# TERRA LATINOAMERICANA



# Folic Acid (vitamin B9) or Inoculation with Beneficial Microbes Enhances Plant Growth in *Phaseolus vulgaris* L. Efectos del Ácido fólico (vitamina B9) o de la Inoculación de Microbios Benéficos Incrementa el Crecimiento de *Phaseolus vulgaris* L.

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# SUMMARY

Folic acid (vitamin B9) plays a crucial role in chlorophyll synthesis, and its exogenous application to plants could positively influence the Dark Green Color Index (DGCI) and plant growth. This study investigated how vitamin B9 supplementation could affect the growth of inoculated Phaseolus vulgaris L. (Fabaceae) and its symbiont. The inoculant used contained both arbuscular mycorrhizal fungi (AMF) and bacteria. We measured DGCI in leaves, plant growth variables and AMF colonization 35 days after seeding. Our results showed that in P. vulgaris treated with vitamin B9, both plant growth and DGCI increased compared to controls. However, the addition of vitamin B9 to P. vulgaris plants, inoculated with a mixture of AM fungi and bacteria, did not further increase plant growth, suggesting that inoculated plants are not limited by vitamin B9. This study is the first to highlight the role of vitamin B9 in inoculated P. vulgaris plants. Independently, both vitamin B9 amendment and inoculation with beneficial microbes increased the measured variables; though, their combined effect was not additive. Therefore, our results suggest that the addition of vitamin B9 does not further increase the measured variables when the plants are inoculated with a mixed AMF and bacterial inoculant.

Index words: arbuscular mycorrhizal fungi, B9, chlorophyll, proline.

# RESUMEN

El ácido fólico (vitamina B9) juega un papel crucial en la síntesis de clorofila y su aplicación exógena a las plantas podría influir positivamente en el índice de verdes obscuros (DGCI) y crecimiento vegetal. Este estudio investigó cómo la suplementación de la vitamina B9 puede afectar el crecimiento de plantas de Phaseolus vulgaris L. (Fabaceae) inoculadas y al simbionte. El inoculante utilizado contenía tanto hongos micorrízicos arbusculares (HMA) como bacterias. Se midió el Índice de Verdes Obscuros (IVO) en las hojas, variables de crecimiento de las plantas y la colonización de HMA, 35 días después de la siembra. Los resultados mostraron que en P. vulgaris tratadas con la vitamina B9, tanto el crecimiento de la planta como el índice de verdes obscuros aumentaron en comparación con el control. Sin embargo, la adición de la vitamina B9 a las plantas de P. vulgaris inoculadas con una mezcla de HMA, no aumentó el crecimiento de estas, lo que sugiere que las plantas inoculadas no están limitadas por la vitamina B9. Este estudio es el primero en destacar el efecto de la adición de la vitamina B9 en plantas de P. vulgaris inoculadas con organismos benéficos en variables de crecimiento. De manera independiente, tanto la adición de la vitamina B9 como del inoculante incrementaron las variables medidas; sin



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**Copyright:** © 2025 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY NC ND) License (https://creativecommons.org/licenses/ by-nc-nd/4.0/). embargo, el efecto combinado no fue aditivo. Por lo tanto, nuestros resultados sugieren que la adición de la vitamina B9 no produce un mayor incremento en las variables medidas cuando las plantas están inoculadas con un inoculante mixto de HMA y bacterias.

Palabras clave: hongos micorrízicos arbusculares, B9, clorofila, prolina.

# INTRODUCTION

Folates are a group of water-soluble B vitamins that play a crucial role in various biological processes, including plant growth and development. They are essential for the formation of purines (Zrenner, Sitt, Sonnewald, and Boldt, 2006), which are critical for cell division, and they influence on the production of plant hormones such as auxins (Li et al., 2023) and ethylene (Wang, Li, and Ecker, 2002); as well as root formation and cell elongation (Hwang et al., 2018). Folates also enhance stress tolerance in plants (Aljuaid et al., 2022; Alsamadany, Mansour, Elkelish, and Ibrahim, 2022), they are necessary for methionine, serine and glycine formation (Basset, Quinlivan, Gregory, and Hanson, 2005), and act as regulators during symbiotic interactions (Bekuzarova, Kozyrev, Shabanova, Lushenko, and Weissfeld, 2020; Banuelos, Martínez-Romero, Montaño, and Camargo, 2021; Piya et al., 2023). Although plants can synthesize folates, they often rely on external sources for an adequate supply. This has been observed in Phaseolus vulgaris L. (Fabaceae), where foliar folic acid (vitamin B9) application (0.1-0.2 mM) significantly increased shoot and root growth, as well as the content of chlorophyll a, b and carotenoids (Alsamadany et al., 2022). Additionally, the folate precursor pABA (4-aminobenzoic acid) enhanced plant growth, particularly by increasing the number of nitrogen-fixing bacteria root nodules in Trifolium rapens L. (Faboideae) and Medicago sativa L. (Fabaceae) (Bekuzarova et al., 2020). Folate synthesis increases during elevated light exposure (Jabrin, Ravanel, Gambonnet, Douce y Rébeillé, 2003), as folates are involved in chlorophyll biosynthesis through their contribution to one-carbon metabolism (Van-Wilder et al., 2009). Accordingly, an increase in folate supply could alter chlorophyll production, but its effect on arbuscular mycorrhizal fungi (AMF) symbiosis is still unknown.

Folates also confer tolerance against plant stress, primarily through regulating plant metabolites. One of these molecules is proline, an amino acid that serves as an osmoregulator for salt and drought stress; and varies during symbiosis with AMF (Bañuelos et al., 2012). Proline is an indicator not only of stress conditions but is connected to several other cellular processes, such as energy status, nutrient availability, and changes in redox balance and its catabolism is linked to photosynthesis and mitochondrial respiration (Alvarez, Savouré y Szabados, 2022). Therefore, it is an important marker of general plant health and metabolic status. Although folates are essential vitamins for most living organisms, AMF does not biosynthesize most vitamins (Ghignone et al., 2012). Therefore, the addition of folates may affect root colonization by AMF and host plant responses. Other plant root symbionts, like Rhizobium, are affected by vitamin supplementation or increase (Yang, Bhuvaneswari, Joseph, King, and Phillips, 2002), and as AMF share a common symbiosis signaling pathway (De Brujin, 2019), this symbiosis also might be affected. The effect of different vitamins has been studied in P. vulgaris (Keshavarz y Moghadam, 2017; Banuelos, Martínez, Montaño y Camargo, 2023) that not only form a symbiosis with rhizobia but also with AMF. We hypothesize that the addition of an external source of stable folate, like vitamin B9, might increase the leaf's DGCI while affecting AMF root colonization or their effect on the plant. The objectives of this study were to determine if the exogenous application of vitamin B9 could change the AMF effect in *Phaseolus vulgaris* L. (Fabaceae), as well as the growth and the Dark Green Color Index (DGCI) of P. vulgaris L. leaves.

# **MATERIALS AND METHODS**

# **Experimental Design**

The experimental design was completely randomized, using a 2×2 factorial scheme with two inoculation treatments (with and without AMF), two vitamin irrigation treatments (with and without vitamin B9), and a combination of both. We considered one plant as an experimental unit. We used 4 replicates per treatment, yielding 16 experimental units.

#### **Experiment Management Conditions**

The experiment was conducted for 35 days under greenhouse conditions before flowering, at the Faculty of Agricultural Sciences, Universidad Veracruzana, Xalapa, México. The greenhouse maintained an average relative humidity of 74% and an average temperature of 20 °C. The plants were grown in one-liter pots and irrigated every other day until the harvest. The substrate used for the study was obtained from the top 10 cm of soil in a coffee-growing area located within a mountain mesophyll forest in Coatepec, Veracruz, Mexico. Soil analysis was performed by the Soil Analysis Laboratory of the National Institute of Ecology (INECOL), Xalapa, Mexico, and presented the following physical and chemical characteristics: 63.5% phosphorus (P) removed (Blakemore); 40.52 mg kg<sup>-1</sup> of available P (PO<sub>4</sub><sup>-1</sup>, Olsen); 34.28 mg kg<sup>-1</sup> ammonium (NH<sub>4</sub>-N) (salicylate method); 36.28 mg kg<sup>-1</sup> nitrates (NO<sub>3</sub>-N); 1016 mg kg<sup>-1</sup> pyrophosphate-extractable iron (Fe) (PeF); 7.13% organic matter (OM), (Walkley-Black); 4.14% organic carbon (OC) (Walkley-Black); 667.71 mg kg<sup>-1</sup> sodium (Na<sup>+</sup>) (atomic absorption spectrometry); 693.38 mg kg<sup>-1</sup> calcium (Ca<sup>2+</sup>) (atomic absorption spectrometry), and 794.77 mg kg<sup>-1</sup> magnesium (Mg<sup>2+</sup>) (atomic absorption spectrometry). The pH<sub>H2O</sub> was 6.7, obtained in a suspension of soil and deionized water (1:2 w/v). The substrate was sterilized for 40 min by autoclaving, at 120 °C, 1.05 kg cm<sup>-2</sup> (Khavazi, Rejali, Seguin y Miransari, 2007; Sheteiwy *et al.*, 2022).

#### Plant Material

"Negro jamapa" is a cultivar of *P. vulgaris* L., a bean variety from Veracruz, México (Voysest, 1983). Negro Jamapa bean seeds were surface disinfected by treating them with 70% ethanol for 1 min, followed by 1.2% Na<sup>+</sup>hypochlorite for 15 min. The seeds were then rinsed four times with sterilized water, once with 2% Na<sup>+</sup>thiosulfate and a final rinsing with water (Rosenblueth y Martínez, 2004). Seeds were germinated in sterilized quartz sand at total water-holding capacity (42.7%) before being planted in 1 L pots (13 cm diameter) with autoclaved soil (120 °C, 1.05 kg cm<sup>-2</sup> for 40 min).

#### Folic Acid (vitamin B9) Supply

We used folic acid, a fully oxidized monoglutamate form of vitamin B9, for this experiment. A stock solution of 50 µM vitamin B9 from Sigma-Aldrich (BioReagent for cell culture) was prepared in deionized sterile water by stirring for 10 min in the dark and stored at 4 °C until use. We added 50 mL of vitamin B9 to the plants, 2 cm from the stem, at night to prevent degradation, five days after mycorrhizal inoculation and every five days until harvest. The control treatments were irrigated with sterile deionized water in the same manner.

#### Arbuscular Mycorrhizal Fungi (AMF) Inoculum

The inoculum was provided by the Laboratory of Beneficial Organisms, Faculty of Agricultural Sciences, Universidad Veracruzana, Mexico. This inoculant contained 15 species of AMF (Table 1) (Banuelos *et al.*, 2023) and diverse bacterial species (Hernández-Álvarez, Peimbert, Rodrìguez, Trejo y Alcaraz, 2023). The inoculum consisted of spores (6500 per 100 g) and propagules within *Brachiaria decumbens* Stapf (Poaceae) roots (87% AMF colonization), with sand as a vehicle. The inoculum was added (10 g) in the transplantation hole below the plantlet. After 35 days, we measured the AMF colonization percentage (Giovannetti y Mosse, 1980) by clearing and staining plant roots (Phillips y Hayman, 1970).

The inoculum was produced in host plants and contains mainly AMF, however, there are associated bacteria in this inoculant (Hernández-Álvarez *et al.*, 2023).

### Dark Green Color Index (DGCI)

We used an indirect method to measure leaf nitrogen (N) content as an average of three fully developed leaves at half the height of the plant. This is a non-destructive method that allows us to use an Android mobile phone camera to measure changes in the leaves by obtaining a DGCI on a scale from 0-1 (Petiole LTD, 2019). DGCI is a relative quantitative measure of greenness, closely related to leaf N concentration (Rorie, Purcell, Karcher, and King, 2011a; Kaler *et al.*, 2020). The procedure relies on a calibration pad to normalize the color among samples, while normalizing the background light changes. The mobile phone was placed on a stationary stand, 5 cm above the leaf sample, in a controlled light environment and a picture was taken to then be used in the Petiole Pro mobile phone application.

Family	AMF species	Synonym
Archaeosporaceae	Archaeospora trappei (R.N. Ames and Linderman) J.B. Morton and D. Redecker	Acaulospora trappei
Acaulosporaceae	Acaulospora sp	
Gigasporaceae	Dentiscutata savannicola (R.A. Herrera and Ferrer) C. Walker and A. Schüßler	Gigaspora savannicola; Fuscutata savannicola; Scutellospora savannicola
	Gigaspora rosea T.H. Nicolson and N.C. Schenck	
	<i>Cetraspora pellucida</i> (T.H. Nicolson and N.C. Schenck) Oehl, F.A. Souza and Sieverding	Scutellospora pellucida
Diversisporaceae	Diversispora varaderana J. Błaszkowski, G. Chwat, Kovács and Góralska	
Claroideoglomeraceae	<i>Claroideoglomus etunicatum</i> (W.N. Becker and Gerd.) C. Walker and A. Schüßler	Glomus etunicatum; Entrophospora etunicata
Glomeraceae	Dominikia bernensis Oehl, Palenz., Sánchez-Castro, N.M.F. Sousa and G.A. Silva	
	Dominikia lithuanica J. Błaszkowski, G. Chwat, A. Góralska	
	Rhizophagus aggregatus (N.C. Schenck and G.S. Sm.) C. Walker	Glomus aggregatum. Rhizoglomus aggregatum
	Glomus macrocarpum T.H. Nicolson and Gerd	Endogone macrocarpa
	Funneliformis mosseae T.H. Nicolson and Gerd	
	Kamienskia bistrata J. Błaszkowski, G. Chwat and Kovács	
	<i>Rhizophagus irregularis</i> Błaszk., Wubet, Renker and Buscot (C. Walker and A. Schüßler comb. Nov)	
	Rhizophagus neocaledonicus D. Redecker, Crossay and Cilia	

# Table 1. List of the 14 species of arbuscular mycorrhizal fungi (AMF) contained in the inoculum mixture added to *Phaseolus vulgaris* L. (Fabaceae) plants.

Taken from Bañuelos et al. (2023).

An affordable leaf N content meter is commonly used to determine leaf greenness and indirectly infer leaf chlorophyll concentration (Kaler *et al.*, 2020). Karcher y Richardson (2003) found that digital image analysis can be used to determine differences among plants fertilized with N by measuring their DGCI. Also, Rorie *et al.* (2011b) demonstrated that the coefficient of determination (R<sup>2</sup>) between DGCI and leaf N concentration (g 100 g<sup>-1</sup>) was 0.81-0.91 and that the DGCI is an effective color-image analysis to assess N concentration in leaves (Rhezali, Prucell, Roberts y Greub, 2018).

# **Proline Content Determination**

Proline content was determined using the method of Bates, Waldren, and Teare (1973), with modifications. A 0.25 g sample of all the leaves was homogenized with liquid N in a mortar, and 5 mL of 3% sulfosalicylic acid was added. The extract was filtered under a vacuum. Aliquots of 0.5 mL of the extract were mixed with 0.5 mL of acid ninhydrin solution, together with 0.5 mL of acetic acid. Samples were heated for 30 min at 90 °C and cooled in an ice bath until room temperature. Toluene was used to extract the amino acid, and samples were incubated for 20 min in a dark environment. The absorbance of the organic phase was measured at 520 nm using a spectrophotometer (Thermo-scientific GENESYS 10S UV-Vis). Proline concentration was calculated using a calibration curve and expressed as micromoles por miligramo.

#### **Plant Growth Variables**

Harvested plants were divided into root and shoot and then oven-dried at 45 °C for three days until reaching a constant weight. We counted the number of trifoliate leaves at harvest. The height of the plant was measured from the base of the stalk at harvest, and the trifoliate leaves were counted manually. Leaf area was measured using the mobile app) Petiole Pro (Petiole LTD, 2019).

#### **Statistical Analysis**

A two-way ANOVA was performed to compare treatments. Results were expressed as means with standard deviation ( $\pm$ ). Significant differences between treatment means were analysed using Tukey's post hoc test (HSD,  $P \le 0.05$ ). Normality and homogeneity of variances were addressed using Shapiro-Wilk and Levene's test. All data analyses were performed in Minitab<sup>®</sup> 18.1 (Minitab, 2017).

### **RESULTS AND DISCUSSION**

#### Plant Growth

As P. vulgaris production moves towards more sustainable production methods, there is a need for novel information regarding the use of biofertilizers and non-synthetic metabolites. In this study, we observed that the addition of the inoculant increased the leaf area significantly (ANOVA, F:13.63,  $P \leq 0.044$ ), whereas vitamin B9 significantly increased DGCI (ANOVA, F:19.27,  $P \le 0.001$ ) (Figure 1, 2). The combination of both factors showed significant differences in plant height (F:13.63,  $P \le 0.004$ ), number of trifoliate leaves (F:17.36,  $P \le 0.002$ ) and leaf area (F:7.74,  $P \leq 0.018$ ). Specifically, we observed a 67% increase in shoot dry weight with the addition of vitamin B9 (F: 5.01,  $P \le 0.047$ ; Figure 1a). This aligns with previous reports in *Capsicum annuum* L. (Solanaceae) and Triticum aestivum L. (Poaceae), where vitamin B9 supplementation also led to biomass increase (Al-Said and Kamal, 2008; Esfandiari, Enayati, Sabaghina, and Janmohammadi, 2012). This increase could potentially translate into higher yields at later stages, as has been suggested in other studies (Rashidi, Yousefi, Pouryusef, and Goicoechea, 2021). Vitamin B9 is essential for processes, such as DNA synthesis, cell division, and amino acid metabolism, all of which play crucial roles in supporting overall plant growth (Gorelova, Ambach, Rébeillé, Stove, and Straeten, 2017). Its affordability and proven effectiveness in crops such as Pisum sativum L. (Fabaceae) (Farouk and Abdul-Qados, 2018) and P. vulgaris (Ibrahim, Ibrahim, and El-Gawad, 2021) position it as a promising tool for improving crop productivity. The positive impact of vitamin B9 supplementation on plant weight indicates that it likely acts as a general growth promoter, contributing to enhanced biomass accumulation in both shoot and root tissues (Burguieres, McCue, Kwon, and Shetty, 2007). Other studies have shown that vitamin B9 deficiency can cause growth abnormalities and reduce overall plant vigour (Li et al., 2023), and by increasing its supply, we can raise its vigour. The increase in plant weight upon vitamin B9 addition could also be attributed to the restoration of folate-related metabolic pathways, leading to improved growth and development (Ayala-Rodríguez, Barrera, Ruiz, and López, 2017). Folates are essential for pyruvate biosynthesis, which is vital for biomass production (Bar-Even, Noor, Flamholz, and Milo, 2013; Kim et al., 2020).

The inoculum significantly increased leaf area (F: 4.36,  $P \le 0.030$ ; Figure 1b), which is consistent with the welldocumented role of AMF in nutrient acquisition and transport (Augé Stodola, Ebel, and Duan, 1995; Kavanova, Grimoldi, Lattanzi, and Schnyder, 2006). The expansion of leaf area may be due to the improved nutrient availability facilitated by the mycorrhizal hyphal network, which enhances photosynthetic capacity and overall plant vigour (Cela *et al.*, 2022; Tao, Dong, Wang, Chen, and Tang, 2022) or by the effect of the associated bacteria (Mashatleh *et al.*, 2024). AMF increased plant height (F: 5.25,  $P \le 0.017$ ); however, when vitamin B9 was added, the inoculated plants had lower height compared to uninoculated ones (F: 13.63,  $P \le 0.0004$ ; Figure 1c). Additionally, plants showed an increase in the number of trifoliate leaves when inoculated (F: 5.92,  $P \le 0.012$ ), but the number of trifoliate leaves did not increase when vitamin B9 was added (F: 0.02,  $P \le 0.888$ , Figure 1d). Interestingly, the absence of an additive effect of vitamin B9 supplementation in AMF-bacteria inoculated plants on these variables, suggests a potential threshold in folate availability, where additional folate does not confer extra benefits. The plant might already optimize vitamin B9 acquisition to a point where the AMF inoculation does not further enhance the physiological processes responsible for plant growth parameters. Moreover, the regulatory mechanisms governing plant development might involve other factors, such as hormonal signaling (Pozo, López, Azcón, and García, 2015). The lack of an additive effect could also indicate that the specific interaction between

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Figure 1. a) *Phaseolus vulgaris* L. dry weight (foliar and root), b) leaf area, c) plant height, and d) the number of trifoliate leaves in inoculated plants (AMF) and not inoculated controls (C) and irrigated with 50  $\mu$ M of folic acid (vitamin B9) or not (control, C); 35 days after mycorrhizal and bacterial mixed inoculation. Means ± standard deviation with n=4. Different letters above bars indicate significant differences (Tukey HSD  $P \le 0.05$ ).



Figure 2. Dark Green Color Index (DGCI) in Phaseolus vulgaris L. inoculated (AMF) and non-inoculated controls (C) and irrigated with 50  $\mu$ M folic acid (vitamin B9), or not (control, C) 35 days after mycorrhizal and bacterial mixed inoculation. Means ± standard deviation with n=4. Different letters above bars indicate significant differences (Tukey HSD  $P \le 0.05$ ).

microbes and the host plant modulates the response to exogenous vitamin B9 differently compared to nonmycorrhizal plants (Seddas *et al.*, 2009). The bacteria present in the inoculum could also be a source of vitamins, in particular B9 (Hernández-Álvarez, *et al.*, 2023), but we found no differences between inoculated plants and inoculated plus vitamin B9 plants (Figure 1). We also measured AMF colonization in all treatments but found no significant differences between them, with no colonization in non-AMF treatments and 25.75±8.5% for the AMF treatment and 21±6.1% for the AMF+B9 treatment.

#### Dark Green Color Index (DGCI)

We measured the DGCI as an indicator of the leaf N content affected by the inoculant and vitamin B9 supplementation. Inoculated plants did not increase the DGCI (F: 1.31,  $P \le 0.276$ ). Usually, AMF inoculation increases chlorophyll content in plants which is related to leaf N content (Kavanova *et al.*, 2006; Kayoumu *et al.*, 2023); although studies with AMF inoculated *Vitis vinifera* L. (Vitaceae) have also shown that chlorophyll content does not vary compared to uninoculated plants (Ye, Wang, and Li, 2022). Irrigation with vitamin B9 increased DGCI (F:19.27,  $P \le 0.001$ ); whereas the combination of vitamin B9 plus inoculant did not differ from the vitamin B9 treatment alone (F:4.40,  $P \le 0.0.06$ ) (Figure 2). The increase in DGCI by vitamin B9 supplementation could be due to folates being involved in N metabolism, mediating its translocation from the root to the shoot (Banuelos *et al.*, 2021). The N availability affects plants' photosynthesis as chlorophyll is associated with leaf N content (Wang *et al.*, 2021), and the involvement of vitamin B9 in N translocation could have caused the increase in DGCI.

#### **Proline Content**

The inoculation reduced proline content in leaves, although the reduction was not significant (F: 4.02,  $P \le 0.07$ ). On the other hand, we observed that vitamin B9 addition significantly decreased the concentration of the metabolite proline, both in control and in AMF-inoculated plants (F: 37.63,  $P \le 0.000$ ; Figure 3). This decrease in proline could be related to the improved water-use efficiency observed with vitamin B9 treatment, which has been reported to reduce the need for osmoregulatory metabolites like proline in plants (Ibrahim *et al.*, 2021; Al-Elwany *et al.*, 2022). For instance, foliar irrigation with 150  $\mu$ M of vitamin B9 can increase water usage efficiency by 29% (Ibrahim *et al.*, 2021). In *P. vulgaris*, vitamin B9 supplementation has been shown to alleviate salt stress, which is often linked to water use efficiency (Alsamadany *et al.*, 2022; Ma, Wei, Liu, Liu, and Liu, 2021). Therefore, the growth-promoting effects of vitamin B9 may also contribute to overall plant fitness, as reflected in lower proline levels.



Figure 3. Proline content in leaves of inoculated (AMF) Phaseolus vulgaris L. and non-inoculated controls (C) and irrigated with 50  $\mu$ M folic acid (vitamin B9) or not (control, C). 35 days after mycorrhizal and bacterial mixed inoculation. Means ± standard deviation with n=4. Different letters above bars indicate significant differences (Tukey H  $P \le 0.05$ ).

# CONCLUSIONS

The positive impact of vitamin B9 on *P. vulgaris* weight aligns with its established role in growth-promoting metabolic pathways. Additionally, this study found that vitamin B9 reduced proline in leaves. The enhanced leaf area with microbial inoculation emphasizes the contribution of symbiosis to an increased leaf greenness related to N content in leaf acquisition and photosynthetic efficiency. The lack of an additive effect of vitamin B9 on leaf area in inoculated *P. vulgaris* L. indicates a complex interplay among nutrient availability, symbiotic interactions, and *P. vulgaris* L. physiological responses. A biological and/or physical restriction might limit how much *P. vulgaris* L. can utilize vitamin B9, and it cannot be further enhanced by the inoculation with the microbes.

#### **ETHICS STATEMENT**

Not applicable.

# **CONSENT FOR PUBLICATION**

Not applicable.

# **AVAILABILITY OF SUPPORTING DATA**

All data generated or analysed during this study is included in this published article.

### **COMPETING INTERESTS**

The authors declare no competing interests.

### FINANCING

Not applicable.

# **AUTHORS' CONTRIBUTIONS**

Conceptualization: J.B. Methodology: J.B., S.L.C.R., N.M.M., and E.M.R. Research: J.B., E.M.R., S.L.C.R., and N.M.M. Resources: E.M.R., N.M.M., and S.L.C.R. Monitoring: S.L.C.R., E.M.R., and N.M.M. Writing, preparing the original draft: J.B. Writing, review and editing: S.L.C.R., N.M.M., and E.M.R.

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