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RESEARCH ARTICLE

Lime and wood ash as amendments in acidic soil can increase the quality of *Phaseolus vulgaris*

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ABSTRACT

Among the consequences of acidity, low fertility significantly impacts agricultural yields. However, it can be mitigated by using amendments to increase the pH and improve nutrient uptake efficiency. This study evaluated the effect of applying lime, dolomite, and wood ash, with phosphate rock, on soil pH, bean (*Phaseolus vulgaris* L.) growth, yield, nutritional quality, and economic viability under controlled conditions. Nineteen treatments were applied to a slightly acidic soil under a completely randomized design. Data were analyzed using Tukey and Kruskal-Wallis tests, revealing differences among the treatments. In the bloom stage, dolomite affected height, diameter, Chl *a*/*b* ratio, and leaf P concentration (56%). At the same time, lime increased pod number, and wood ash increased leaf biomass by 47% *vs*. the control. At physiological maturity, all three amendments increased the pH, while lime increased width and the P (257%), K (314%), Ca (180%), Mg (233%), Fe (240%), and Mn (167%) in seeds, compared to the control. These results suggest that amendments, especially wood ash, can increase bean yield up to 1290.8 kg ha^{-1} and seed quality in slightly acidic soils, resulting in a net gain of US$369.3 per hectare.

Key words: Acidity, dolomite, micronutrients, phosphate rock, phosphorus.

INTRODUCTION

Emphasizes the Worldwide, approximately 30% of soils are acidic primarily found in the northern temperate zones and humid tropical regions (von Uexküll and Mutert, 1995). In Mexico, 15% of soils are slightly to strongly acidic in temperate regions, such as the state of Hidalgo, and tropical regions, such as Chiapas and Veracruz (Tosquy-Valle et al., 2019). The largest area corresponds to volcanic soils; whose chemical characteristics differ from those of soil in temperate zones. Acidity affects the nutritional quality of crops by decreasing the efficiency of nutrients absorption such as P and reducing their availability; consequently, it reduces the yield of staple crops, including maize, rice, and beans (Tosquy-Valle et al., 2019). Nutrient use efficiency can be improved by applying amendments that enhance the pH and the availability of nutrients such as P, Ca, Mg, and K (Holland et al., 2019). Despite the beneficial effects of liming, an excessive dose can adversely affect the absorption of nutrients so the amount of lime applied must be controlled (Olego et al., 2021).

Acidic soils can be amended by applying materials that contain $CaCO_3$ or an alkaline-reaction compound. The materials used include finely ground seashells and pulverized rocks such as calcium hydroxide $Ca(OH)_2$, dolomite [$CaMg(CO)_3$], and wood ash (Enesi et al., 2023). Dolomitic lime has a greater capacity to neutralize soil acidity and provides a higher amount of Mg to the soil than calcite. Wood ash has been used to manage the fertility of acidic soils in tropical areas (Baloch et al., 2024). Depending on its origin, ash contains a mixture of carbonates, oxides, and hydroxides of alkaline and alkaline earth elements [CaO, MgO, K_2O, K(OH)], silicates, and low N contents (Baloch et al., 2024). Soil amendments promote the adaptation and increase the yield of basic crops (Hartina et al., 2025).

On the other hand, for beans grown in acidic soils, phosphate rock has been proposed as an alternative source of P to conventional fertilizers (Savini et al., 2016) and is also characterized by an alkaline residual reaction. However, little is known about the effect of liming on the dissolution of phosphate rock, so it was included as a variable to be evaluated in this study.

Due to its high protein content, the common bean is a significant nutritional source for more than 300 million people in regions of Africa and Latin America, contributing substantially to the dietary supply of protein (65%) and energy (32%) (Blair et al., 2010). Beans are the second staple crop in Mexico. Bean production reached 1.02 million tons in 2024, with an average yield of 770 kg ha^{-1} (SIAP, 2025), which is low compared to the world average (1270 kg ha^{-1}). To meet the demand for beans, 313 and 688 thousand tons were imported in 2023 and 2024, respectively (Redacción Espejo, 2024). In acidic soils, yields are low, ranging from 620 to 650 kg ha^{-1} (Tosquy-Valle et al., 2019). Consequently, new acidity-tolerant varieties have been developed and soil amendments are currently applied, resulting in increased yields (Tosquy-Valle et al., 2019). The Oti bean variety was used in this study because it is productive (up to 2750 kg ha^{-1}) disease-tolerant, and resistant to root rot which makes it a potential alternative for this type of soil (Estrada-Gómez et al., 2022).

Given the importance of beans as a staple crop, it is essential to increase their yield and evaluate their nutritional composition in the human diet, which is directly influenced by soil nutrient bioavailability (Fluit et al., 2025). This study aimed to evaluate the effects of lime, dolomite, and wood ash amendments on the soil pH and phosphate rock-solubilization, using bean growth variables, yield, and nutritional quality as indicators. In addition, we analyzed the economic profitability associated with the application of each amendment.

MATERIALS AND METHODS

Soil and amendment characteristics

The sampling site is located in Santo Domingo Agua Zarca, Huasca de Ocampo (20°9'47.86" N, 98°29'40.82" W; 2282 m a.s.l.), Hidalgo, Mexico. The soil (0-30 cm) was dried, homogenized, and then sieved through a mesh (#10). The pH and electrical conductivity (EC) were measured in a 1:2.5 (soil: water) ratio using a potentiometer (701A digital Ionalyzer/pH-mV meter, Orion Research Incorporated, Cambridge, Massachusetts, USA) and a conductivity bridge (24 h after equilibrium; CL3, Conductronic). Cation exchange capacity (CEC), Ca, Mg, Na, K, P, inorganic N, soluble sulphates, and exchangeable Al (Al, titration with 0.1 N NaOH) were determined using the procedure of Rowell (1994). The micronutrients Fe, Mn, Zn, and Cu were extracted using the double-acid method of Nelson et al. (1953) and quantified by atomic absorption (AA). The Ca, Mg, and K total concentrations in the amendments were determined using the procedure suggested by Ure (1995): Digestion with aqua regia, filtration, and quantification.

Soil equilibrium experiment

Three doses of calcium hydroxide (lime), dolomite, and wood ash were applied to the slightly acidic soil (pH = 6.3). Doses were calculated based on previous equilibrium experiments at 20 °C. A total of 19 treatments were applied using a completely randomized design (Table 1) to the bean under greenhouse conditions. Each treatment was homogenized using a mechanical mixer for 15 min. Afterward, 3.5 kg homogenized soil were placed in each pot per treatment (n = 3). To promote neutralization reactions, water was added to each pot up to 80% of field capacity, followed by incubation. The equilibrium time was 4 wk until the pH stabilized (≈ 7).

Table 1. Treatments of the *Phaseolus vulgaris* nutritional quality experiment in amended acidic soil. PR: Phosphate rock; LM: lime; WA: wood ash; D: dolomite; 1: 1 t ha^{-1}; 2: 2 t ha^{-1}; 4: 4 t ha^{-1}.

		PR application		Without PR application	
Amendment	Dose (t ha^{-1})	Treatment	Stabilized pH	Treatment	Stabilized pH
Calcium hydroxide (lime)	1	LM1PR	6.3	LM1	6.3
	2	LM2PR	6.8	LM2	6.8
	4	LM4PR	6.9	LM4	6.9
Ash wood	1	WA1PR	6.6	WA1	6.6
	2	WA2PR	6.5	WA2	6.6
	4	WA4PR	6.9	WA4	6.9
Dolomite	1	D1PR	6.4	D1	6.4
	2	D2PR	6.7	D2	6.7
	4	D4PR	6.9	D4	6.9
Without amendment	0	Control	6.3		

Experimental setting

The experiment was conducted in a greenhouse from July to September 2024, at a mean temperature of 25 °C (minimum 14 °C; maximum 40 °C) and a mean photoperiod of 14:10 h. The bean (*Phaseolus vulgaris* L.) 'Oti' used was provided by the Postgraduate College Seed Program. Seeds were treated with carbendazim (methyl *N*-(1H-benzimidazol-2-yl)carbamate) to eliminate potential microbial contamination; two seeds were sown in each pot. To meet the phosphate requirements in the crop, the treatments were fertilized by adding 15.5 kg P ha^{-1} of phosphate rock (PR) at stage V2 of the crop cycle. The experiment was evaluated in the first stage (R5; flowering; at 50 d) on the first ten treatments fertilized with PR (Table 1). In the second stage, plants were collected at physiological maturity from the treatments with and without PR application to the soil.

Foliar nutrient concentration and growth variables

When bean plants reached the flowering stage (R5), leaves were cut and stove-dried at 65 °C to constant weight; leaf biomass was quantified, and leaves were pulverized with a mortar. Samples were subjected to wet digestion using a 3:1 mixture of nitric and perchloric acids (Jones et al, 1990); afterward, Ca, Mg, P, K, S, Mn, Cu, Zn, and Fe were measured. For N, samples were digested in a mixture of sulfuric acid and perchloric acid. The elements were measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Varian Liberty Series II, Agilent Technologies, Palo Alto, California, USA).

Plant height, stem diameter, and the number of pods and leaves were recorded on a weekly basis. Chlorophyll *a*, chlorophyll *b*, and carotenoids were also measured in leaves using the method of Lichtenthaler and Buschmann (2001). The photosynthetic pigments were extracted by maceration: Five circles (7 mm in diameter) were obtained from the fourth trifoliate leaf; 5 mL 80% acetone were added to these circles, which were left to stand in the dark at 4 °C for 1 wk. The absorbance was read in the supernatant using a UV-visible spectrophotometer at a wavelength of 663 nm for chlorophyll *a* (Chl *a*), 646 nm for chlorophyll *b* (Chl *b*), and 470 nm for carotenoids. The concentrations of the pigments were calculated with the following Equations 1-4.

$$\text{Chl } a\ (\text{mg mL}^{-1}) = 12.25A_{663.2} - 2.79A_{646.8} \quad (1)$$

$$\text{Chl } b\ (\text{mg mL}^{-1}) = 20.50A_{646.8} - 5.10A_{663.2} \quad (2)$$

$$\text{Chl } a{+}b\ (\text{mg mL}^{-1}) = 7.15A_{663.2} + 18.71A_{646.8} \quad (3)$$

$$\text{Chl } x{+}c\ (\text{mg mL}^{-1}) = \frac{1000A_{470} - 1.82C_a - 85.02C_b}{198} \quad (4)$$

Yield (kg ha^{-1}) was calculated from the weight of 100 seeds (g), number of seeds per plant (NSP), and number of plants per hectare (plants ha^{-1}) = 191 235. The equations used were as follows (Equations 5 and 6).

$$\text{Number of seeds in 1 kg} = \frac{1000\text{ g kg}^{-1}}{\text{Weight of 100 seeds (g)}} \times 100 \quad (5)$$

$$\text{Yield kg ha}^{-1} = \frac{\text{NSP} \times \text{Nº plants per ha}}{\text{Nº seeds in 1 kg}} \quad (6)$$

Nutrients in beans

The harvest was carried out after 77 d in the R9 stage. For each plant, the total number of pods (empty and full) were recorded, and their length and width were measured. The pods were dried for 8 d; then, NSP was counted, and 100 seeds were weighed. The length, width, and area of each seed were measured using the ImageJ image recognition software (Rueden et al., 2021). Bean seeds were stove-dried at 65 °C to 12% humidity and then ground with a mortar and pestle for subsequent wet digestion. The nutrients N, Ca, Mg, P, K, Mn, Cu, Zn, and Fe were quantified by spectroscopy as described by Jones et al. (1990). Proteins were obtained from N analysis (USDA, 2016).

Statistical and economic analysis

An ANOVA was performed on the plant growth variables. The Tukey test ($P < 0.05$) was used to compare means between treatments. The nutrients in seeds were evaluated using the non-parametric Kruskal-Wallis test. To detect differences between treatments, the Dunn test with Bonferroni adjustment was performed. A principal component analysis (PCA) was carried out on the seed nutrient content data. To this end, the data were entered in a matrix format, ($n = 5$, $N = 95$). Confidence ellipses were generated for each treatment. The PCA calculations were performed using the prcomp function, and visualizations were generated using the fviz_pca_ind function of the package factoextra V. 1.0.7 in R (R Foundation for Statistical Computing, Vienna, Austria). The economic analysis was performed using the economic indicators described by Dhaliwal et al. (2022) and calculated using Equations 7-9. The production cost was estimated based on the 2025 market price of pinto bean (US$1.4 per kg), according to data from SNIIM (2025). The cost of application per hectare of lime, dolomite, wood ash, and phosphate rock was US$174.1, US$519.8, US$52.0, and US$415.8, respectively; these values were obtained through direct quotations from nearby local suppliers in the study area (personal communications, June 2025).

$$\text{Gross income} = \text{Yield (kg ha}^{-1}\text{)} \times \text{Grain price} \quad (7)$$

$$\text{Profitable return} = \text{Gross income} - \text{Production cost} \quad (8)$$

$$\text{Profitability } vs. \text{ Control} = \text{Profitable return - control} \quad (9)$$

RESULTS

Soil characterization and amendments

The soil was slightly acidic (6.3 ± 0.1), with no salinity problems (EC = 0.009 ± 0.01 S m^{-1}) (Hazelton and Murphy, 2007). Nutrient concentrations in the soil were 160.1 ± 0.3 for Ca, 20 ± 0.2 for Mg, and 14± 0 $mmol_{(+)}$ kg^{-1} for K. The ionic ratios were 8 for Ca/Mg, 1.4 for Mg/K, and 11.4 for Ca/K. These values indicated an imbalance of nutrients, with a higher Ca concentration and a lower Mg concentration, while K was balanced according to the optimal 5:2:1 Ca:Mg:K ratios (Yang et al., 2024). The micronutrients Fe (18.5 ± 0.01), Mn (41.8 ± 0.01), and Zn (2.1 ± 0.01 mg kg^{-1}) were found in high concentrations, except for Cu (0.19 ± 0.02 mg kg^{-1}) (Lopes and Cox, 1977). Cation exchange capacity (12.04 ± 0.03 $cmol_{(+)}$ kg^{-1}), P (11.6 ± 0.02 mg kg^{-1}), inorganic N (3.5 ± 0.03 mg kg^{-1}), and sulphates (13.9 ± 0.02 mg kg^{-1}) were found at low concentrations (Hazelton and Murphy, 2007). Al (14.7 ± 0.1 mg kg^{-1}) was found within the toxic range for plants sensitive to Al toxicity (Hazelton and Murphy, 2007). Regarding the amendments, the pH of lime and ash was strongly alkaline (11.5 ± 0.1 and 11.3 ± 0.1, respectively). Dolomite had a slightly alkaline pH (7.9 ± 0.1), contrasting with a neutral pH of phosphate rock (PR) (7.2 ± 0.1). The percentage of Ca in lime (75.9%) was 1.7, 1.6, and 1.8 times higher than in dolomite (42.5%), ash (45%), and PR (40.9%), respectively. K concentration (48.07 ± 0.2 $cmol_{(+)}$ kg^{-1}) in ash was 39, 90 and 9 times higher than in lime (1.2 ± 0.2), dolomite (0.5 ± 0.1), and PR (5.4 ± 0.2 $cmol_{(+)}$ kg^{-1}), respectively. Finally, Mg concentration was higher in dolomite (10%), followed by PR (2.9%), and ash (1.3%); this macronutrient was not detected in lime.

Growth variables, photosynthetic pigments, and nutrient concentration in the flowering stage

The D1PR treatment showed the highest stem height and diameter compared to the control (Figures 1A and 1B), but no differences were observed with the other treatments. The LM1PR treatment had the highest number of pods (10) compared to the control; this parameter decreased with increasing lime content (Figure 1C). Leaf biomass was higher in the WA1PR treatment compared to the control (Figure 1D).

The highest Chl *a/b* ratio was found in the D4PR treatment (Table 2). Regarding chlorophylls *a* and *b,* carotenoids (*x+c*) content, total chlorophyll (Chl *a+b*), and the (Chl *a+b*)/carotenoids ratio, no differences were observed compared to the control.

The concentrations of N, K, Ca, Mg, S, Fe, Cu, Zn, and Mn did not show differences between the treatments; in contrast, P increased by 56% compared to the control with the application of dolomite plus PR fertilization (D2PR; Table 3). The application of dolomite had a positive effect on leaf P content.

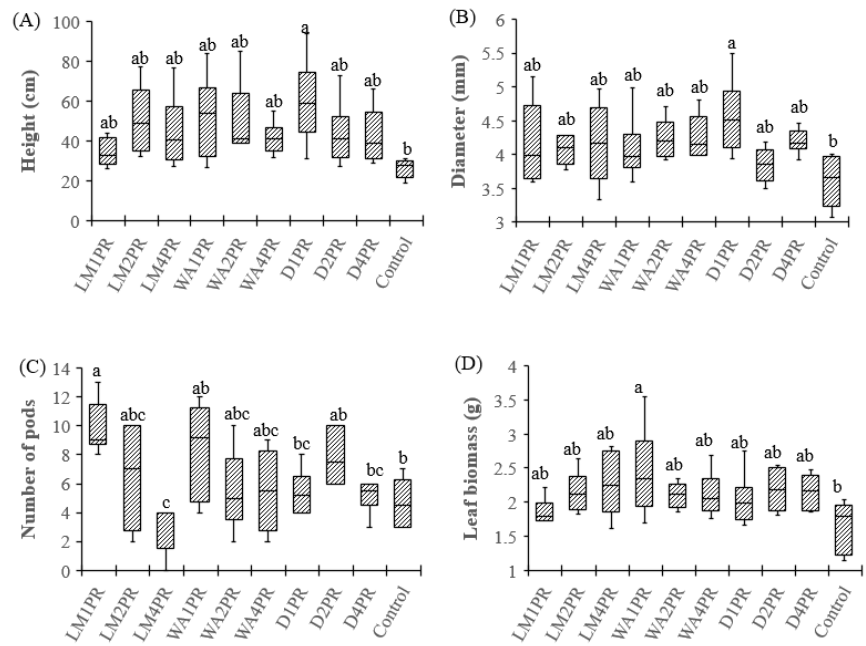

Figure 1. Plant height (A), Plant diameter (B), number of pods (C), and leaf biomass of *Phaseolus vulgaris* (D). Mean ± standard deviation (n = 6). Different letters indicate a significant difference (P < 0.05; Tukey). LM: Lime; D: dolomite; WA: wood ash; PR: phosphate rock; 1: 1 t ha^{-1}; 2: 2 t ha^{-1}; 4: 4 t ha^{-1}.

Table 2. Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (*x+c*) in *Phaseolus vulgaris* during the flowering stage as a response to the application of amendments and PR to the soil. Mean ± standard deviation (n = 6). Different letters indicate a significant difference (P < 0.05; Tukey). LM: lime; D: dolomite; WA: wood ash; PR: phosphate rock; 1: 1 t ha^{-1}; 2: 2 t ha^{-1}; 4: 4 t ha^{-1}.

Treatment	Chl *a*	Chl *b*	Chl *a/b*	Chl *a+b*	(*x+c*)	(Chl *a+b*)/(*x+c*)
	μg cm^{-1}					
LM1PR	26.1 ± 2.2^{ab}	8.0 ± 1.1^{a}	3.2 ± 0.5^{ab}	34.1^{a}	6.0 ± 0.6^{abc}	5.7 ± 0.4^{ab}
LM2PR	26.8 ± 3.3^{ab}	8.5 ± 4.3^{a}	3.2± 1.5^{ab}	35.3^{a}	5.8 ± 0.6^{abc}	6.1 ± 0.5^{ab}
LM4PR	22.8 ± 3.6^{b}	8.0± 2.2^{a}	2.8 ± 0.9^{b}	30.8^{a}	4.9 ± 0.2^{c}	6.3 ± 1.1^{a}
WA1PR	25.5 ± 3.5^{ab}	8.7 ± 2.5^{a}	2.9 ± 0.7^{ab}	34.2^{a}	5.6 ± 0.6^{abc}	6.1 ± 0.6^{ab}
WA2PR	24.4 ± 4.3^{ab}	8.2 ± 1.6^{a}	3.0 ± 0.1^{b}	32.6^{a}	5.4 ± 0.8^{bc}	6.1 ± 0.3^{ab}
WA4PR	24.7 ± 1.7^{ab}	7.7 ± 0.7^{a}	3.2 ± 0.4^{ab}	32.4^{a}	5.5 ± 0.6^{abc}	5.9 ± 0.3^{ab}
D1PR	23.9 ± 2.9^{b}	8.1 ± 1.2^{a}	3.0 ± 0.1^{b}	32.0^{a}	5.5 ± 0.6^{abc}	5.8 ± 0.2^{ab}
D2PR	22.6 ± 3.4^{b}	8.6 ± 2.4^{a}	2.6± 0.7^{b}	31.2^{a}	5.4 ± 0.7^{bc}	5.8 ± 0.3^{ab}
D4PR	28.0 ± 0.9^{ab}	6.6 ± 2.3^{a}	4.2 ± 1.5^{a}	34.6^{a}	6.5 ± 0.6^{a}	5.3 ± 0.5^{b}
Control	30.0 ± 1.5^{a}	10.8 ± 1.3^{a}	2.8 ± 0.2^{b}	40.8^{a}	6.4 ± 0.4^{ab}	6.4 ± 0.3^{a}

Table 3. Concentration of some nutrients in *Phaseolus vulgaris* during the flowering stage in response to the application of amendments plus PR to the soil. Mean ± standard deviation (n = 6). Different letters indicate a significant difference ($P < 0.05$, Tukey). LM: Lime; D: dolomite; WA: wood ash; PR: phosphate rock; 1: 1 t ha^{-1}; 2: 2 t ha^{-1}; 4: 4 t ha^{-1}. [1]Jones (2012).

Treatment		N	P	K	Ca	Mg	S
LM1PR	Macronutrients, %	3.3 ± 0.3^{a}	0.06 ± 0.0^{d}	0.3 ± 0.1^{b}	1.3 ± 0.2^{b}	0.2 ± 0.0^{c}	0.07 ± 0.0^{c}
LM2PR		3.3 ±0.1^{a}	0.12 ± 0.0^{abc}	0.7 ± 0.2^{ab}	1.6 ± 0.1^{ab}	0.3 ± 0.0^{abc}	0.13 ± 0.0^{ab}
LM4PR		3.1 ± 0.5^{a}	0.13 ± 0.0^{ab}	0.9 ± 0.1^{a}	1.7 ± 0.1^{a}	0.4 ± 0.0^{a}	0.15 ± 0.0^{a}
WA1PR		3.1 ± 0.2^{a}	0.11 ± 0.0^{abc}	0.7 ± 0.4^{ab}	1.7 ± 0.2^{a}	0.3 ± 0.1^{ab}	0.13 ± 0.0^{ab}
WA2PR		3.4 ± 0.3^{a}	0.11 ± 0.0^{abc}	0.7 ± 0.3^{ab}	1.7 ± 0.0^{a}	0.3 ± 0.0^{ab}	0.14 ± 0.0^{ab}
WA4PR		3.0 ± 0.3^{a}	0.11 ± 0.0^{abc}	0.8 ± 0.1^{ab}	1.7 ± 0.0^{a}	0.3 ± 0.0^{ab}	0.15 ± 0.0^{a}
D1PR		3.2 ± 0.4^{a}	0.07 ± 0.0^{cd}	0.4 ± 0.1^{ab}	1.5 ± 0.2^{ab}	0.2 ± 0.0^{bc}	0.09 ± 0.0^{bc}
D2PR		3.2 ± 0.2^{a}	0.14 ± 0.0^{a}	1.0 ± 0.3^{a}	1.7 ± 0.1^{a}	0.4 ± 0.0^{a}	0.17 ± 0.0^{a}
D4PR		3.5 ± 0.3^{a}	0.12 ± 0.0^{abc}	0.8 ± 0.3^{ab}	1.8 ± 0.2^{a}	0.4 ± 0.0^{a}	0.15 ± 0.0^{a}
Control		3.3 ± 0.2^{a}	0.09 ± 0.0^{bcd}	0.7 ± 0.3^{ab}	1.6 ± 0.3^{ab}	0.3 ± 0.1^{abc}	0.13 ± 0.0^{ab}
Reference[1]		4.0-5.0	0.2-0.5	1.5-5.0	0.35-2.0	0.25-1.0	0.2-0.4
		Mn	Cu	Zn	Fe		
LM1PR	Micronutrients, mg kg^{-1}	45.4 ± 9.0^{b}	3.4 ± 1.0^{c}	15.1 ± 6.7^{a}	224.9 ± 106.4^{a}		
LM2PR		64.9 ± 10.0^{a}	7.3 ± 2.6^{abc}	16.6 ± 1.8^{a}	286.6 ± 96.4^{a}		
LM4PR		73.8 ± 5.6^{a}	7.9 ± 0.7^{ab}	16.8 ± 1.7^{a}	355.0 ± 96.4^{a}		
WA1PR		63.5 ± 10.2^{ab}	7.0 ± 2.6^{abc}	16.3 ± 2.9^{a}	296.5 ±107.2^{a}		
WA2PR		73.1 ± 11.4^{a}	6.4 ± 2.8^{abc}	20.8 ± 3.8^{a}	297.2 ± 60.6^{a}		
WA4PR		70.9 ± 8.7^{a}	7.1 ± 1.6^{abc}	17.0 ± 3.2^{a}	276.6 ± 81.0^{a}		
D1PR		57.1 ± 15.6^{ab}	3.7 ± 0.4^{bc}	15.8 ± 2.2^{a}	242.3 ± 105.1^{a}		
D2PR		64.4 ± 6.4^{ab}	9.7 ± 2.9^{a}	16.7 ± 2.1^{a}	330.7 ± 99.3^{a}		
D4PR		67.2 ± 11.3^{a}	7.1 ± 2.6^{abc}	19.3 ± 1.6^{a}	269.0 ± 88.5^{a}		
Control		59.8 ± 9.4^{ab}	5.5 ± 3.2^{abc}	18.6 ± 3.4^{a}	187.5 ± 13.4^{a}		
Reference[1]		20-100	10-30	20-50	50-350		

pH and bean yield at physiological maturity

pH values exceeded neutrality (7.0) in all treatments except for the control (Table 4). No differences in pod parameters were detected (data not shown). In the LM2 treatment, wider seeds (8.2 mm) were produced compared to the control. Regarding the number of seeds per plant (NSP) and yield, no differences were observed compared to the control. It should be noted that the WA1 treatment had the highest yield (1290.8 kg ha^{-1}), while the WA2PR treatment produced the lowest yield (915 kg ha^{-1}). A lower yield was also observed as the amount of lime applied plus PR increased.

Table 4. Quality parameters, pH, and yield of *Phaseolus vulgaris* as a response to the application of amendments with and without PR application in the soil. Mean ± standard deviation (n = 4). Different letters indicate a significant difference (P < 0.05, Tukey). NSP: Number of seeds per plant; LM: lime; D: dolomite; WA: wood ash; PR: phosphate rock; 1: 1 t ha^{-1}; 2: 2 t ha^{-1}; 4: 4 t ha^{-1}; P100: weight of 100 seeds (g).

Treatment	NSP	pH	P100	Length	Width	Area	Yield
			g	mm	mm	cm^2	kg ha^{-1}
LM1PR	18.7 ± 2.9^{ab}	7.0^{a}	27.5 ± 2.0^{a}	12.0 ± 0.4^{a}	7.2 ± 0.1^{a}	0.7 ± 0.0^{ab}	980.5 ± 88.5^{ab}
LM2PR	18.7 ± 1.7^{ab}	7.0^{a}	27.3 ± 1.6^{a}	12.1 ± 0.8^{a}	7.2 ± 0.4^{a}	0.7 ± 0.0^{ab}	977.2 ± 81.0^{ab}
LM4PR	19.0 ± 2.8^{ab}	7.1^{a}	26.0 ± 0.8^{a}	12.0 ± 0.5^{a}	7.1 ± 0.1^{a}	0.6 ± 0.0^{ab}	948.0 ± 159.2^{ab}
WA1PR	22.0 ± 3.1^{ab}	7.0^{a}	27.6 ± 1.3^{a}	11.6 ± 1.1^{a}	7.1 ± 0.4^{a}	0.6 ± 0.1^{ab}	1161.0 ± 159.6^{ab}
WA2PR	16.7 ± 1.5^{b}	7.1^{a}	28.6 ± 3.1^{a}	11.7 ± 0.2^{a}	7.3 ± 0.1^{a}	0.6 ± 0.0^{ab}	915.0 ± 116.7^{b}
WA4PR	19.7 ± 4.9^{ab}	7.1^{a}	29.8 ± 1.8^{a}	12.0 ± 1.1^{a}	7.3 ± 0.5^{a}	0.7 ± 0.1^{ab}	1122.0 ± 251.4^{ab}
D1PR	20.0 ± 1.8^{ab}	7.1^{a}	27.2 ± 0.9^{a}	12.0 ± 0.5^{a}	7.2 ± 0.1^{a}	0.7 ± 0.0^{ab}	1044.1 ± 120.4^{ab}
D2PR	22.0 ± 2.1^{ab}	7.1^{a}	28.8 ± 2.1^{a}	12.1 ± 0.0^{a}	7.2 ± 0.2^{a}	0.7 ± 0.0^{ab}	1207.6 ± 100.9^{ab}
D4PR	19.2 ± 3.3^{ab}	7.2^{a}	28.2 ± 1.5^{a}	12.4 ± 0.4^{a}	7.6 ± 0.3^{a}	0.7 ± 0.0^{ab}	1044.6 ± 227.6^{ab}
LM1	20.5 ± 3.8^{ab}	7.0^{a}	28.5 ± 3.3^{a}	12.1 ± 0.9^{a}	7.2 ± 0.5^{ab}	0.7 ± 0.1^{ab}	1100.5 ± 82.1^{ab}
LM2	20.0 ± 4.5^{ab}	7.0^{a}	29.1 ± 4.0^{a}	12.6 ± 0.9^{a}	8.2 ± 0.6^{a}	0.8 ± 0.1^{a}	1114.4 ± 88.5^{ab}
LM4	20.2 ± 1.5^{ab}	7.2^{a}	28.8 ± 0.6^{a}	12.3 ± 0.8^{a}	7.4 ± 0.9^{ab}	0.7 ± 0.1^{ab}	1108.2 ± 73.0^{ab}
WA1	24.5 ± 1.5^{a}	7.0^{a}	27.5 ± 0.4^{a}	12.0 ± 0.5^{a}	7.2 ± 0.3^{ab}	0.7 ± 0.0^{ab}	1290.8 ± 76.6^{a}
WA2	23.2 ± 1.2^{ab}	7.1^{a}	26.6 ± 0.6^{a}	12.2 ± 0.4^{a}	7.6 ± 0.3^{ab}	0.7 ± 0.0^{ab}	1180.8 ± 63.8^{ab}
WA4	19.7 ± 1.5^{ab}	7.2^{a}	29.7 ± 0.7^{a}	12.6 ± 0.5^{a}	7.9 ± 0.1^{ab}	0.7 ± 0.0^{ab}	1120.1 ± 72.9^{ab}
D1	22.7 ± 1.4^{ab}	7.0^{a}	25.0 ± 0.9^{a}	11.7 ± 0.4^{a}	7.4 ± 0.1^{ab}	0.7 ± 0.0^{ab}	1083.3 ± 60.5^{ab}
D2	19.7 ± 2.1^{ab}	7.1^{a}	29.4 ± 0.8^{a}	12.0 ± 0.4^{a}	7.8 ± 0.2^{ab}	0.7 ± 0.0^{ab}	1093.8 ± 103.4^{ab}
D4	24.5 ± 1.7^{a}	7.1^{a}	27.1 ± 0.5^{a}	12.2 ± 0.2^{a}	7.7 ± 0.2^{ab}	0.7 ± 0.0^{ab}	1258.8 ± 94.0^{ab}
Control	19.2 ± 2.0^{ab}	6.3^{b}	29.1 ± 0.3^{a}	11.8 ± 0.1^{a}	7.4 ± 0.2^{b}	0.7 ± 0.0^{ab}	1065.6 ± 115.1^{ab}

Nutrient concentration in bean seed and PCA

The contents of proteins, macronutrients (P, K, Ca, and Mg), and micronutrients (Fe, Cu, Zn, and Mn) were below the reference values (Table 5). Proteins did not show a significant difference between treatments. The LM4PR treatment induced the highest nutrient concentrations, which were as follows (mg 100 g^{-1} DM): 211 for P, 484.6 for K, 127.9 for Ca, 99.4 for Mg, 5.1 for Fe, and 0.8 for Mn. These increases were 257%, 314%, 180%, 233%, 240%, and 167%, respectively, compared to the control. Cu and Zn concentrations were not significantly different compared to the control.

The PCA allowed the combination of variables to explain the variability of the results from a holistic approach, due to the effect of amendments on nutrient accumulation in seeds. The first two components explained 80.9% of the variance (Figure 2). The treatments LM4PR, WA2PR, and D4PR were positioned in the direction of the Zn, Cu, Mn, K, Mg, Fe, P, and Ca vectors (quadrants I and IV), while the vector corresponding to proteins was not clearly associated with any treatment, positioned outside the clusters (quadrant IV). The control, treatments, WA1, WA1PR, D1, D1PR, D2PR, D4, LM1, LM2, and LM4 did not show a clear association with any vector, positioned in quadrants II and III, without preference for any specific nutrient. The remaining treatments were positioned at the center of the graph, without a clear association with any of the vectors evaluated.

Table 5. Nutrients in *Phaseolus vulgaris* seed from the use of amendments with and without PR application in the soil. Mean ± standard deviation (n = 5). Different letters indicate a significant difference ($P < 0.05$, Kruskal-Wallis-Dunn with Bonferroni's adjustment). LM: Lime; D: dolomite; WA: wood ash; PR: phosphate rock; 1: 1 t ha^{-1}; 2: 2 t ha^{-1}; 4: 4 t ha^{-1}. [1]USDA (2016).

Treatment	Protein	P	K	Ca	Mg	Fe	Cu	Zn	Mn
	%	mg 100 g^{-1}							
LM1PR	13.5 ± 1.0[a]	124.1 ± 16.9[a]	252.6 ± 32.5[a]	100.5 ± 10.7[a]	61.7 ± 7.2[a]	3.7 ± 0.7[a]	0.3 ± 0.1[a]	1.2 ± 0.1[a]	0.6 ± 0.1[a]
LM2PR	14.8 ± 1.8[a]	129.7 ± 5.4[a]	250.5 ± 12.8[a]	92.9 ± 8.9[a]	57.6 ± 3.7[a]	3.1 ± 0.3[a]	0.3 ± 0.1[a]	1.2 ± 0.1[a]	0.5 ± 0.0[a]
LM4PR	14.5 ± 1.6[a]	211.0 ± 7.6[a]	484.6 ± 23.9[a]	127.9 ± 18.3[a]	99.4 ± 7.1[a]	5.1 ± 0.5[a]	0.4 ± 0.1[a]	1.5 ± 0.2[a]	0.8 ± 0.1[a]
WA1PR	12.8 ± 0.6[a]	84.1 ± 17.4[b]	160.9 ± 35.8[b]	67.5 ± 8.0[b]	34.8 ± 3.1[b]	3.4 ± 1.1[a]	0.1 ± 0.1[b]	1.1 ± 0.2[a]	0.3 ± 0.1[b]
WA2PR	13.9 ± 2.0[a]	136.2 ± 12.9[a]	314.5 ± 45.7[a]	112.8 ± 23.4[a]	70.6 ± 6.3[a]	4.4 ± 0.6[a]	0.4 ± 0.2[a]	1.5 ± 0.1[a]	0.7 ± 0.2[a]
WA4PR	14.1 ± 2.0[a]	117.1 ± 16.6[a]	233.7 ± 29.1[a]	69.1 ± 11.3[a]	50.1 ± 9.6[a]	3.0 ± 0.7[a]	0.3 ± 0.1[a]	1.4 ± 0.5[a]	0.5 ± 0.1[a]
D1PR	12.1 ± 1.3[a]	47.9 ± 4.3[b]	106.3 ± 7.3[b]	40.1 ± 6.7[b]	24.6 ± 2.0[b]	1.6 ± 0.2[b]	0.1 ± 0.0[b]	0.7 ± 0.2[b]	0.2 ± 0.0[b]
D2PR	12.9 ± 0.9[a]	86.2 ± 8.7[b]	162.9 ± 7.4[b]	68.0 ± 9.3[a]	39.1 ± 1.8[b]	2.9 ± 1.2[a]	0.2 ± 0.0[a]	1.1 ± 0.5[a]	0.5 ± 0.2[a]
D4PR	14.1 ± 2.1[a]	146.4 ± 7.3[a]	285.6 ± 13.9[a]	97.7 ± 8.8[a]	67.3 ± 3.3[a]	3.9 ± 0.2[a]	0.4 ± 0.1[a]	1.5 ± 0.4[a]	0.6 ± 0.1[a]
LM1	13.9 ± 1.4[a]	96.6 ± 10.7[a]	217.1 ± 15.8[a]	80.0 ± 14.7[a]	48.7 ± 9.3[a]	3.3 ± 0.6[a]	0.2 ± 0.1[a]	1.0 ± 0.2[a]	0.4 ± 0.1[a]
LM2	13.7 ± 1.7[a]	89.3 ± 10.5[b]	139.1 ± 9.0[b]	50.6 ± 11.1[b]	35.7 ± 8.0[b]	2.3 ± 0.6[b]	0.1 ± 0.0[b]	1.0 ± 0.2[a]	0.3 ± 0.0[b]
LM4	13.4 ± 1.3[a]	106.9 ± 4.2[a]	215.6 ± 9.4[a]	81.3 ± 10.2[a]	48.3 ± 1.5[a]	2.2 ± 0.2[b]	0.2 ± 0.0[a]	0.9 ± 0.2[a]	0.4 ± 0.1[a]
WA1	14.7 ± 1.0[a]	90.6 ± 8.7[b]	202.3 ± 11.7[a]	75.3 ± 9.6[a]	42.9 ± 9.5[a]	2.8 ± 1.0[a]	0.2 ± 0.0[a]	1.1 ± 0.3[a]	0.4 ± 0.0[a]
WA2	13.3 ± 1.3[a]	122.1 ± 1.2[a]	262.9 ± 19.2[a]	89.9 ± 1.7[a]	62.1 ± 1.4[a]	3.2 ± 0.2[a]	0.2 ± 0.0[a]	1.2 ± 0.1[a]	0.5 ± 0.0[a]
WA4	13.8 ± 2.3[a]	124.0 ± 12.8[a]	281.0 ± 17.7[a]	90.4 ± 2.5[a]	58.0 ± 9.3[a]	3.2 ± 0.3[a]	0.2 ± 0.0[a]	1.3 ± 0.1[a]	0.6 ± 0.1[a]
D1	13.7 ± 1.1[a]	82.7 ± 13.3[b]	163.1 ± 8.4[b]	76.7 ± 7.9[a]	40.6 ± 6.2[b]	2.4 ± 0.4[b]	0.2 ± 0.1[a]	1.1 ± 0.2[a]	0.4 ± 0.1[a]
D2	15.0 ± 1.6[a]	134.3 ± 11.4[a]	279.8 ± 16.3[a]	98.2 ± 11.2[a]	63.4 ± 4.3[a]	3.6 ± 0.2[a]	0.3 ± 0.0[a]	1.3 ± 0.3[a]	0.6 ± 0.0[a]
D4	15.4 ± 1.1[a]	108.8 ± 28.0[a]	164.9 ± 17.2[b]	82.3 ± 17.5[a]	51.5 ± 26.3[a]	4.2 ± 0.1[a]	0.2 ± 0.0[a]	1.2 ± 0.3[a]	0.4 ± 0.2[a]
Control	12.3 ± 1.6[a]	59.1 ± 5.2[b]	116.8 ± 4.7[b]	45.6 ± 6.2[b]	29.8 ± 3.7[b]	1.5 ± 0.1[b]	0.2 ± 0.0[a]	1.0 ± 0.2[a]	0.3 ± 0.1[b]
Reference[1]	23.58	407	1406	143	140	8.2	0.95	2.79	1.02

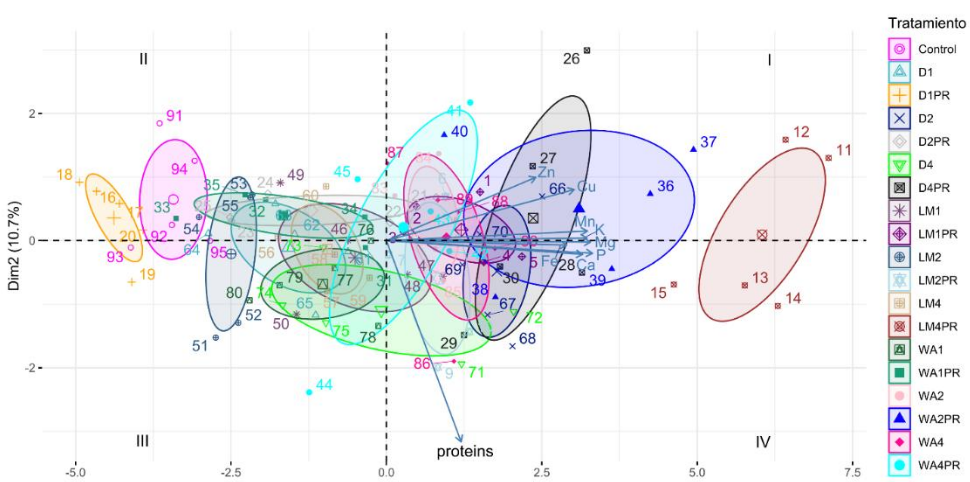

Figure 2. Principal component analysis for the nutrients evaluated in *Phaseolus vulgaris*. LM: Lime; D: dolomite; WA: wood ash; PR: phosphate rock; 1: 1 t ha^{-1}; 2: 2 t ha^{-1}; 4: 4 t ha^{-1}.

Cost-effectiveness of the amendments on bean cultivation

Dolomite was the most expensive amendment, followed by lime, and wood ash was the least expensive amendment (Table 6). Treatments WA1 and WA2 demonstrated cost-effectiveness over the control. The application of the WA1 treatment translates into an additional income of US$369.3 per hectare for the producer.

Table 6. Economic profitability of lime, ash, and dolomite amendments associated with the yield of *Phaseolus vulgaris.* LM: Lime; D: dolomite; WA: wood ash; PR: phosphate rock; 1: 1 t ha^{-1}; 2: 2 t ha^{-1}; 4: 4 t ha^{-1}; US$1.00-US$19.24 (as of 22 June 2025).

Treatment	Gross income	Production cost	Profitable return	Profitability *vs*. control
	US$	US$	US$	US$
Control	1500.9	104.3	1396.6	
LM1PR	1381.1	184.4	1196.7	-199.9
LM2PR	1376.4	359.1	1017.2	-379.4
LM4PR	1335.3	708.6	626.7	-769.9
WA1PR	1635.3	61.8	1573.6	177.0
WA2PR	1288.8	114.0	1174.9	-221.7
WA4PR	1580.4	218.3	1362.1	-34.5
D1PR	1471.3	531.3	940.0	-456.6
D2PR	1700.9	1052.9	648.0	-748.6
D4PR	1471.3	2096.2	-624.9	-2021.5
LM1	1550.1	174.8	1375.3	-21.3
LM2	1569.6	349.5	1220.1	-176.5
LM4	1560.9	699.0	861.8	-534.8
WA1	1818.1	52.2	1765.9	369.3
WA2	1663.2	104.3	1558.9	162.3
WA4	1577.7	208.7	1369.0	-27.6
D1	1525.8	521.6	1004.2	-392.4
D2	1540.6	1043.3	497.4	-899.2
D4	1500.9	2086.6	-585.7	-1982.3

DISCUSSION

Variation on growth variables and photosynthetic pigments

Variations in bean plant height are related to differences in the response to soil management practices, particularly regarding the use of amendments and nutrients. The application of dolomite plus phosphate rock (PR) produced significant increases in plant height, stem diameter, and chlorophyl *a/b* ratio (Chl *a/b*). These effects are attributed to Mg enrichment and the increase in soil pH, which improved the availability and absorption of nutrients; greater nutrition by plants is reflected in more vigorous growth (Minardi et al., 2021). Plant height is a significant variable because it directly affects yield components (Etana and Nebiyu, 2023). In contrast to the control, Chl *a/b* was associated with leaf biomass; this finding differs from Li et al. (2024), who associated better growth and yield when Chl *a/b* was low. Likewise, a higher Chl *a/b* is also related to the adaptation of plants to high luminosity (Lichtenthaler and Buschmann, 2001).

The higher number of pods produced in the flowering stage in lime-amended treatments was associated with the yield obtained, which decreased as the dose increased. This is most likely due to excessive soil scaling, which affects the availability of essential nutrients such as Zn and B (Olego et al., 2021). In contrast Etana and Nebiyu (2023) obtained a higher number of pods in bean plants grown in lime-amended soil at 4.8 t ha^{-1}. Leaf biomass was affected by the availability of nutrients such as K, which improves photosynthesis rates and results in higher total dry biomass (Huang et al., 2023). Wood ash provided higher K concentrations than the other amendments, which led to a 47% increase in leaf biomass compared to the control. This increase is consistent with Baloch et al. (2024), who reported a higher leaf biomass grown in soil amended with wood ash.

Effect of amendments on nutrients in the flowering stage

In contrast to the findings reported in other studies, the application of the amendments did not consistently improve leaf nutrient absorption in the flowering stage, except for P, which increased with dolomite amendment (D2PR). Although P concentration did not reach the sufficiency indicated by Jones (2012), it increased compared to the control. Low P availability may be associated with P fixation by iron and aluminum oxides and hydroxides on the soil surface. Therefore, the increase in P observed in the present study is indicative of the positive effect of dolomite in acidic soils associated with improved growth and yield.

Regarding the low concentrations of K, N, S, Cu, and Zn, according to Jones (2012), the higher Ca accumulation in leaf tissue may have limited the concentration of K due to an antagonistic effect (Xie et al., 2021). Higher K accumulation stimulates plant growth and improves development because it participates in the activation of more than 60 enzymes (Johnson et al., 2022). Separately, N and S act synergistically; S deficiency in leaf tissue and soil limited N availability (Zenda et al., 2021). Maintaining adequate S levels leads to higher bean yields and improved quality, as the crop demands large amounts of S due to its high protein content. Regarding the low concentrations of Cu and Zn, their availability can increase with foliar applications (Kachinski et al., 2020). Therefore, these nutrients should be considered for crop management in acidic soils to avoid asymptomatic deficiency conditions.

In contrast, the Ca, Mg, Fe, and Mn levels recorded in this study were within the reference values according to Jones (2012) despite the nutritional imbalance of Ca and Mg observed in the soil. The application of lime and PR (LM4PR) produced 2.5- and 1.3-fold increases Fe (355 mg kg^{-1}) and Mn (73.8 mg kg^{-1}), respectively, relative to previously reported values (Fe = 139.8 mg kg^{-1}, Mn = 56.6 mg kg^{-1}; Kachinski et al., 2020). This increase demonstrated the effectiveness of lime amendment on Fe and Mn levels in the plant.

Effect of amendments on pH, bean quality and yield

In this study, the three amendments neutralized the slightly acidic soil to values close to neutral (7.0), which promotes optimal growth of *Phaseolus vulgaris*. This is attributed to the alkaline reaction of the amendments that contribute OH^- ions. Agronomic practices such as the application of amendments are known to improve seed quality attributes, including width, length, and weight (Etana and Nebiyu, 2023). In the present study, the amendments did not improve seed attributes, except for lime, which increased seed width but not weight. Based on width, bean seeds are considered medium-sized, falling within the previously reported range of 6.1 to 11.3 mm (Espinosa-Pérez et al., 2015). Larger seeds mean larger reserves during germination and better consumer acceptance for use in salads.

The yield was influenced by a set of parameters, including soil fertility management practices that involve the use of amendments. Our results showed no differences between treatments with the use of the amendments. However, the application of wood ash (WA) and PR (WA1PR) increased the yield 1.85 times relative to non-amended acidic soils (1290.8 kg ha^{-1} *vs*. 650 kg ha^{-1}, respectively) (Tosquy-Valle et al., 2019). Phosphate rock did not impact yield when combined with the amendments, likely due to the low dissolution of PR at higher pH levels. At an application level of 15.5 kg P ha^{-1}, the solubility of PR is very low; additionally, if calcium phosphate is formed, it is also poorly soluble, resulting in antagonism in P availability for the plant. This outcome may be related to the gradual and long-term release of P. It is suggested to apply higher PR levels to better observe their effect on yield in bean crops. Alternatively, combining them with soluble P may be an effective strategy to improve bean nutrition in P-deficient soils (Coelho and Resende, 2023).

Effect of nutrient amendments on bean seeds

The low concentrations of N, P, and K in the seed indicated that the deficiency of these nutrients in the soil had a significant impact on seed development. The deficiency in Ca and Mg is attributed to the low mobility of these nutrients in the phloem, which prevents their translocation in the seeds (Maillard et al., 2015). The application of LM4PR increased the concentration of P, K, Ca, Mg, Fe, and Mn relative to the control. Previous studies have also found that liming increased P in bean seeds (Etana and Nebiyu, 2023). Similar concentrations of 100.1, 113.7, 4.8 and 1.1 mg 100 g^{-1} have been reported for Ca, Mg, Fe and Mn, respectively (Espinoza-García et al., 2016). It is worth mentioning that K, Ca, Mg, Fe and Mn concentrations showed an even greater difference compared to the control, demonstrating the positive effect of the applied treatment in improving the nutritional quality of the bean seed. Regarding Cu and Zn, there were no differences between treatments, likely due to factors such as the dose applied or a baseline Cu deficiency in the soil. Other studies have also reported no differences in Cu content in *P. vulgaris* seeds with the application of different amounts of lime to the soil (Rosemary et al., 2020).

PCA in bean seeds and profitability

The results of the PCA suggest that treatments LM4PR, WA2PR, and dolomite plus PR (D4PR) favor the accumulation of nutrients: P, K, Ca, Mg, Mn, Fe, Cu and Zn in bean seeds due to the neutralization of acidity with the application of the amendments and PR. These results confirmed the effectiveness of the three amendments at doses of 4 t ha^{-1} (lime), 2 t ha^{-1} (wood ash), and 4 t ha^{-1} (dolomite). In contrast, lower doses were insufficient to promote adequate accumulation of nutrients in the seed. Although amendments may improve seed quality in terms of nutrient concentration, inadequate doses may have a limited effect, mainly due to the ionic interaction of nutrients. For example, the use of lime in the soil resulted in higher leaf concentrations of P, Ca (Domingues et al., 2016), Mg, K, Fe and Mn; however, Cu and Zn availability did not increase, which can lead to a nutritional disorder in certain crops. While Jian et al. (2025) showed that wood ash increases the concentration of P, K, Ca and Mg and reduces the concentration of Zn and Mn in the soil.

Wood ash at 1 and 2 t ha^{-1} without PR application is a cost-affordable option for producers in the region who seek to increase bean yield and improve seed quality. Lime amendments are more expensive than wood ash, so the producer is advised not to select lime amendments to increase yield but to improve quality in terms of higher concentrations of macro and micronutrients in the seed. The use of dolomite is a suitable option for farmers who have sufficient resources, as its cost is higher than that of the previous amendments. However, the addition of nutrients should be balanced for optimum results.

CONCLUSIONS

The three amendments increased soil pH at the three doses evaluated. However, each of them provided different nutrients to the soil, affecting the growth, yield, and nutritional quality of *Phaseolus vulgaris*. Dolomite increased height, diameter, Chl *a/b* ratio, and P content in the plant during the flowering stage, which was associated with better seed quality. However, it is worth mentioning that its cost is high. Lime increased the number of pods but was ineffective in increasing yield. However, it improved concentrations of Fe and Mn during flowering stage, along with better seed quality in terms of seed width and concentrations of P, K, Ca, Mg, Fe, and Mn during physiological maturity. Ash increased leaf biomass during flowering and was the most affordable amendment to increase yield, also contributing to improve seed quality at 2 t ha^{-1}. Fertilization with phosphate rock did not influence the nutrition of either the plants or seeds of common bean.

Author contribution

Conceptualization: M.F.C., R.C.G., D.S.F.R. Methodology: M.F.C., D.S.F.R. Validation: D.H.D., H.M.O.E., J.L.G.R. Formal analysis: M.F.C., R.C.G. Investigation: M.F.C., D.S.F.R. Resources: R.C.G., D.S.F.R. Data curation: D.H.D., H.M.O.E., J.L.G.R. Writing-original draft: M.F.C., R.C.G. Writing-review & editing: D.S.F.R., A.E.R.S., J.E.C.S. Visualization: A.E.R.S. Supervision: D.S.F.R. All co-authors reviewed the final version and approved the manuscript before submission.

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