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Research Article

Co-inoculation of *Bradyrhizobium lupini* and *Azospirillum brasilense* Enhances the Agronomic Performance of *Lupinus mutabilis* Sweet in Two Agroecological Zones of Southern Peru

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ABSTRACT

Lupinus mutabilis Sweet (tarwi) is a high-protein Andean legume adapted to marginal environments; however, its productivity is often limited by frost and poor agricultural management practices in high-altitude regions. Plant growth-promoting rhizobacteria (PGPR) offer a promising approach to enhance growth, nitrogen fixation, and yield under stressful conditions. The present study evaluated the effect of co-inoculation with *Bradyrhizobium lupini* and *Azospirillum brasilense* on growth, root development, yield components, and grain quality of two tarwi ecotypes, Yunguyo (270-day cycle) and Andenes (180-day cycle), cultivated under field conditions. Results showed that co-inoculation significantly improved shoot growth parameters, including plant height, biomass accumulation, and stem diameter, compared with uninoculated controls. Root development was also markedly enhanced, with increases in root length, nodule number, and root and nodule dry weight, indicating improved biological nitrogen fixation and nutrient uptake. Reproductive traits such as pod length, pod number, stem number, and pod weight with seeds were significantly higher in inoculated plants. Grain yield components also improved substantially, with increases in seed weight, grains per pod, and overall yield. Yield gains reached up to 167.5 kg/ha in the Yunguyo ecotype and exceeded 1000 kg/ha in the Andenes ecotype, which benefited from its shorter phenological cycle and reduced exposure to frost. Co-inoculation also promoted a positive trend in protein content, reflecting enhanced nitrogen assimilation. In conclusion, co-inoculation with *B. lupini* and *A. brasilense* is an effective strategy to enhance growth, yield, and nutritional quality of tarwi under high-altitude conditions, supporting its sustainable production.

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Introduction

Lupinus mutabilis Sweet, commonly known as “tarwi” or “chocho,” native to the Andean region of Peru, Bolivia, and Ecuador, is considered a superfood due to its high content of protein, essential fatty acids, peptides, carbohydrates, fiber, calcium, and iron,

surpassing any other lupine species (Guilengue et al., 2020; Rodríguez et al., 2023; MIDAGRI, 2024; Tian et al., 2024). In Peru, it is estimated that tarwi was domesticated approximately 1,500 years ago, based on findings of ceramics, textiles, and paintings on ceremonial vessels from the Tiahuanaco culture (500-1000 AD), as well as seeds found in tombs of the Nasca culture (100-500 BC) (MIDAGRI, 2021; AgroPerú, 2024). In 2021, Peru ranked eighth in the world in the production of this grain (MIDAGRI, 2023). However, *L. mutabilis* continues to be an undervalued crop due to its high alkaloid content and low yields (Gulisano et al., 2019). This problem is exacerbated by the presence of frost, which compromises its yield (Tapia, 2015).

A sustainable alternative to address these problems is the use of Plant Growth-Promoting Rhizobacteria (PGPR), such as *Azospirillum brasilense* and *Bradyrhizobium lupini*. *Azospirillum* promotes the production of phytohormones, which cause physiological and morphological changes in the plant, generating greater absorption of water and nutrients that help increase grain production (Sangoquiza-Ciza et al., 2024). *Bradyrhizobium,* a bacterium isolated and selected from *lupinus,* forms root nodules and fixes atmospheric nitrogen so that the plant can assimilate it and promote its growth, thereby increasing its productivity and grain quality (Ramírez-Bahena et al., 2016). Beyond these primary functions, PGPR also contribute to plant resilience by conferring tolerance to biotic and abiotic stress, improvement of soil fertility, production of antibiotics (Chamki et al., 2021; Msaadak et al., 2023; Kashyap et al., 2024). In addition, many PGPR synthesize secondary metabolites, lytic enzymes, and volatile organic compounds that suppress pests and pathogens through mechanisms such as antibiosis, parasitism, and modulation of plant immune responses (Harmsen et al., 2024). Among the most significant plant-protective strategies mediated by PGPR is the activation of Induced Systemic Resistance (ISR), a defense mechanism analogous to Systemic Acquired Resistance (SAR), but generally dependent on jasmonic acid and ethylene signaling pathways rather than salicylic acid (Sowmiya et al., 2025). ISR primes the plant immune system, enabling it to respond more rapidly and effectively to pathogen attack (Rabari et al., 2023).

The co-inoculation of *Azospirillum* and *Bradyrhizobium* represents a viable alternative to enhance the productivity of *lupinus*, protect the environment and ensure food security. Therefore, the objective of this study was to compare the effect of the co-inoculation of *B. lupini* and *A. brasilense* in Tarwi Andenes and Yunguyo ecotypes in relation to non-inoculated ones, evaluating the agronomic performance, yield and quality of the grain in two Peruvian localities.

Materials and Methods

Experimental site

The experimental fields were located in the department of Ayacucho, province of Huamanga, district of Chiara. The study was conducted during the 2020-2021 growing season. The site at Chupas, situated at an altitude of 3600 m (longitude -74.237; latitude -13.263), recorded maximum temperatures ranging from 15.47 to 21.94°C and minimum temperatures from -3.41 to 1.75°C, with relative humidity levels between 64.21% and 82.92% (NASA, 2026). The second site, Llachoccmayo, at an altitude of 3800 m (longitude -74.221; latitude -13.401), registered maximum temperatures between 15.47 and 22.24°C and minimum temperatures ranging from -3.41 to 1.75°C, with relative humidity ranging from 64.21% to 82.92% (NASA, 2026). During late summer and early autumn (February, March, and April), minimum temperatures of -0.80°C, -0.75°C, and -0.35°C were recorded, respectively, with relative humidity ranging from 78% to 83%. Subsequently, during the transition from autumn to winter (May, June, and July), minimum temperatures reached -0.51°C, 0.27°C, and -3.41°C, with relative humidity between 69% and 75%. These thermal and humidity conditions favored the occurrence of frost events during these periods.

Regarding precipitation, an average of 600 mm was recorded, according to data from the Allpachaka meteorological station that belongs to the Universidad Nacional de San Cristóbal de Huamanga.

Soil sampling for physicochemical analysis

In the experimental areas, 20 subsamples were collected per site following a zigzag sampling pattern. These subsamples were combined to form a composite sample, from which 1000 g were taken and sent to the AGROLAB S.A.C. laboratory for analysis.

Physicochemical characterization of agricultural soil and fertilization

The physicochemical characterization of the experimental field in Chupas indicated a sandy loam texture, moderate organic matter content (1.26%), a moderately acidic pH (5.86), and a very slightly saline condition (0.33 dS/m). Total nitrogen (Nt) was low (0.23%), available phosphorus

(P) was very high (39.3 ppm), and potassium (K) was also high (360 ppm). The cation exchange capacity (CEC) was moderate, 13.78 Cmol(+)/kg, with calcium (Ca++) in good quantity (8.03 Cmol(+)/kg), magnesium (Mg++) at adequate levels (2.84 Cmol(+)/kg), potassium (K+) balanced (0.67 Cmol(+)/kg), sodium (Na+) very low (0.12 Cmol(+)/kg), and aluminum (Al+++) absent (0.00 Cmol(+)/kg). Base saturation was high (85%).

In contrast, the experimental site in Llachoccmayo also showed a sandy loam texture and a moderate organic matter content (1.48%), with a moderately acidic pH (6.0), very slightly saline (0.41 dS/m), very low total nitrogen (0.07%), very high phosphorus (46.1 ppm), and high potassium (340 ppm). The cation exchange capacity (CEC) was 14.5 Cmol(+)/kg, with calcium (Ca++) in good quantity (9.76 Cmol(+)/kg), adequate Mg++ (2.67 Cmol(+)/kg), balanced K+ (0.74 Cmol(+)/kg), very low Na+ (0.14 Cmol(+)/kg), and aluminum (Al+++) absent (0.00 Cmol(+)/kg), with a high base saturation of 92%.

Based on these values and the expected yield, base fertilization consisted of the application of 100 kg/ha of poultry manure, 50 kg/ha of compound fertilizer (15N-25P_2O_5-15K_2O-2MgO-3S), 100 kg/ha of diammonium phosphate (DAP), and 2 kg/ha of sulfur flower in each experimental area.

Tarwi seeds ecotypes: Andenes and Yunguyo

Two seed ecotypes of tarwi were selected, provided by the Company Solid Perú. Their selection was based on adaptability and potential yield in the region.

Ecotype Andenes

Sourced from the Experimental Center "Andenes" INIA-Cusco, this ecotype produces light pink flowers and large, round, white-colored grains. It is early-maturing and can yield up to 2.5 t/ha (Garay, 2015; Tapia, 2015).

Ecotype Yunguyo

Originating from the Puno region and released by INIA, this ecotype is characterized by blue flowers and round, white-colored grains. It has a late maturity and an average yield of 1.8 t/ha (Garay, 2015; Tapia, 2015).

Inoculants *Bradyrhizobium lupini* (Bl) and *Azospirillum brasilense* (Ab)

The inoculants used were developed under the Institutional Strategic Project titled "Strengthening and Implementation of Research Capacity on PGPR Microorganisms in the AD-305 and AD-306 Laboratories of the School of Agronomy", as part of the agreement FOCAM-Universidad Nacional de San Cristóbal de Huamanga (Ayacucho, Peru).

For inoculation, a peat-based formulation of *B. lupini* was applied at a rate of 200 g/ha with a concentration of 1×10^9 CFU/g, and a liquid formulation of *A. brasilense* was applied at 150 ml per 35 kg of tarwi seeds per hectare, with a concentration of 1×10^8 CFU ml.

Experimental design and plot distribution

A randomized complete block design (RCBD) was used across two localities, with six replicates, two treatments, and two ecotypes. Experimental units were arranged in plots, totaling 48 experimental units. Seeds were sown with 0.80 m spacing between rows and 0.3 m between hills, with 2 seeds per planting hole. Each plot consisted of 10 rows, each 9 m long, resulting in a net plot area of 81 m^2 per experimental unit.

Agronomic management

Land preparation, harrowing, and plot marking were conducted in October 2020. The selected tarwi seeds showed a germination rate of 94% and high purity. Seeds were weighed and inoculated using the pelletization method, with recommended doses of *B. lupini* and *A. brasiliense* inoculants. The adhesive solution was 40% gum arabic, and the coating material was 18 kg/ha of dolomite.

Fertilization was carried out based on soil analysis and expected yields, using 200 g of seeds per plot.

Hilling was performed 1.5 months after sowing, which also helped with weed control. Pest control occurred between January and February using organic agriculture-approved products. For *Agroti*s sp., a nuclear polyhedrosis virus was used as the active ingredient, and for damping off fungal disease, copper sulfate pentahydrate was applied. Irrigation was not required due to adequate rainfall ensuring optimal plant development. Harvesting took place once plants reached physiological maturity. After, drying and threshing were performed to ensure grain quality.

Evaluation methodology

To assess the effect of Bl + Ab, six complete plants (shoot and root systems) were selected per locality and ecotype, both inoculated and uninoculated, according to the appropriate phenological stage.

The onset of flowering was identified as the optimal stage to measure inoculation effects on shoot traits (plant height, fresh and dry shoot weight, stem diameter) and root traits (root length, fresh and dry root weight). Nodulation parameters, such as number of nodules and fresh and dry nodule weight, were also assessed as key indicators of inoculation efficiency,

following standard protocols for inoculant trials (García-Blásquez and Sato, 2019).

In addition, pod length and number per plant, number of stems per plant, pod weight with grains per plant, grain yield, grain weight per plant, grain number per pod, 100-grain weight, total nitrogen content, and crude protein content (in percentage) were measured once the plants completed their vegetative cycle.

Evaluated variables

Height (cm)

Height was measured using a measuring tape, from the base of the plant collar to the terminal bud.

Number of stems and pods per plant, and number of grains per pod

The parameters were counted manually by selecting plants randomly.

Fresh and dry shoot weight and dry root weight (g)

A cut was made at the base of the plant collar, separating the shoot from the root system. Using a precision balance (BOECO model BPS40PLUS), the weight of the shoot part of each plant was recorded. Subsequently, the experimental units were placed in kraft paper bags and dried in an oven (MENMERT model UN55) at 80°C for three days until a constant weight was achieved, allowing for the determination of shoot and root dry weight.

Stem diameter (mm)

Using a vernier caliper (Mitutoyo, with an accuracy of ±0.0001″), the stem diameter was measured 5 cm above the plant base.

Root length (cm)

Root length was measured using a measuring tape, taking the distance from the plant collar to the apical end of the main root.

Number of nodules

Nodules located on the main root (primary nodules) and lateral roots (secondary nodules) were counted manually.

Nodule dry weight (g)

Nodules from each experimental unit were weighed using a precision balance (BOECO model BPS40PLUS), placed in kraft paper bags, and dried in an oven (MENMERT model UN55) at 50°C for two days until a constant weight was reached.

Pod length (cm)

Measurements were taken using a graduated ruler.

Pod weight (g) with grains per plant, grain weight (g) per plant, and 100-grain weight (g)

All samples were weighed using a precision balance (BOECO model BPS40PLUS).

Yield (kg/ha)

Grain yield was calculated using a platform scale, based on the harvested grains from the usable plot area. Results were expressed in Kg/ha once plants reached physiological maturity.

Total nitrogen (%) and protein content (%)

Grains previously dried to a moisture content of 12-13% were ground, bagged, labeled, and sent to the Agricultural Chemistry Laboratory, Valle Grande, Cañete, Lima for total nitrogen determination using the MEF-001 method and the Dumas technique. Protein content was calculated by multiplying the total nitrogen percentage by the standard conversion factor of 6.25 (Krul, 2019).

Statistical analysis

A 2 × 2 factorial experiment was conducted under a Randomized Complete Block Design (RCBD). The two factors were: treatment (uninoculated and co-inoculated) and ecotype (Yunguyo and Andenes), resulting in four treatment combinations with six blocks. The experiment was replicated in two locations (Chupas and Llachoccmayo). A two-way combined ANOVA was performed across both sites, followed by Tukey's test for multiple comparisons. Statistical analyses were carried out using Infostat software version 2008 (Di Rienzo et al., 2008), with significance considered at $p \leq 0.05$.

Results and Discussion

During the study period, sub-zero temperatures were recorded, especially in late summer, autumn, and winter (NASA, 2026), which limited open-field agriculture. The Andenes ecotype (180 day cycle) experienced minimum temperatures of -0.80 °C, -0.75 °C, and -0.35 °C (82.92%, 80.92%, and 78.61% relative humidity, respectively) during the grain-filling and maturation stages. Over the same timeframe, the Yunguyo ecotype (270 day cycle), characterized by late maturation, was already established and in the vegetative stage; therefore, these low temperatures could have affected its early development. Subsequently, the grain was exposed again to frost (-0.51°C, 0.27°C, and -3.41°C; 75.07%, 71.36%, and 69.24% relative humidity) during grain filling and maturation. This sequential exposure suggests cumulative effects of frost, supporting its negative influence on production parameters (Tapia, 2015).

Effect of co-inoculation on shoot parameters in *L. mutabilis* Sweet plants

Table 1 showed that tarwi plants of the Yunguyo and Andenes ecotypes inoculated with Bl + Ab exhibited

significantly greater growth and development than uninoculated plants ($p \leq 0.05$). Inoculation improved plant height (up to 57 cm), biomass accumulation (up to 915 g), and stem diameter (up to 3.67 mm) in both locations, confirming the positive impact of PGPR on shoot development. These improvements are consistent with the ability of these bacteria to stimulate root hair formation and nodulation, thereby facilitating nitrogen fixation and increasing physiological efficiency (Kunert et al., 2016). Furthermore, the strains demonstrated phosphate solubilization, auxin and siderophore production, and high nitrogen fixation efficiency, all of which contribute to improved plant growth (Chaddad et al., 2023). These findings align with previous studies on lupin (Benintende et al., 2010; Sulewska et al., 2019; Sarmiento et al., 2020; Monroy-Guerrero et al., 2022;), which highlight the synergistic effect of co-inoculation, positively impacting the evaluated parameters.

Table 1. Shoot characteristics of tarwi (*L. mutabilis*) ecotypes inoculated with Bl + Ab, and uninoculated, evaluated in the locations of Chupas and Llachoccmayo.

Treatment	Height (cm)	Fresh weight (g)	Dry weight (g)	Stem diameter (mm)
Ecotype Yunguyo at Chupas site				
Uninoculated	122.50 cd	381.67 de	65.33 c	5.83 f
Inoculated	168.83 a	1161.47 b	172.67 b	9.29 bc
Ecotype Yunguyo at Llachoccmayo site				
Uninoculated	100.00 e	369.33 de	69.17 c	6.67 ef
Inoculated	133.33 bc	678.33 cd	170.17 b	8.67 bcd
Ecotype Andenes at Chupas site				
Uninoculated	109.50 de	297.00 e	61.00 c	8.00 cde
Inoculated	166.50 a	1195.00 b	193.83 b	11.67 a
Ecotype Andenes at Llachoccmayo site				
Uninoculated	105.50 de	703.00 c	90.83 c	7.17 def
Inoculated	143.00 b	1618.33 a	256.00 a	10.00 ab

Different letters in columns indicate significant differences ($p \leq 0.05$).

Effect of co-inoculation on root development parameters in *L. mutabilis* plants

Table 2 showed that inoculating the Yunguyo and Andenes ecotypes with Bl + Ab significantly promoted root and nodule development compared to uninoculated plants ($p \leq 0.05$). Root length increased by over 20 cm, while nodule numbers rose more than fivefold, along with higher dry weights of roots and nodules, indicating greater underground biomass. Clear differences in root nodulation between inoculated and non-inoculated plants are evident in Figure 1. These improvements demonstrate how *Bradyrhizobium* and *Azospirillum* stimulate root system expansion and nodulation, thereby improving nitrogen fixation. *Azospirillum* produces auxin, a key factor in promoting root growth and density, which aids water and nutrient uptake (Prassad et al., 2019; Licea-Herrera et al., 2020). Its interaction with *Rhizobium* further enhances biological nitrogen fixation and nodule formation (Gadal, 1997). Moreover, *Azospirillum* has been shown to improve plant resistance under water stress (Cassan and García de Salamone, 2008; Osses et al., 2017). This beneficial activity could be crucial, as it has been widely demonstrated that water and heat stress are among the primary limiting factors for biological nitrogen fixation in the tropics, significantly affecting root infection, nodule formation, and nitrogen fixation capacity (Hungria et al., 2015). These findings align with previous studies on lupin and soybean, where co-inoculation notably increased nodulation and nodule biomass (Hungria and Mendes, 2015; Sulewska et al., 2019; Bais et al., 2023; da Silva et al., 2024; Prando et al., 2024), emphasizing the synergistic effect of combining *Bradyrhizobium* and *Azospirillum*.

Effect of co-inoculation on yield component traits in *L. mutabilis* plants

Table 3 showed that inoculation with *B. lupini* and *A. brasilence* (Bl + Ab) significantly enhanced pod formation and reproductive traits in both Yunguyo and Andenes

ecotypes compared to uninoculated plants ($p \leq 0.05$). In Yunguyo, pod length reached 7.88 cm and the number of pods more than doubled, accompanied by increases in stem number and pod weight with seeds. In Andenes, the effects were even more pronounced, with pod length exceeding 8.4 cm, pod number doubling, and pod weight increasing by over 70 g, almost twice that of the controls. The differential response between ecotypes is explained by climatic adversity during grain filling in Yunguyo, which has a longer phenological cycle (270 days) and is thus exposed to frost and drought, whereas Andenes, with an earlier maturity (180 days), completes reproductive development before these stress events. Despite these abiotic challenges, inoculation proved effective in enhancing reproductive performance, likely due to the ability of microbial bio-stimulants to promote growth and activate tolerance mechanisms to stress, improving nutrient uptake and overall plant health (Msaadak et al., 2023; Kashyap et al., 2024). These findings are consistent with previous studies on this group of legumes, where co-inoculation significantly increased pod set and yield (Sulewska et al., 2019; Villafañe et al., 2024; da Costa et al., 2025), underscoring the synergistic effect of combining *Bradyrhizobium* and *Azospirillum.*

Table 2. Belowground characteristics (roots and nodules) of Yunguyo and Andenes tarwi ecotypes, with and without Bl + Ab inoculation, evaluated in Chupas and Llachoccmayo.

Treatment	Root length (cm)	Number of Nodules	Nodules dry weight (g)	Root dry weight (g)
Ecotype Yunguyo at Chupas site				
Uninoculated	16.83 b	65.33 c	0.57 d	10.35 e
Inoculated	44.83 a	439.33 a	3.88 ab	36.40 ab
Ecotype Yunguyo at Llachoccmayo site				
Uninoculated	16.67 b	65.00 c	0.57 d	11.67 e
Inoculated	39.00 a	343.33 b	5.00 a	46.17 a
Ecotype Andenes at Chupas site				
Uninoculated	19.17 b	38.33 c	0.58 d	7.92 e
Inoculated	46.83 a	373.83 ab	2.47 c	22.50 cd
Ecotype Andenes at Llachoccmayo site				
Uninoculated	23.00 b	58.33 c	0.58 d	18.00 de
Inoculated	40.67 a	363.00 ab	3.00 bc	29.17 bc

Different letters in columns indicate significant differences ($p \leq 0.05$).



Figure 1. Nodulation of *L. mutabilis* at the flowering stage. (A) Control and Andenes; (B) Control and Yungugo.

Table 3. Yield component traits of Yunguyo and Andenes ecotypes of tarwi co-inoculated with Bl + Ab compared to non-inoculated plants, evaluated in the locations of Chupas and Llachoccmayo.

Treatment	Pod length (cm)	Number of pods per plant	Number of stems per plant	Weight of pods with grains per plant (g)
Ecotype Yunguyo at Chupas site				
Uninoculated	7.32 b	36.17 d	11.50 c	75.47 cd
Inoculated	7.88 ab	84.17 b	18.00 b	87.83 bc
Ecotype Yunguyo at Llachoccmayo site				
Uninoculated	6.33 c	37.17 d	13.17 c	65.00 d
Inoculated	7.83 ab	87.50 b	19.00 ab	92.00 b
Ecotype Andenes at Chupas site				
Uninoculated	7.84 ab	54.50 c	11.50 c	81.67 bc
Inoculated	8.41 a	116.50 a	21.77 a	154.00 a
Ecotype Andenes at Llachoccmayo site				
Uninoculated	7.33 b	56.33 c	11.50 c	82.33 bc
Inoculated	8.42 a	115.00 a	22.17 a	154.00 a

Different letters in columns indicate significant differences ($p \leq 0.05$).

Effect of co-inoculation on grain yield parameters in *L. mutabilis* plants

In Table 4, grain yield and yield components showed significant improvements ($p \leq 0.05$) in plants inoculated with Bl + Ab compared to uninoculated controls. In the Yunguyo ecotype, inoculation enhanced seed weight, grains per pod, and yield, with increases of up to 167.5 kg/ha, while in the Andenes ecotype, the effects were more pronounced, with yield gains exceeding 1000 kg/ha and notable improvements in seed weight and grain production per plant. These results confirm the positive impact of PGPR co-inoculation on reproductive performance, particularly in Andenes, whose shorter maturity cycle (180 vs 270 days) reduced exposure to frost, a major limiting factor in tarwi production that often causes empty grains and yield losses (Tapia, 2015). Similar studies in lupin have reported yield increases of up to 36% due to co-inoculation (Ferchichi et al., 2019), and comparable evidence exists in soybean, where *Bradyrhizobium* and *Azospirillum* co-inoculation enhances nitrogen fixation, yield components, and grain quality, often eliminating the need for fertilization (Hungria et al., 2015; Queiroz et al., 2018; Zilli et al., 2020; Martín et al., 2023; da Silva et al., 2024). Yield increases of up to 80% have been documented (Prando et al., 2024), along with improvements in seed weight and grain yield index (Benintende et al., 2010; Bais et al., 2023). Therefore, the present study demonstrates that both tarwi ecotypes, particularly Andenes, benefit significantly from Bl + Ab inoculation, reinforcing the role of PGPR co-inoculation as a biotechnological strategy to improve seed quality and yield in Andean legumes.

Table 4. Grain yield and yield component traits of Yunguyo and Andenes ecotypes of tarwi co-inoculated with Bl + Ab compared to non-inoculated plants, evaluated in the locations of Chupas and Llachoccmayo.

Treatment	Weight of 100 seeds (g)	Number of grains per pot	Grain weight per plant (g)	Yield (kg/ha)
Ecotype Yunguyo at Chupas site				
Uninoculated	24.40 c	4.67 cd	39.50 b	2855.17 bc
Inoculated	31.68 a	5.29 abc	41.87 b	2929.17 b
Ecotype Yunguyo at Llachoccmayo site				
Uninoculated	23.92 c	4.50 d	38.17 bc	2749.00 c
Inoculated	29.47 a	5.67 a	40.33 b	2916.50 bc
Ecotype Andenes at Chupas site				
Uninoculated	28.57 ab	4.92 abcd	35.25 c	2445.67 d
Inoculated	31.20 a	5.58 ab	50.83 a	3574.17 a
Ecotype Andenes at Llachoccmayo site				
Uninoculated	24.67 bc	4.83 bcd	34.33 c	2474.83 d
Inoculated	30.99 a	5.67 a	50.83 a	3505.17 a

Different letters in columns indicate significant differences ($p \leq 0.05$).

As shown in Figure 2, co-inoculation with Bl + Ab exerted a positive influence on nitrogen assimilation and protein content, with increases recorded across all cases, although these differences were not statistically significant ($p \leq 0.05$). In ecotype Yunguyo at Llachoccmayo, the highest protein increment reached 1.05%, whereas in ecotype Andenes, the most notable effect was observed in Chupas, with a 3.6% increase. This general trend indicates that co-inoculation enhances nitrogen availability, thereby promoting protein synthesis in tarwi. Considering that proteins represent the costliest and often least accessible nutritional component in the human diet, plant-based sources are crucial despite their variability in composition depending on species, environment, and maturation stage (Sazonova et al., 2022). In another legume, Bais et al. (2023) reported that *Bradyrhizobium* alone achieved slightly higher protein yields compared to its co-inoculation with *A. brasilense*, a finding corroborated by Fávero et al. (2024), who noted no significant improvement in soybean nutritional status under co-inoculation. In contrast, the present study demonstrates that, for tarwi, particularly in ecotypes Andenes and Yunguyo, co-inoculation with Bl + Ab produced a consistent and biologically relevant positive effect on protein content, highlighting species-specific responses and the potential of microbial associations to enhance nutritional quality in Andean crops.

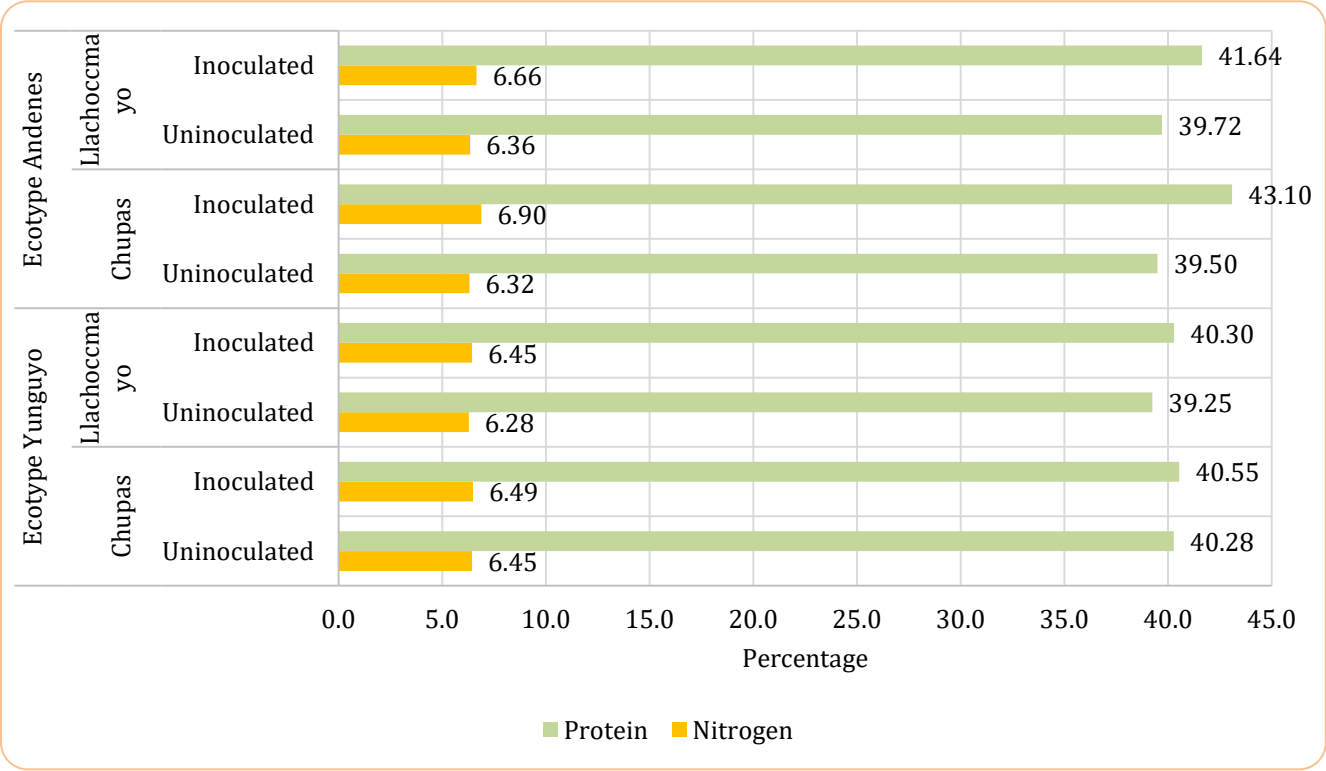


Figure 2. Total nitrogen and protein content in grains of *L. mutabilis* ecotypes (Yunguyo and Andes) in the locations of Chupas and Llachoccmayo ($p \leq 0.05$).

Conclusions

Plants of tarwi *L. mutabili* Sweet co-inoculated with the bacteria *B. lupini* and *A. brasilense* exhibited significantly superior shoot and root characteristics compared to uninoculated plants across both locations and ecotypes. Regarding yield, the Andenes ecotype performed significantly better at both sites, whereas Yunguyo did not show significant differences, likely due to the occurrence of frosts during the late vegetative cycle. As for seed quality, no statistically significant differences were observed; however, nitrogen and protein percentages were higher in inoculated plants compared to controls.

Based on these findings, co-inoculation with *B. lupini* and *A. brasilense* in tarwi represents an effective agronomic strategy to improve crop yield and quality. This practice not only enhances biological nitrogen fixation, but also

contributes to reducing agrochemical inputs, thus promoting more sustainable agriculture.

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Authors' Contribution
JSGBM and CGBM conceptualized and designed the study, and provided the inoculants. SMAR and CGBM conducted field surveys, acquired the seeds, carried out the investigation, and prepared the initial draft of the manuscript. SMAR and TARC compiled the dataset. TARC and JAQT contributed to data analysis, critical revision, and improvement of the manuscript structure and presentation. JSGBM and TARC supervised the execution of the research. All authors reviewed, corrected, and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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Conflict of Interest
The authors declare no conflict of interest.

Sustainable Development Goals Targeted
SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 13: Climate Action

Policy Addressed
This study aligns with global and national policy frameworks promoting climate-smart and sustainable agriculture, including the FAO Climate-Smart Agriculture strategy and Peru's National Agrarian Policy (MIDAGRI).

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