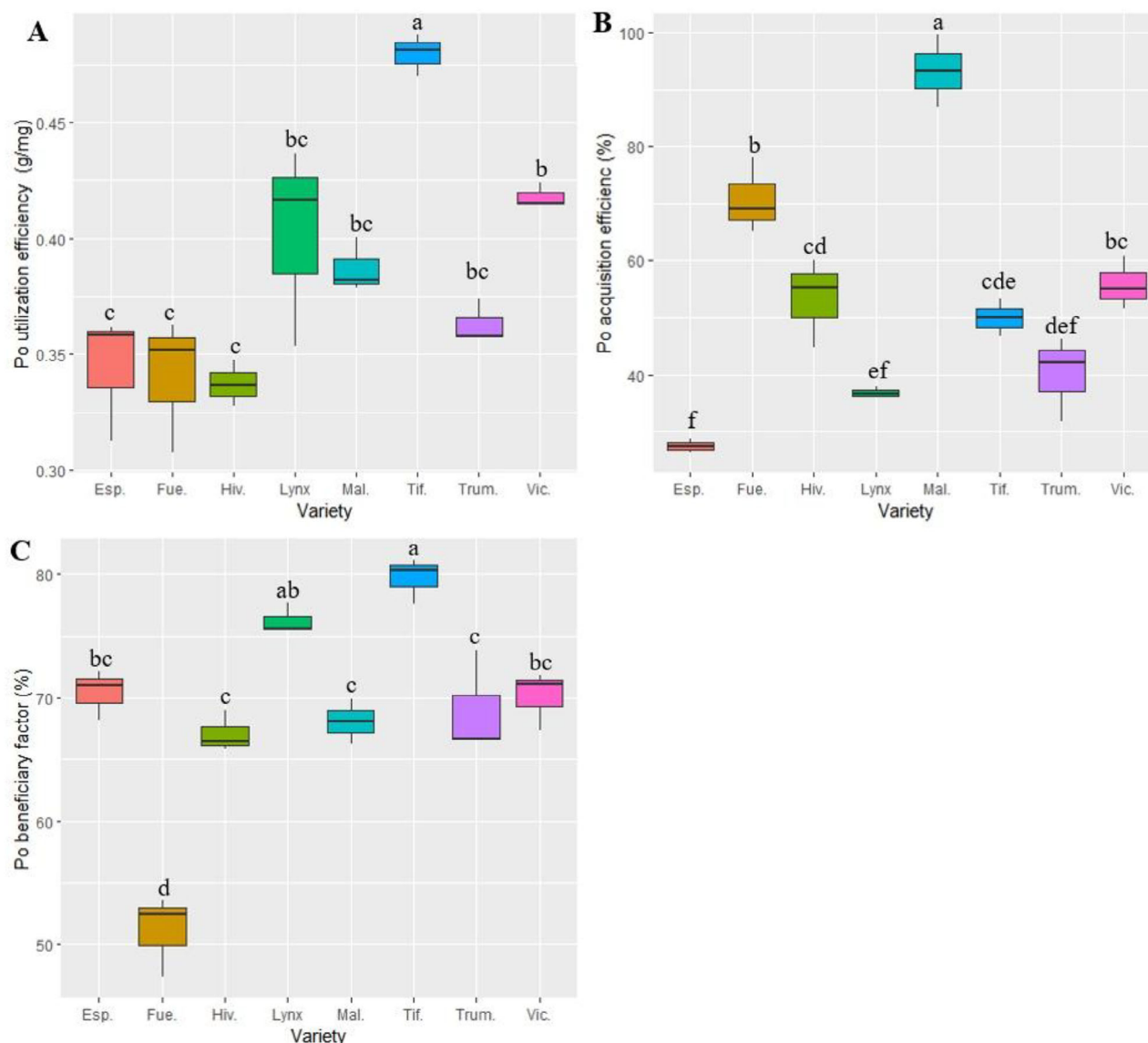


FIGURE 5 Organic phosphorus-related traits: (A) organic P utilization efficiency (g mg^{-1}), (B) organic P acquisition efficiency (%), and (C) organic P beneficiary factor (%) of only phytic acid-treated plants of faba bean varieties under symbiotic conditions. Different letters indicate significant differences between varieties and are separated by Tukey's honestly significant difference (HSD) ($p < 0.05$).

to be higher (738%) when compared with $-P$. Under Po supply, the SP_{Cont} ranged from 34.53 to 46.58 mg P plant^{-1} . Trumpet and Malory varieties supplemented with Po exhibited the highest and lowest P contents (668.7% and 551.5%), respectively, compared with their respective varieties under $-P$. Interestingly, $P_{V \times T}$ interaction effects were observed to be highly significant ($p < 0.001$) in shoot P uptake.

Root P content (RP_{Cont}) showed similar results, with highly significant differences between treatments (Table S1). The significant variation between P treatments (Pi vs. Po) with respect to $-P$ is increased by 329.2% and 201.1%, respectively. In comparison with $-P$, Espresso plants treated with Po obtained the highest (522.4%) in RP_{Cont} , whereas Fuego under the same condition observed the lowest (87.6%) root P uptake under Po. Meanwhile, SP_{Cont} under Po was several-folds higher than RP_{Cont} (Table S1). Statistically informative interactions between $P_{V \times T}$ were observed and showed a highly

significant ($p < 0.001$) effect. P content directly relies on biomass accumulation (DM) and P concentration, so observing a solid association between them was highly anticipated in our results. SP_{Cont} , for instance, correlated positively and significantly with RDM and NDM ($r = 0.44$ and 0.80 , respectively) (Figure 1). By contrast, RSR was negatively significant and strongly correlated with RP_{Cont} and SP_{Cont} ($r = -0.68$ and -0.63 , respectively) in all varieties and treatments (Figure 1).

3.6 | P-acquisition and -utilization efficiencies

In contrast to the Pi varieties that were significantly enhanced in terms of their P contents ahead of Po, the effects of Po treatments were highly significant ($p < 0.001$) regarding the PUE and PAE (Table 4).

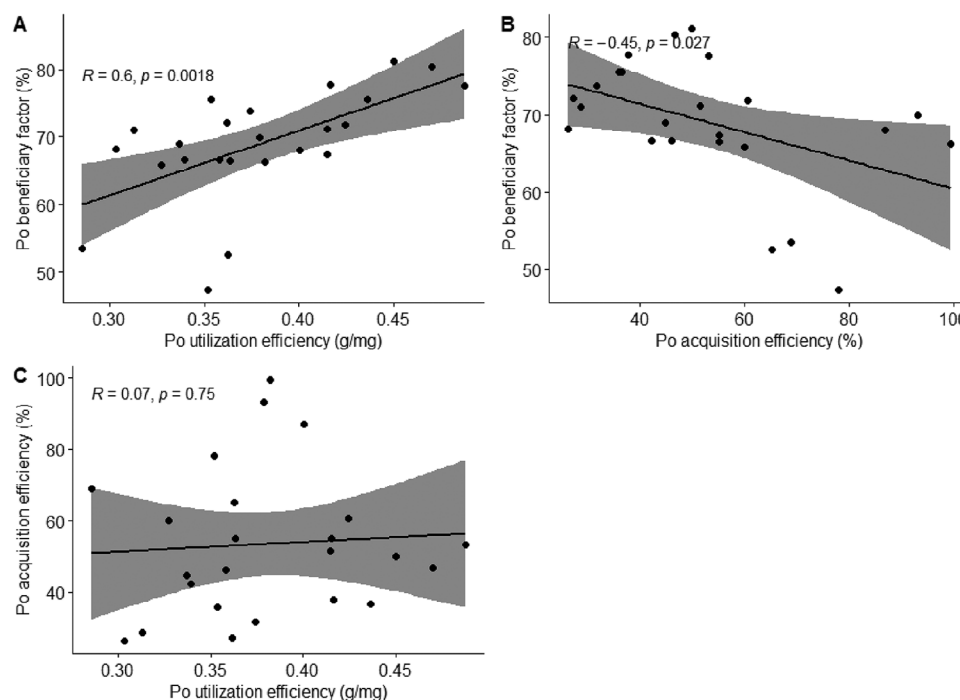


FIGURE 6 Pearson correlations strictly based on only organic phosphorus (phytic acid) treatments for eight faba bean varieties (A) between organic P beneficiary factor and organic P utilization efficiency, (B) between organic P beneficiary factor and organic P acquisition efficiency, and (C) between organic P acquisition efficiency and organic P utilization efficiency under symbiotic conditions.

The average P uptake (PAE) of plants treated with Po was significantly ($p < 0.001$) higher than plants treated with Pi. The maximum and minimum PAE were observed in Mallory and Lynx with 65.58% and 19.26% mean values under Pi, respectively. Interestingly, Mallory variety recorded the best PAE followed by Fuego, whereas Espresso

exhibited the least records under Po treatment. In addition, the $P_{V \times T}$ interaction was highly significant at $p < 0.001$ (Table 4). Nevertheless, PAE correlated negatively, but not significant, with PUtE under Pi and Po ($r = -0.03$, $p = 0.80$) (Figure 7C). Meanwhile, a negative correlation existed between PAE and TDM ($r = -0.06$, $p = 0.68$), (Figure 7A).

TABLE 4 P utilization efficiency and acquisition efficiency of eight faba bean varieties inoculated with *Rhizobium leguminosarum* bv. *viciae* 3841 under different phosphorus (P) treatments (no P, KH_2PO_4 , and phytic acid) over a period of 8 weeks.

Variety	P utilization efficiency (g mg ⁻¹)			P acquisition efficiency (%)	
	–P	KH_2PO_4	Phytic acid	KH_2PO_4	Phytic acid
Espresso	0.77 ± 0.02 ^a	0.15 ± 0.02 ^e	0.33 ± 0.03 ^{b-d}	22.87 ± 3.72 ^{h-j}	27.50 ± 1.21 ^{g-i}
Fuego	0.81 ± 0.06 ^a	0.17 ± 0.02 ^e	0.33 ± 0.08 ^{b-d}	32.63 ± 1.16 ^{f-i}	70.73 ± 6.59 ^b
Hiverna	0.78 ± 0.11 ^a	0.15 ± 0.01 ^e	0.35 ± 0.02 ^b	36.28 ± 1.47 ^{fg}	53.38 ± 7.81 ^{cd}
Lynx	0.88 ± 0.02 ^a	0.20 ± 0.03 ^{c-e}	0.39 ± 0.05 ^b	19.26 ± 1.35 ^j	36.84 ± 1.01 ^{fg}
Mallory	0.75 ± 0.14 ^a	0.18 ± 0.00 ^e	0.37 ± 0.05 ^b	65.58 ± 3.25 ^{bc}	93.20 ± 6.30 ^a
Tiffany	0.76 ± 0.10 ^a	0.17 ± 0.01 ^e	0.42 ± 0.08 ^b	20.33 ± 2.24 ^{ij}	49.97 ± 3.29 ^{de}
Trumpet	0.80 ± 0.33 ^a	0.19 ± 0.01 ^{de}	0.36 ± 0.01 ^b	34.05 ± 1.18 ^{f-h}	40.04 ± 7.48 ^{ef}
Victus	0.77 ± 0.05 ^a	0.17 ± 0.01 ^e	0.41 ± 0.01 ^b	34.09 ± 4.24 ^{f-h}	55.76 ± 4.61 ^{cd}
Mean	0.79 ± 0.10 ^A	0.17 ± 0.01 ^C	0.37 ± 0.04 ^B	33.14 ± 2.33 ^B	53.43 ± 4.79 ^A
P_V	NS	NS	NS	***	***
P_T	***	***	***	***	***
$P_{V \times T}$	NS	NS	NS	***	***

Note: Data presented means, standard deviations, and ANOVA for varieties (V), treatment (T), and $V \times T$ interaction of four replicates. Different capital letters indicate significant differences between the P treatments for all varieties (Tukey's test; $p < 0.05$). Different small letters indicate significant differences among the varieties at each P treatment (Tukey's test; $p < 0.05$). Asterisks indicate significant differences as determined by Tukey's test (** $p < 0.001$).

Abbreviations: NS, nonsignificant; –P, no phosphorus.

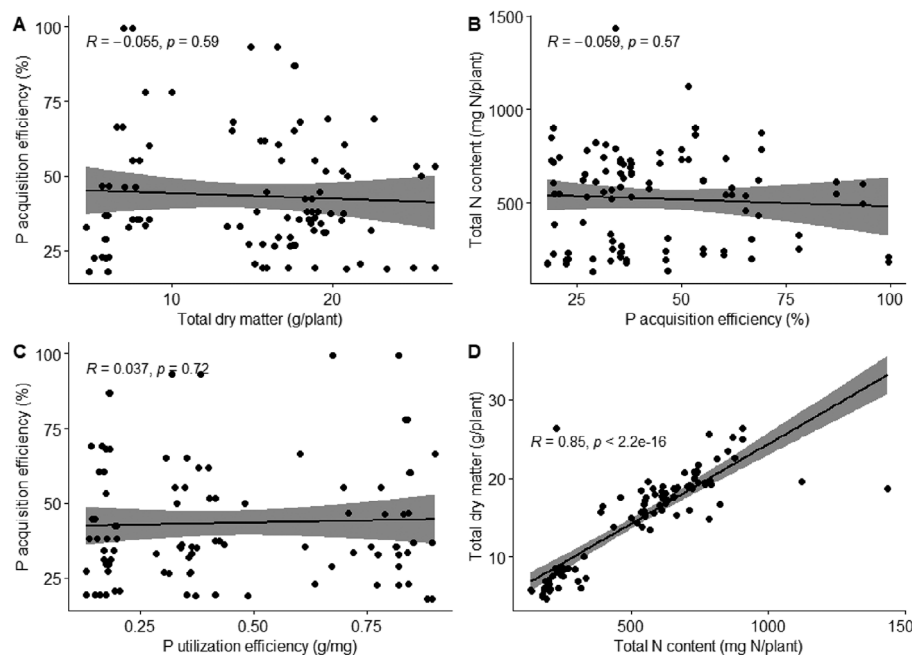


FIGURE 7 Pearson correlation between (A) total dry matter and P acquisition efficiency (B) total N content and P acquisition efficiency (C) P utilization efficiency and P acquisition efficiency (D) total dry matter and total N content of eight faba bean varieties fertilized with only KH_2PO_4 (Pi) and phytic acid (Po) under symbiotic conditions. Regression equations R^2 and p -values are given, $n = 4$ with four biological replicates.

Intriguingly, a negatively nonsignificant correlation occurred between PAE and total N content (TNcont) ($r = -0.07$, $p = 0.64$; Figure 7B).

Plants treated with Po displayed greater PUE than Pi, whereas P untreated plants showed the highest values in this regard. Under Po supplementations, the faba bean varieties showed significant differences in PUE, with the variety Tiffany exhibiting the highest values and both Fuego and Espresso displaying the lowest records, respectively (Table 4). When grown under P deprivation, Lynx and Mallory varieties showed the greatest and lowest PUE, respectively (Table 4). On the contrary, the $P_{V \times T}$ interaction displayed no significant variations on the PUE of the faba bean varieties (Table 4). Interestingly, RSR significantly correlated ($r = 0.80$) with PUE in all treatments (Figure 1). However, PUE correlated negatively and significantly with SPC and SPcont ($r = 0.88$ and 0.88 ; Figure 1), respectively.

A principal component analysis (PCA) was conducted as an alternative method to evaluate and confirm variations on the various treatments and traits. This approach characterized a highly significant variation across treatments and traits (Figures 8 and 9). The first two principal components (PC) explained 49.4% and 14.6% (64%) of the variation, respectively, in all three treatments (Figure 8) and second PC explaining 52.4% and 11% (63.4%) of the variation for traits (Figure 9). ANOVA was carried out on each PC to evaluate if treatments differed among traits. The results, however, indicated highly significant differences between P treatments (Pi and Po) and untreated plants as depicted (Figure 8). PC1 and PC2 largely separated $-P$ treatments from P treatments (Pi and Po), indicating that Pi and Po are closely related and nonsignificant. The PCA again showed overlaps relationship among traits as depicted (Figure 9).

3.7 | Nitrogen concentration in plant tissues

Significant differences in plant-N concentration between P treatments and faba bean varieties were noticed (Table S2). As anticipated, N concentration was relatively higher in nodules than in the other plant organs. When plants were supplemented with Po, nodule N concentration (NNC) significantly increased ($p < 0.001$) relative to other P treatments. Tiffany and Mallory varieties accounted for the highest and lowest (7.3% and 4.6%) NNC under Po, respectively (Table S2). For $-P$ treatment, the maximum and minimum N concentrations in nodules were recorded by Espresso and Victus varieties (4.5% and 3.3%), respectively. However, Mallory displayed the least NNC under Pi supply, whereas Lynx showed the highest records (Table S2).

The root N concentration (RNC) was relatively lower in faba bean varieties; with no significant variations between P treatments as well as varieties (Table S2). In accordance with the NNC data, the RNC was significantly ($p < 0.001$) higher in response to Po than Pi treatments, although no difference existed. In addition, under P deprivation, varieties Mallory and Trumpet displayed the highest and lowest RNC (3.3% and 2.8%), whereas, under Pi supply, Hiverna and Lynx varieties exhibited the greatest and least values, respectively (Table S2). Specifically, Fuego recorded the highest RNC (3.2%) with Po supply, whereas the Espresso variety showed the lowest (2.9%) records under the same conditions. The effects of P supply on the shoot N concentration (SNC) were not statistically significant between treatments and varieties when compared to the negative control ($-P$) (Table S2). At various P treatments, the SNC of the faba bean varieties ranged from 2.7% to 4.8%, with Victus varieties showing the highest

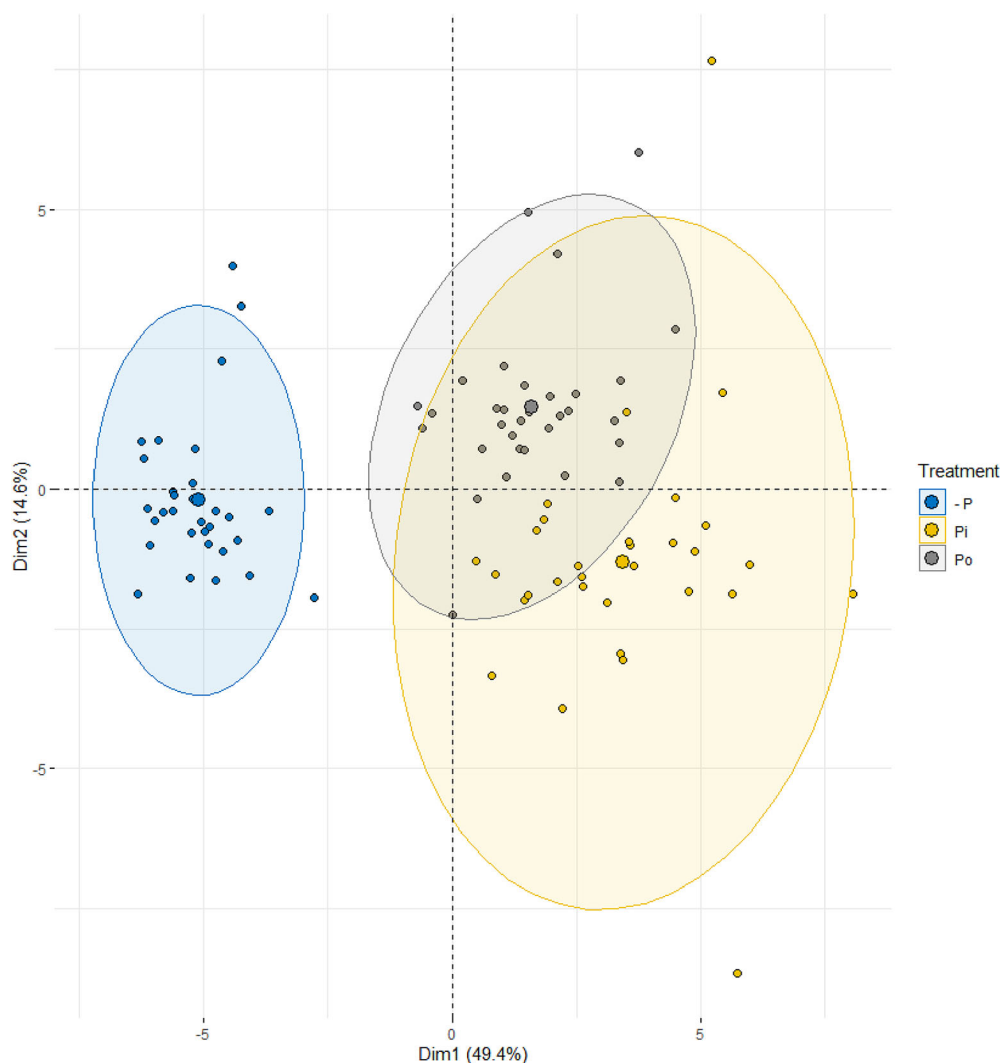


FIGURE 8 Principal component analysis (PCA) score plot of various measured traits under the three P sources (no P, KH_2PO_4 , and phytic acid denoted as $-P$, P_i , and P_o) supplied to eight faba bean varieties grown under symbiotic conditions for 8 weeks. Each treatment represents a color in different shapes.

N concentrations, whereas Espresso varieties display the lowest values under P_o treatments, respectively (Table S2). Meanwhile, the $P_{V \times T}$ interactions revealed no significant effects on the nodule, root, and SNCs of the faba bean varieties, respectively.

3.8 | Nitrogen and carbon contents in faba bean varieties

The ANOVA testing the effects of P supply on nodule N content (NNcont) of faba bean varieties indicated highly significant ($p < 0.001$) differences between P treatments (Table S3). Although variety Tiffany recorded the least NNcont under P deprivation but observed the highest values under P_i and one of the best under P_o supply (49.82 and 26.96 mg N nodule $^{-1}$, respectively). Under P_o application, the faba bean varieties displayed significant variations ($p < 0.001$) in NNcont,

with Hiverna and Mallory scoring the highest and lowest values, respectively (Table S3).

Regarding root and shoot components, both P_i and P_o were not statistically different from each other but differed significantly from the negative control (Table S3). However, the maximum and minimum RNcont accumulations under $-P$ were observed in Hiverna and Tiffany (99.42 and 51.02 mg N root $^{-1}$), respectively. Under P_i , the root of Hiverna accumulated the most significant N content (23.7%), with Mallory observing significant reduction relative to its corresponding $-P$ (29%), respectively. Fuego had the least N content under P_o , with Espresso recording the highest RNcont. In comparison, the shoot N contents (SNcont) were higher than those in the NNcont and RNcont (Table S3). The effect of P treatment on the SNcont ranged from 119.15 to 729.73 Nmg shoot $^{-1}$, with variety Trumpet under P_i exhibiting the most outstanding value (307%). Under $-P$ treatment, Fuego and Espresso varieties recorded the

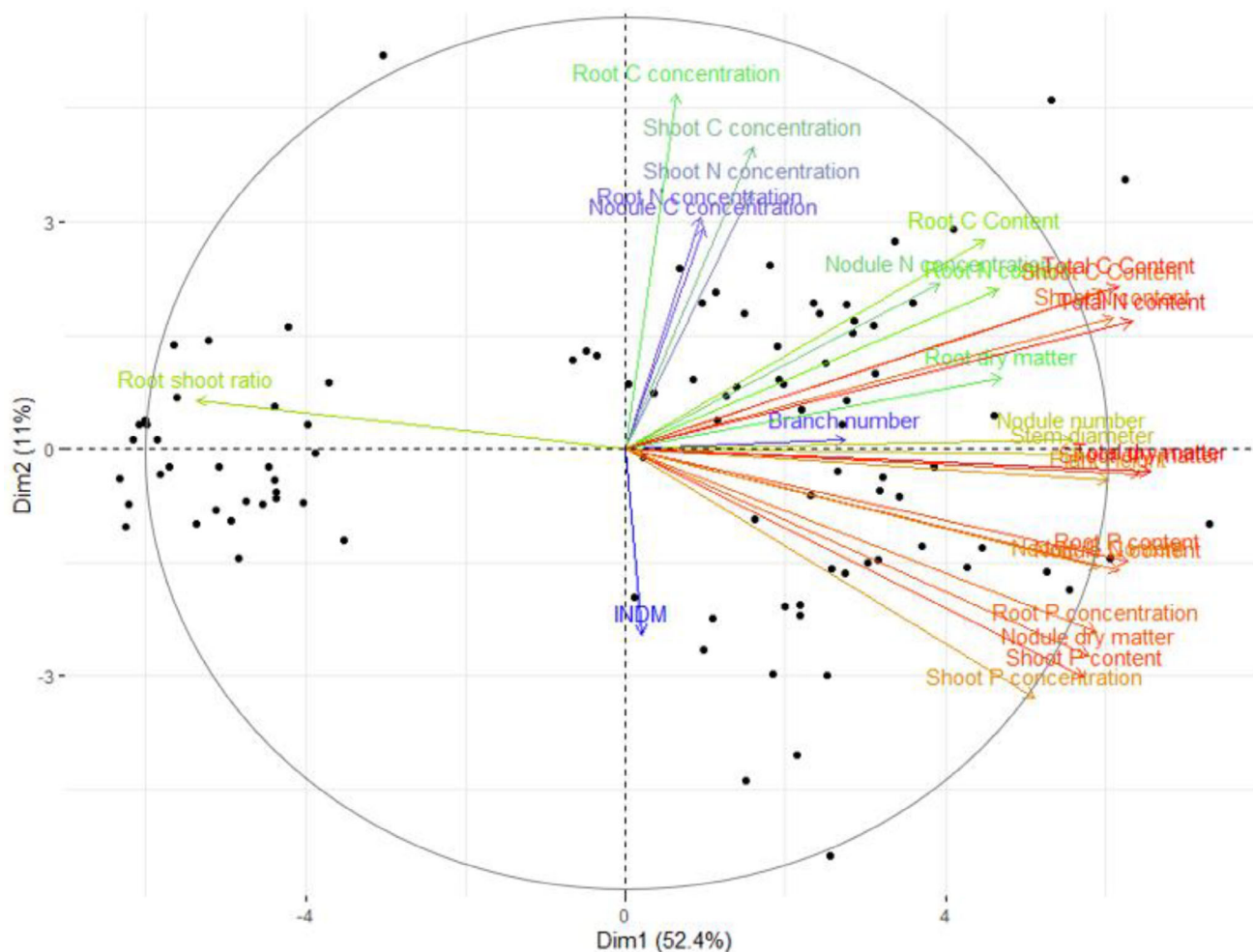


FIGURE 9 Principal component analysis (PCA) score plot of individual traits measured under the three P sources (no P, KH_2PO_4 , and phytic acid denoted as $-P$, P_i , and P_o) supplied to eight faba bean varieties grown under symbiotic conditions for 8 weeks. INDM, individual nodule dry matter.

maximum ($193.42 \text{ mg N shoot}^{-1}$) and minimum ($119.15 \text{ N mg shoot}^{-1}$) SNcont. However, a highly significant interactive effect ($p < 0.001$) was observed in nodule and root N contents, but not in shoots N content (Table S3). Figure S1, however, presents contrasting varietal responses when grand nitrogen and carbon contents in tissues were estimated. Significant variations were observed among varieties and treatments, with both P treatments (P_i vs. P_o) differing significantly from $-P$. Tiffany variety under both P treatments accounted for the highest nitrogen and carbon contents relative to other varieties (Figure S1A,B).

4 | DISCUSSIONS

4.1 | Growth, nodulation, and biomass accumulation in response to P supply

P limitation is a major nutritional obstacle impeding legume growth and crop yields, especially during symbiotic conditions (Nasr Esfahani

et al., 2016). A wide array of established shreds of evidence for P acquisition and utilization among crop species, cultivars, and varieties within and between similar species is documented (Abbas et al., 2018; Akhtar et al., 2016; Aziz et al., 2014; Fageria et al., 2011; Irfan et al., 2017, 2020). However, there is not much direct information regarding the potential mechanisms underlying nodulating plants' differential P_o uptake and utilization (Suliman & Mühling, 2021). To meet these goals, we need to focus not just on the dynamics of soil organically bound P but also to explore the various regulatory pathways of the symbiotic nodules at the whole-plant level.

Screening for PUE is a promising strategy that has been employed in the selection of varieties that have the propensity to acquire and assimilate P efficiently and to adapt to harsh environmental conditions such as P starvation (Aziz et al., 2011; Irfan et al., 2020). This paper presents evidence that P_o availability imposes strong effects on the growth and development of faba bean when grown under symbiotic conditions. Remarkable differences were observed among faba bean varieties in response to P availability. The results of this study indicated that traits such as root and shoot DM, TDM, NN, NB, PH, and SD

increased tremendously upon P supply and did not vary significantly between Pi and Po but differed substantially from untreated plants (Tables 1–3). The non-significance variation between Pi and Po in these traits indicates similar and equal utilization efficiencies and responses by varieties. When the different varieties under Pi and Po treatments were compared, Tiffany was observed to be an outstanding variety in traits such as SDM, PH, SD, and TDM (Tables 1–3). These traits, coupled with other parameters, obviously show that Tiffany under P supplementation performed better and was declared the best variety under the aforementioned traits in both Pi and Po treatments. Jillani et al. (2022) accurately observed what is reported in this current work that Tiffany is very efficient in Po utilization and produces greater biomass compared to other varieties. These results are in accordance with what was reported by Adams and Pate (1992) that inositol-P was the best P source to white lupin and could be confirmed by Feng et al. (2003), who also reported similar shoot growth for red clover when supplemented with Pi and Po sources under mycorrhizal.

Nodule growth is severely affected by P starvation (Nasr Esfahani et al., 2016; Sulieman et al., 2019). This finding was affirmed in this current study, as NDM under –P was severely affected and decreased markedly from the two P treatments (Table 2). Intriguingly, the results indicate apparent significant differences between Pi and Po in terms of NDM; nevertheless, Po supplementation differed significantly from –P, suggesting the effect and response of nodule growth to Po supply in terms of NDM (Table 2). Contrary, Po supplemented varieties observed a general significant reduction in all varieties relative to Pi and –P under INDM (Table 2). This development could indicate Po produced higher NN but could not reflect its biomass accumulation on the individual nodule level. However, the progressively higher NN could have masked the overall INDM in Po treatment in that sizes of some nodules formed under this treatment (compared with Pi and –P) were numerous and not uniform. The differential nodule size and number were the absolute cause of the reduction in INDM in Po fertilized plants. The positive correlations observed between INDM and NDM, and the negative correlation between INDM and NN (Figure 1), reflect the reduction in Po treatment under INDM. This development was confirmed in this study, as it is quite plausible to postulate that NN did not necessarily contribute to nodule biomass production under each variety. Although NDM differed between the two treatments, nonsignificant differences occurred in NN, with Po recording the highest. Comparisons among varieties, however, indicate that the Po-fed varieties such as Hiverna, Victus, Mallory, and Trumpet produced the highest NN relative to their Pi counterparts, suggesting the nodulation ability of faba bean plants under organic P fertilization facilitated by rhizobia. Notwithstanding, the deleterious effect of P starvation on nodulation was perceived in this study when P treatments were compared with –P. The reason is that nodulation requires a higher amount of energy and goes through a series of metabolic processes (Nasr Esfahani et al., 2016; Olivera et al., 2004; Sulieman et al., 2019; Valentine et al., 2011). And so, the deprivation of P was obviously going to impose negative effect. The exciting part is that it has been evident in this study that Po could perfectly support nodulation, N₂-fixation, and

metabolism as could be seen in the NN, NDM and supported by its high N concentration.

Root characteristics (e.g., biomass) play an essential role under P limitation for better P scavenging and absorption (Bates & Lynch, 2001; Ma et al., 2001). A similar trend was observed in the present study, with no significant variation between Pi and Po but differed significantly from the negative control (Table 1). However, RDM in Hiverna under –P condition responded better and equivalent to almost all the varieties under both Pi and Po (Table 1), suggesting that P starvation enhanced root biomass and partitioning in the variety Hiverna (Irfan et al., 2020). Generally, the findings of this study show that those faba bean varieties supplied with phytic acid (Po) responded competently were at least equal to inorganic P (Pi) and were by far better than varieties under P starvation (–P). Adding to this, Espresso plants treated with Po recorded the highest RDM and even better than the best variety under Pi (i.e., Tiffany). The results opine that Po improved root biomass without any impairment and could be a good P source for plant nutrition. The similarities in growth and biomass accumulation exhibited between Pi and Po treated varieties could be attributed to the fact that an effective phosphate hydrolyzable substrate was available to enhance Po solubilization and mobilization, as faba bean plants have being evident to perform better when inoculated with rhizobia (Tarafdar & Claassen, 1988). The partitioning of DM into different growing and developmental organs is vital to evaluate varieties' efficiency and P utilization (Iqbal et al., 2019). Interestingly, in this study, it was evident that Pi-treated varieties produced similar biomass as Po in traits such as SDM, RDM, and TDM, suggesting that Po supplementation effectively partitioned these developmental traits similar to Pi. Tiffany showed greater allocation and accumulation under Po and Pi relative to –P. Generally, the current findings buttress the higher competence of Po and propose that the variety Tiffany was relatively more efficient than others in biomass partitioning at the whole-plant level. Hence, the hypothesis that Po in the form of phytic acid could support the growth and nodulation of faba bean plants grown in association with rhizobia is accepted.

4.2 | Varietal differences for PoUE

Screening faba bean varieties for PoUE is a promising strategy in the current dispensation where an urgent alternative to Pi is required to ensure a sustainable supply of P in the uncertain foreseeable future. Three conditions, as elaborated earlier, were used in determining the best and worst faba bean variety for PoUE in the current study. A variety meeting at least two of these conditions with intermediate (average) PoAE value was considered most efficient and was based on modified conditions proposed by Irfan et al. (2020). In this study, however, among the Po-fed varieties, Tiffany plants supplemented with phytic acid produced the greatest PoUE (Figure 5A), as could be evident and confirmed from traits such as SDM, PH, SD, and TDM (Tables 1–3). Tiffany significantly differed from other varieties, with Fuego obtaining the least PoUE (Figure 5A,C). Interestingly, the higher

P-utilization efficiency of Tiffany was further supported by its higher PoBF value (Figure 5C) in comparison to other varieties under the same treatment. Although Tiffany acquired an intermediary (an average) in terms of PoAE, with Mallory acquiring the best acquisition efficiency (Figure 5B), notwithstanding, Tiffany accumulated the highest DM. This suggests that the higher PoAE acquired by Mallory could not be translated into biomass production, but Tiffany did. It has been proposed that a variety producing higher biomass with an average or low PAE could be associated with its high internal utilization/remobilization efficiency, and its ability to convert the little acquired P into higher biomass production (Irfan et al., 2020; Akhtar et al., 2016). It could be elucidated that not all acquired P is utilized or converted into higher biomass production, and this could be explained by the nonsignificant and negative correlation between biomass (DM) and PAE (PoAE; Figure 7A). Another justification for this assertion is the higher production of PoAE by Fuego variety but could only record the least PoBF (Figure 5B,C) compared with Tiffany. Adding to this was the nonsignificant correlation between PoAE and PoUE ($r = 0.11$, $p = 0.55$; Figure 6B,C). Remarkably, the negative correlation between PoAE and PoBF attests to the fact that higher PAE does not always translate to biomass (Figure 6B). All these assertions could be factual in the sense that Tiffany obtained one of the fewest acquisition efficiencies but was, however, observed to have amassed the greatest in terms of PoUE, PoBF, and biomass accumulation. This literally means that the little P acquired by Tiffany was efficiently utilized and translated into higher biomass compared with Mallory and Fuego. This is an apparent indication of its (i.e., Tiffany) competency and responsiveness to Po fertilization facilitated by rhizobia, as evident by other workers (Richardson et al., 2001; Singh & Satyanarayana, 2011) that Po solubilization is enhanced by rhizobia inoculation. Tiffany was selected as the best ahead of other varieties because of their respective differential PoAE capabilities; as reported (Jillani et al., 2022). The results of this study could hypothesize that higher P acquisition does not necessarily translate into higher biomass production, but rather its internal P utilization efficiencies.

Consequently, Fuego was selected as the worst performed variety because it produced the lowest in terms of PoUE and PoBF compared to other varieties (Figure 5A–C). These two conditions (i.e., PoUE and PoBF) were paramount in declaring the best and worst varieties in this present study. Although Fuego produced the second highest PoAE (Figure 5B) in this sense, however, PoAE has been expatiated above and found not to consistently correlate with biomass production. Based on these three conditions, coupled with traits-related results (i.e., SDM, PH, SD, and TDM), variety Tiffany was selected as the best variety of PoUE and was, however, supported by its intermediate PoAE value. In conclusion, Tiffany and Fuego were chosen as the best (efficient) and worst (less efficient) performed varieties, respectively, in their response to phytic acid supplementation facilitated by bacteria inoculation. To this end, we concluded that the factors and mechanisms that ascertained the variation in PoUE among faba bean varieties were high internal P utilization and effective remobilization efficiencies. The hypothesis that faba bean varieties would exhibit differential PoUE and PoAE under symbiotic conditions is therefore supported. To

the best of our knowledge, these criteria for selection are the first of its kind in faba bean and could serve as a basis for selecting PoUE in different varieties, cultivars, and other plant species.

4.3 | Supply of Po promotes N-fixation and assimilation in plant organs

Various research presenting that P application increased the N content under symbiotic conditions have been chronicled in legumes (Drevon & Hartwing 1997; Kolawole & Kang 1997; Olivera et al., 2004). In this present study, the N concentration in the nodule, shoot, and root varied and significantly increased among treatments, especially in nodules of various faba bean varieties (Table S2, Figure S1A). NNC was higher than root and shoot, with no P-treated varieties accumulating lowest N (Table S2). The higher N concentration in nodules is attributed to N_2 -fixation under symbiotic conditions, which ensures that P and N are always optimum in nodules compared to other organs. Faba bean varieties exhibited differential responses to P treatments (Pi versus Po) regarding N concentration and contents in the various compartments. Data in root and shoot tissues generally indicate comparable N concentrations and contents between Pi- versus Po-treated varieties, except in NNC and contents, where contrasting significances were observed. The patterns of the results generally indicate higher N accumulation in both Po and Pi relative to $-P$ under symbiotic (Tables S2 and S3). However, TNcont showed equal N accumulation and contrasting patterns among Pi and Po (Figure S1A), suggesting comparable performance in N assimilation. This was highly anticipated due to the differential genetic make-up when dealing with different varieties of plants.

The significantly higher N contents and concentrations in all fractions of faba bean tissues supplemented with phytic acid (Po) suggest that Po supply enhanced higher N contents in faba bean and improved assimilation better or equivalent to Pi under the same conditions. This could indicate that intracellular N assimilation and utilization after nodulation and N_2 -fixation might be pretty effective under Po and support the premise that Po could be an excellent alternative to Pi in agriculture P nutrition (Suliman & Mühling, 2021). Similar reports have been ascertained (Jillani et al., 2022), where faba bean varieties supplemented with Ca-glycerol-phosphate resulted in a higher N accumulation than inorganic P. This was attributed to higher symbiotic pursuit of plants under Po treatment. However, nitrogenase activity was not determined in the present study but could be inferred. So, it is quite plausible to deduce that N accumulation in plants was spawned from nodule symbiosis (Míguez-Montero et al., 2020; Pérez-Fernández et al., 2019). This present result perfectly agrees with what was reported (Adams & Pate, 1992) that N concentrations were more significant in roots of *Lupinus albus* when grown with inositol-P than with other sources (*ortho*-P).

Adams and Pate (1992) further reported no significant differences in N contents among cultivars; however, the highest values under this condition were recorded for plants treated with inositol compared to other sources. However, NNcont was lower than that of root and shoot

in this study, with similar results previously reported (Rose et al., 2018). Additionally, the nodule is a P sink and a preferential organ for P accumulation and would eventually improve nodulation and N_2 -fixation in all P-treated varieties compared to untreated ones, as observed in this present study. The higher NNC indicates that nodule function requires more P than plant growth in general. This study reveals that the supply of P improved N_2 -fixation compared with no P. We can confidently report that improved N_2 -fixation in plants contributed enormously to the higher biomass production in this present study. This could be explained by the highly significant and positive correlation observed between TDM and TNcont ($r = 0.85$, $p < 2.2e - 16$; Figure 7D), suggesting the role of fixed N in the growth and development of faba bean plants. The study concurs with the results of Rose et al. (2018), who observed higher NNCs in chickpea, white lupin, field pea, and faba bean cultivars under P supply, respectively.

5 | CONCLUSIONS

The study demonstrates significant genetic variation among the tested Faba bean varieties for organic P (Po) use efficiency. The results provide strong evidence that nodulating Faba bean varieties have capacities to utilize Po competently, thus asserting that Po could be used as an alternative to mineral P fertilizers for legume production. Based on the conceivable findings, Tiffany was found to be the most responsive to Po supplementation, whereas Fuego was the least. To the best of our knowledge, this present study is the first to characterize Faba bean varieties for PoUE under symbiotic conditions. Putting together, we can confidently accept the tested hypothesis of this study, with the reason that Po support growth and biomass accumulation of Faba bean similar to Pi-fertilized plants, and the varieties have substantial and highly contrasting PoUE under symbiotic conditions. This study provides solid bases for the screening of other genotypes, leguminous species, and microsymbionts applying similar criteria.

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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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