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**RESEARCH ARTICLE**
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# Combination of two bacterial strains *Bradyrhizobium* sp and *Bacillus* sp as Biofertilizer and Biocontrol in the Cultivation of Tarwi (*Lupinus mutabilis* Sweet) in the Peruvian Highlands

## Combinación de Cepas Bacterianas *Bradyrhizobium* sp. y *Bacillus* sp. como Biofertilizante y Biocontrolador en el Cultivo de Tarwi (*Lupinus mutabilis* Sweet) en la Región Andina del Perú

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### Abstract

It is very important to know adequately the association of beneficial microorganisms of soil with crops, in order to learn on its biofertilizer and biocontrol effects, which will allow to improve the growth and yield of the crops. The evaluation of the co-inoculation of bacteria that promote plant growth by different mechanisms in plants challenged by pathogens would contribute to increase the knowledge of the interactions of plants with different microorganisms. The aim of this research was to evaluate the biofertilizer and biocontroller effects through the bioinoculations of *Bradyrhizobium* and *Bacillus* spp. strains association. Both effects were measured during plant growing through the variable response against to Anthracnose disease on yield and seed nutrient content, using Altagracia variety. The experiment was carried out in Paltash locality at 3100 m.a.s.l. in the District of Marcará, Carhuaz, Ancash Region. Three treatments were tested: i) *Bradyrhizobium* + *Bacillus bioinoculants*, ii) a chemical treatment, and iii) a control (without bioinoculants or chemicals). The field experiment was carried out under randomized complete block design (RCBD) with four blocks. The following parameters were evaluated: *1*) The fresh and dry weight of the aerial part, root and nodules at 60 days; *2*) Anthracnose severity during flowering, pod filling and harvesting; and *3*) Seed nutrient content. Statistical analysis was performed using Duncan's test (95 % CI). Results obtained indicate that the bioinoculated plants presented the best nodule characteristics; likewise, bioinoculation reduced the degree of Anthracnose severity in the different phenological stages. It is concluded that bioinoculations improved tarwi growth and anthracnose control.

***Keywords:*** *Lupinus mutabilis*; *plant growth promoters; biocontrollers; biofertilizers; biostimulants*

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**Resumen**

Los rendimientos de los cultivos son importantes para la seguridad alimentaria y el nivel de vida de las personas, por lo que es muy importante conocer adecuadamente sobre la asociación de microorganismos con los cultivos, para conocer sus efectos biofertilizantes y biocontroladores, que permitirán el desarrollo de bacterias del suelo, colonizadoras de raíces. de las plantas después de la inoculación en las semillas y mejorar el crecimiento de las plantas. La evaluación de la co-inoculación de bacterias que promueven el crecimiento vegetal por diferentes mecanismos, en plantas afectadas por patógenos, contribuiría a aumentar el conocimiento las interacciones de las plantas con diferentes microorganismos. El objetivo de esta investigación fue evaluar los efectos biofertilizante y biocontrolador a través de las bioinoculaciones de *Bradyrhizobium* y *Bacillus* spp. asociación de cepas. Ambos efectos se midieron durante el crecimiento de la planta a través de la variable respuesta frente a la Antracnosis sobre el rendimiento y el contenido de nutrientes de la semilla, utilizando la variedad Altagracia. El experimento se llevó a cabo en la localidad de Paltash a 3100 m s.n.m. en el Distrito de Marcará, Carhuaz, Ancash, en el campo del pequeño agricultor. Se ensayaron tres tratamientos: i) bioinoculantes Bradyrhizobium + *Bacillus*, ii) un tratamiento químico, y iii) un testigo (sin bioinoculantes ni químicos). El experimento de campo se llevó a cabo bajo un diseño de bloques completos al azar (RCBD) con cuatro bloques. Se evaluaron los siguientes parámetros: 1) El peso fresco y seco de la parte aérea, raíz y nódulos a los 60 días; 2) severidad de la antracnosis durante la floración, llenado de vainas y cosecha; y 3) Contenido de nutrientes de la semilla. El análisis estadístico se realizó utilizando la prueba de Duncan (IC del 95 %). Los resultados obtenidos indican que las plantas bioinoculadas presentaron las mejores características de nódulos; asimismo, la bioinoculación redujo el grado de severidad de la Antracnosis en los diferentes estados fenológicos y provocó los valores más altos en contenido de nutrientes. Se concluye que las bioinoculaciones mejoran el crecimiento del tarwi y el control de la antracnosis.

***Palabras clave:*** *Lupinus mutabilis; promotores del crecimiento de las plantas; biocontroladores; biofertilizantes; bioestimulantes.*

## Introduction

The tarwi (*Lupinus mutabilis* Sweet), known as "Chocho" in the Ancash Region, which is cultivated in small areas mostly for self-consumption. The grains of this legume have a high nutritional value, e.g. among other components: protein, oil and fiber contents over 40 %, 13 % - 23 % and 7 % respectively (Camarena et al., 2012), Also, this crop is an important source of income for small farmers, which is growing with low inputs. The national harvest has remained stable at low yield (800 kg/ha - 1200 kg/ha) (Castañeda et al., 2008). In Ancash, the yield does not reach 50 % of this figure; due to the use traditional varieties with low productivity and nutritional value, susceptibility to diseases such as anthracnose (*Colletotrichum gloeosporoides*) (Falconi, 2012). Those constraints not only cause severe damage to the plant, but also increase production costs and damage the environment. A sustainable alternative for these problems is to use symbiotic bacteria such as *Bradyrhizobium*, which actively participates in biological nitrogen fixation (BNF) in legumes (Taco-Taype & Zúñiga-Dávila, 2020) and a biocontrol bacterium such as *Bacillus* for its biocidal activity that acts on different species of fungi phytopathogens (Sosa et al., 2005). The importance of BNF is due to agricultural soils with around 200 million tons of N per year (Shamseldin, 2022). Therefore, current farming systems need sustainable intensification by including leguminous crops (Kakraliya et al., 2018). For example, *Bacillus* B02 reduces the growth of phytopathogenic fungi through the production of antimicrobial peptides, siderophores, and hydrolytic enzymes such as cellulases and proteases that cause lysis of cell wall components (Memenza-Zegarra & Zúñiga-Dávila, 2019). At the field level, the same bacterium reduced the incidence of the disease caused by *Sclerotinia sclerotiorum* in common bean plants by 94 % (Memenza et al., 2016). The use of plant probiotics as an alternative source of soil fertilization has been the focus of several studies; its use in agriculture improves the supply of nutrients and preserves the management of the field and does not cause adverse effects (de Souza Vandenberghe et al., 2017). In another study on oat crop, Hashmi et al. (2019) found that a consortium of vegetative and endospore cells, successfully colonized the roots and rhizosphere of oat plants, without modifying the general structure of indigenous soil microbial communities.

The objectives of this study were to evaluate the effect of bioinoculations of *Bradyrhizobium* sp. and *Bacillus* sp. strains on fresh and dry weight of the aerial part of the plant, root and plant nodules at 60 days after sowing (DAS); the degree of severity according to the scale shown in (Table 1), caused by anthracnose at flowering, pod filling and at harvest; the seed yield components and its nutrient content of the variety Altagracia.

## Materials and methods

The study was carried out in the rural community of Vicos, Paltash locality, Marcará district, Carhuaz province, Ancash region; at an altitude of 3100 meters above sea level, with geographic coordinates of 9° 20 ‘ 6.2” S and 77° 32 ‘ 53.9” W (Figure 1). The genetic material was seeds of the variety “Altagracia Selection” from the “Programa de Investigación y Proyección Social de Leguminosas de Grano y Oleaginosas” of the “Universidad Nacional Agraria La Molina” (UNALM), and a co-inoculation of two *Bradyrhizobium strains* (LMHZ L8 and LMHZ L3) and a Bacillus B02 (BA) strain (Memenza et al., 2016) from the collection of the Laboratory of Microbial, Ecology and Biotechnology, Marino Tabusso-UNALM (Taco-Taype & Zúñiga-Dávila, 2020). The treatments used were:

i) *Bradyrhizobium* + *Bacillus* (C.Br+Ba), the bioinoculation of biofertilizers were at sowing time and reinoculation of biofertilizers and biocontrollers after sowing, additionally it was used in agronomic management, *Trichoderma harzianum*, *T. viride* and *T. asperellum*, *Bacillus thuringiensis* var. *kurstaki* and *Bacillus thuringiensis* var. *halotolerans*, for the control of pests and diseases.

ii) Chemical Treatment (Chem.trt), in this treatment also was used urea 45 % N as nitrogenous fertilizer and the pests and diseases control were using agrochemicals, which have as actives ingredients: Tebuconazole and Trifloxystrobin, Azoxystrobin and Difenoconazole.

iii) Control Treatment without bacteria or agrochemicals.

Those treatments were established in demonstration plots under a Randomized Complete Block Design with four blocks. The BR inoculum (CFU of *Bradyrhizobium* sp. were $1x10^8$ /mL) was applied to the seed (Zhao et al., 2013) before sowing and 30 days later on the neck of the seedlings. In addition, BA *Bacillus* ($1x10^8$ CFU/ml) was applied three doses of 15 ml, 30 ml and 60 ml, at 30 ml, 60 ml and 90 ml days after sowing (DAS) respectively; those treatments were made on the neck and leaves of the plant.

During the trial, a monthly average maximum temperature of 18.6 °C and a minimum of 5 °C were recorded. Soil characteristics were: sandy loam texture, strongly acid pH 4.40. The electrical conductivity of 0.25 dS/m which means free of salts, 1.03 ppm of organic matter (OM), indicating low proportion, low content of P (10.5 ppm) and medium content of K (198 ppm).

Data were recorded during flowering time, the fresh and dry weight of the aerial part, root and nodules of the plant and the degree of severity caused by anthracnose according to the scale expressed in Table 1. The yield and its components were recorded after harvesting and driying the seeds. The proximal analysis was carried out by “La Molina Calidad Total Laboratorios” - UNALM in order to know the nutritional value of the harvested seeds. The macro and micronutrient content of seeds were carried out by the Soil Laboratory – UNALM. The statistical analysis was performed with the R (R Core Team, 2022) and RStudio (Posit Team, 2022) Software Program and libraries “agricolae” (de Mendiburu, 2021) to perform the ANOVA and comparisons of means with 95 % CI, “FactorMineR” (Lê et al., 2008), “factoextra” (Kassambara & Mundt, 2020), “GGally” (Schloerke et al., 2021) and programming codes in R base using for graphs of descriptive statistics and multivariate analysis

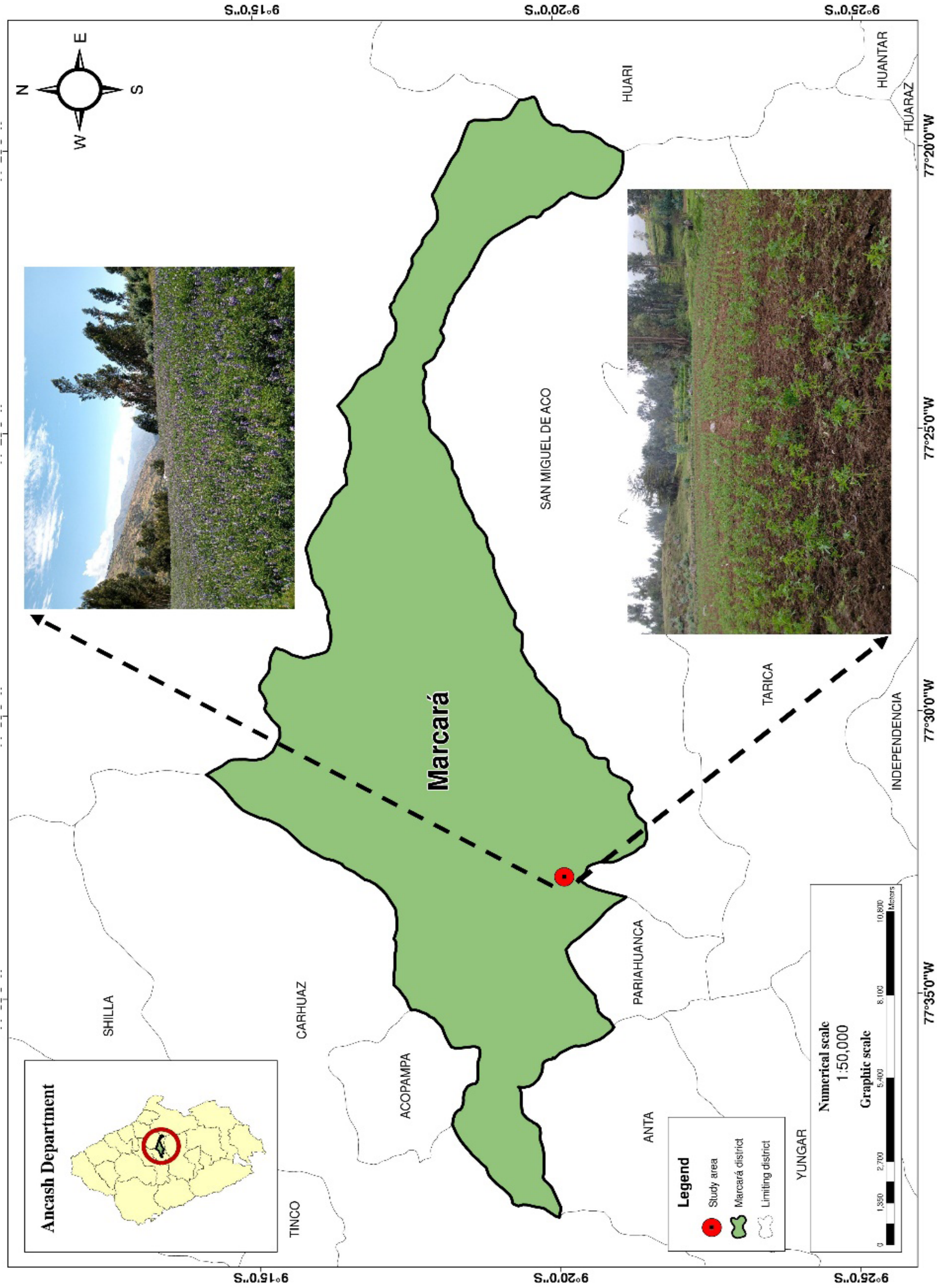


**Figure 1.** Location of the experimental plots in the Vicos rural community , Paltash locality, Marcará District, Carhuaz Province, Ancash Region, Peru.

**Table 1.** Degree of severity and disease percentage caused by Anthracnose in tarwi.

| Degree of severity | Description |
|---|---|
| **0** | Plants without any lesions. |
| **1** | Very small lesions (5mm) on leaves and stems, some wrinkles on leaves, without sporulation; isolated lesions. |
| **2** | Local infections on leaves and stems: lesions of 0.5 cm to 1 cm, little sporulation. |
| **3** | Frequent infections in leaves and stems and/or pods, accompanied by necrotic tissues (Sporulation). |
| **4** | Presence of large lesions (more than 3 cm), on stems, branches and pods with necrotic tissue, accompanied by the collapse of the tissues (sporulation), bent terminal shoots are observed. |
| **5** | Heavily affected necrotic plant, dead plants, small pod shape, orange-pink tissue sporulation. |

Source: Adapted by Falconí (2012).

## Results and Discussion

### Fresh and dry biomass analysis

In the evaluation carried out on three plants per treatment of each block at 60 DAS, it can be observed that the bioinoculated treatment (C.Br+Ba) is higher for the fresh weight of foliar compared to chemical treatments (Chem. trt) and Control (Figure 2 a-b), the same occurs for the root weight characteristic as indicated in Figure 2 c-d. The C.Br+Ba treatment presented the highest fresh foliar weight (19.26 ± 7.71 g) and dry foliar weight (3.80 ± 1.47 g) of the aerial part, but statistically no-significant difference was observed in first (Mean Sq: 40.04, F value: 1.071) and second variable (Mean Sq: 1.796, F value: 1.392). Likewise, Mollinedo et al. (2018), found similar results with the inoculation of rhizobia strains isolated from the cultivated *Lupinus*, these allowed a better percentage of dry matter of 17.76 % compared to the control treatment that had 10.5 %. In such a context, Huasasquiche et al. (2020), as an effect of the inoculation of tarwi seeds in the greenhouse and when evaluating the fresh and dry weight of the inoculated seedlings; found that these presented the highest values, however, finding no statistical significance between the averages with respect to the control (*p*-value = 0.204 for fresh weight and *p*-value = 0.275 for dry weight). Likewise, Steinberga et al. (2008), reports that the effects of inoculation increased by 27 % of dry matter.

On the other hand, the fresh and dry weight of the roots did show significant differences for the treatments studied (*p*-value: 0.04126 *and 0.03546 *) as observed in the ANOVA for root fresh (Mean Sq: 0.2923, F value: 3.552) and dry (Mean Sq: 0.020933, F value: 3.740). When making the comparison of means in the fresh and dry root weight, the highest means were for the C.Br-Ba treatment with 0.75 g ± 0.36 g and 0.25 g ± 0.08 g respectively and those with the lowest response were in the Chem.trt treatment 0.44 g ± 0.20 g and 0.17 g ± 0.04 g in both cases, as can be seen in (Figure 2 c-d). Recent studies show, that the inoculated plants of Tarwi, for the treatment *Bradyrhizobium* spp. + *Bacillus* sp., with respect to fresh weight of root with nodules (41.72 g) in comparison with the control that reports (15.55 g) a statistical difference (Monroy-Guerrero et al. 2022). Likewise, Taco-Taype & Zúñiga-Dávila (2020), pointed out that inoculation with wild strains has the best results compared to the control strain, in terms of the number of nodules, which are related to the greater root weight. However, controlled greenhouse conditions could facilitate bacterial colonization (Diaz et al., 2019), which is not necessarily the case when carried out under field conditions where plants are exposed to biotic and abiotic stress (Ahmad et al., 2019). This could explain the non-significant results of the inoculants on fresh weight of nodules, fresh weight and dry weight of leaves. However, controlled greenhouse conditions could facilitate bacterial colonization (Diaz et al., 2019), which is not necessarily the case when done under field conditions where plants are exposed to biotic and abiotic stress (Ahmad et al., 2019). This could explain the non-significant results of the inoculants on nodules fresh weight, fresh weight and dry weight of leaves.

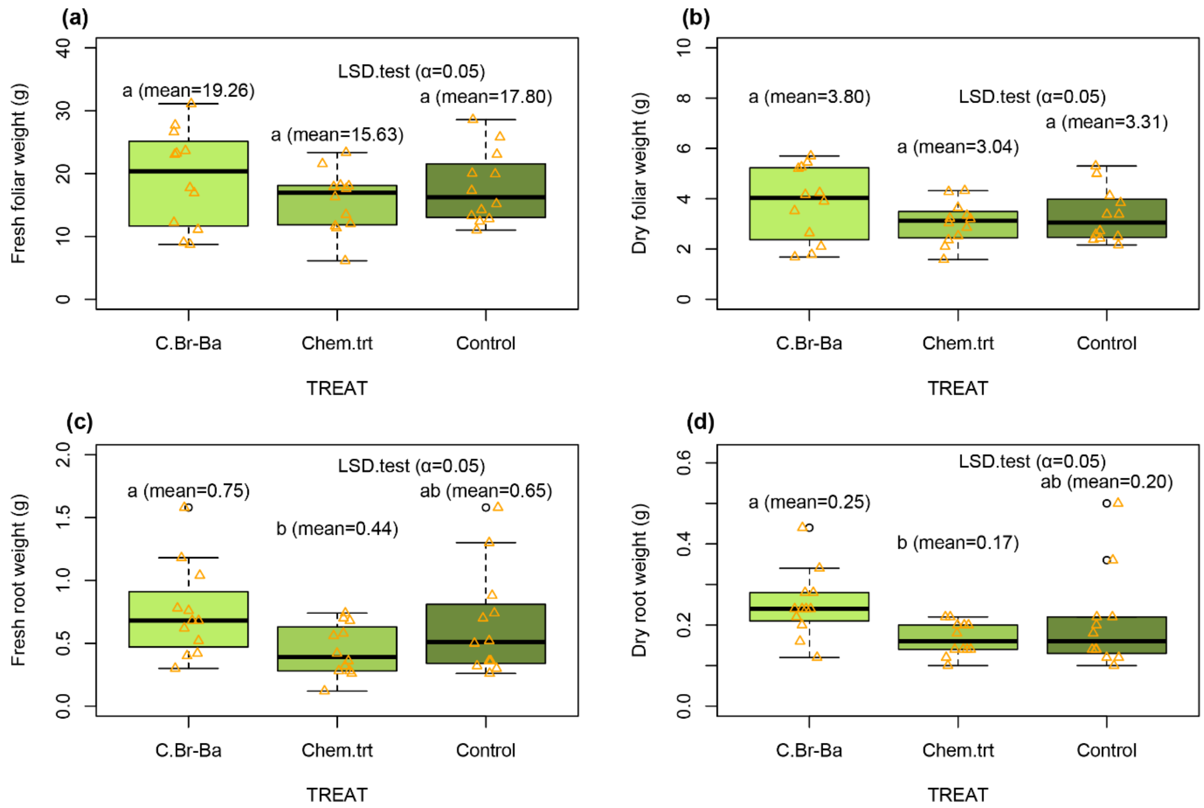


**Figure 2.** Variability of field data with boxplot plus strip chart and means compared using LSD.test ($p < 0.05$), for variables: Fresh foliar weight (a), Dry foliar weight (b), Fresh root weight (c) and Dry root weight (d) at 60 DAS.

## Nodules

The plants inoculated with *Bradyrhizobium* presented nodules with the best characteristics, with the highest fresh weight (0.68 g ± 0.32 g) and dry weight (0.25 g ± 0.096 g) for control treatment, but don't have statistically significant difference in the ANOVA when nodules fresh (Mean Sq: 0.08366, F value: 0.941) and dry (Mean Sq: 0.010811, F value: 1.574) and internal red color with a greater number of nodules, followed by the control. Both treatment (C.Br-Ba and control) superior to the Chem.trt treatment, which presented, nodules of lower fresh weight (0.53 g ± 0.21 g), dry weight (0.19 g ± 0.06 g) and in a small nodule amount (Figure 3 a-b), but it was statistically non-significant differences with C.Br-Ba. Monroy-Guerrero et al. (2022), when evaluating the flowering the Tarwi plants inoculated with *Bradyrhizobium* spp., found that the highest values for fresh weight of nodules (0.258 g), number of nodules (3.33) and size of nodules (4.26 mm) and Internal red color compared to the control that presented fresh weight of nodules (0.017 g), Number of nodules (0.67 g), size (1.57 mm) and internal whitish color. Likewise, Taco-Taype & Zúñiga-Dávila (2020) indicate showed significant differences in the number of primary nodules, where they found a variation from 9 to 12.4 nodules/plant in the inoculated plants. It is also worth indicating the reason for the equality of dry weight of nodules both in the treatment inoculated with *Bradyrhizobium* and control treatment, in this context Chincheros (1996), indicates that the presence of nodules in the roots of the control, is due to the existing native population of the soil, which can be highly competitive in the formation of nodules, but have low nitrogen-fixing efficiency. In this context, Taco-Taype & Zúñiga-Dávila (2020) indicated the effect of native *Bradyrhizobium* strains on tarwi under controlled conditions; resulting in greater plant growth and the number of nodules. However, Monroy-Guerrero et al. (2022) points out that the efficiency of this symbiotic relationship depends on biotic and abiotic factors.

There are few publications on the effect of symbiotic bacteria such as Bradyrhizobium, which actively participates in biological nitrogen fixation (BNF) in legumes (Taco-Taype & Zúñiga-Dávila, 2020), and biocontrol bacteria such as Bacillus in tarwi cultivation, despite its nutritional relevance. Taco-Taype & Zúñiga-Dávila (2020) indicated the effect of native strains of Bradyrhizobium on tarwi under controlled conditions; resulting in increased plant growth and the number of nodules.

that when nodule dry matter increases, the number of pods also increases, an aspect highly related in the performance. In addition, the positive correlations between different variables evaluated; this is because the LMHZ L8 and LMHZ L3 consortium strains of *Bradyrhizobium* spp. are effective strains for nodule formation (Monroy-Guerrero et al., 2022). Mollinedo et al. (2018) states that the number of nodules is an indicator of the infectivity of the bacteria in the roots. In this sense, by having a greater number

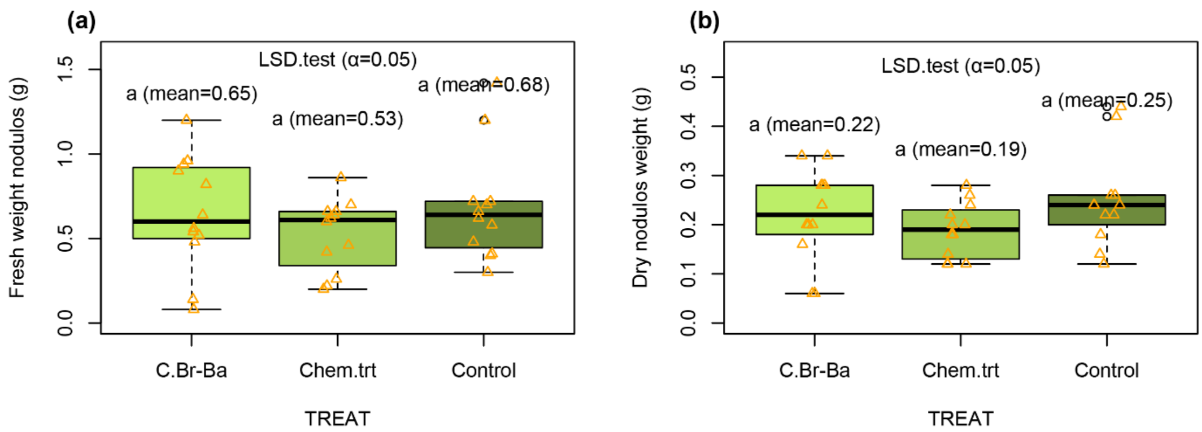


**Figure 3.** Variability of field data with boxplot, stripchart and means compared using LSD.test ($p < 0.05$): Fresh weight nodules (a), Dry nodules weight, (b) at 60 DAS.

The multiple correlation analysis of the quantitative variables at 60 DAS (Figure 4), on the fresh and dry weight of the nodules have a highly significant positive correlation with the variables; leaf fresh weight, leaf dry weight and root dry weight (0.709*** > $r$ > 0.605***), observing the maximum correlation between fresh weight of nodules and dry weight of roots with $r$ = 0.956***. On the other hand, no significant correlations were observed between plant height and roots and nodules, both fresh and dry (0.188 n.s. > $r$ > 0.141 n.s.). In the present work, finding similar correlations to what Taco-Taype & Zúñiga-Dávila (2020) report, they pointed out that there is a 43 % correlation between root dry weight and total nodules, likewise they emphasize that there is a correlation between length and nodules number (42 %). In this context, Mollinedo et al. (2018), added that there is a positive and significant correlation ($p$ = 0.0057), between nodule dry matter and number of pods, also pointing out

of effective nodules in the plant, it makes it store good levels of nitrogen, which directly affects the different characteristics of the plant. This can be explained with the positive correlations found (Figure 4).

The multivariate analysis of quantitative and qualitative data at 60 DAS (Figure 5) showed that, the two first Principal components (PC) explain 49.2 % of the total variance (Dim1 35.19 %, Dim2 14.01 %). For other side, the FAMD analysis (Figure 5 a, b and d), in which we had three qualitative variables (red color) and seven quantitative variables (black color), these being the last ones that show the variance in the Dim1 of FAMD and those used for a PCA analysis (Figure 5c), increasing the variance explained to 79.9 % with its first two dimensions (Dim1 63.3 %, Dim2 16.6 %). However it was not observed contrasting differences between the cluster formed by the treatments, it shows slight increase in magnitudes for Control and C.Br.Ba treatments with respect to Chem.trt.

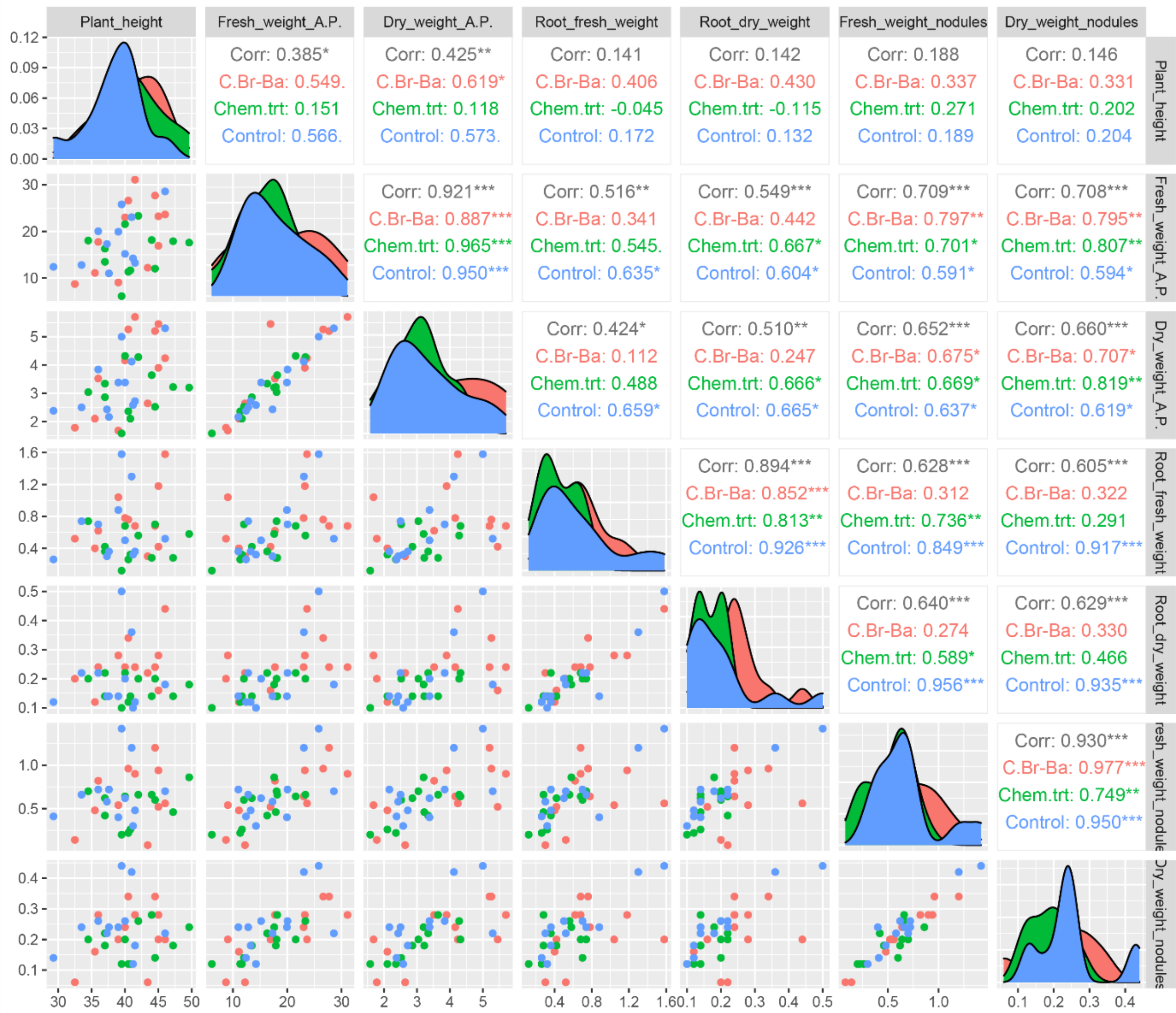


**Figure 4.** Multiple correlation analysis Pearson-r with statistical significance, density graph and dispersion plot for all data and each treatment, in the seven quantitative variables at 60 DAS.

## Severity degree of Anthracnose

Evaluations of Anthracnose (*Colletotrichum gloeosporioides*) were carried out at 60, 110 and 240 days (harvest), the results are showed in Table 2. The bioinoculated treatment C.Br+Ba was presented an attack of 15.5% of Anthracnose at 60 days, of 25 % at 110 days and 45.5 % at 240 days; these values are lower compared to the Chem. trt and control treatments. It should be noted the results found by Monroy-Guerrero et al. (2022) inoculating strains of *Bradyrhizobium* spp., plus *Bacillus* sp., that anthracnose severity decreased by 38.4 % compared to the control treatment at 110 DAS. The *Bacillus* biocontrol acted as a bioprotective bacterium and promoter of plant growth (Obrera et al., 2005), by reducing the degree of severity of Anthracnose. The Bacillus BO2 strain has been reported as a biocontroller (Memenza-Zegarra & Zúñiga-Dávila, 2019) due to its ability to produce antifungal compounds such as hydrolytic enzymes such as proteases, cellulases and amylases that were used to break down the components of the cell wall of fungal cells. In addition, siderophores, antimicrobial peptides (Castillo-Reyes et al., 2015) and other substances that could inhibit the invasion of plants by Colletotrichum were identified, reducing the degree of attack of anthracnose. On the other hand, in the field, the recurrence of the disease caused by *S. sclerotiorum* was significantly reduced (94 %) applying B02 in common bean crops (Memenza, et al., 2016). This strain isolated from common bean plants produced non-volatile antifungal compounds capable of suppressing the mycelial growth of *R. solani*. In addition, the inoculation with Bradyrhizobium guarantees the absorption of nitrogen by the plant and improves the response to diseases.


**Figure 5.** Multivariate analysis, with Factor Analysis of Mixed Data (FAMD) (a, b and d) with quantitative and qualitative data and Principal Component Analysis (PCA), for quantitative data (c) and nodulation at 60 DAS.

**Table 2.** Effect of treatments on the intensity attack percentage of Anthracnose

| | 60 days | 110 days | 240 days |
|---|---|---|---|
| **C.Br-Ba** | 15.5 | 25.0 | 45.5 |
| **Chem.trt** | 17.5 | 27.5 | 60.5 |
| **Control** | 41.5 | 47.5 | 85.5 |

The *Bacillus* biocontroller acted as a bioprotective bacterium and promoter of plant growth (Obrera et al., 2005), by reducing the degree of severity of Anthracnose. Bacillus BO2 strain has been reported as a biocontroller (Memenza-Zegarra & Zúñiga-Dávila, 2019) due to its ability to produce antifungal compounds such as hydrolytic enzymes such as proteases, cellulases, and amylases that were used to break down the cell wall components of fungal cells. In addition, siderophores, antimicrobial peptides (Castillo-Reyes et al., 2015) and other substances that could inhibit the invasion of plants by Colletotrichum were identified, reducing the degree of anthracnose attack. On the other hand, in the field, the recurrence of the disease caused by *S. sclerotiorum* was significantly reduced (94 %) by applying B02 in common bean crops (Memenza, et al., 2016), less. This strain isolated from common bean plants produced nonvolatile antifungal compounds capable of suppressing mycelial growth of *R. solani*. In addition, inoculation with *Bradyrhizobium* guarantees the nitrogen absorption by the plant and improved disease response.

## Grain production and yield components

The ANOVA analysis and SNK.test (p <0.05) on the data recorded in 10 plants for each treatment within block at 240 days showed a significant statistical differences for the number of pod locules, with 4.43 value for the C.Br+Ba treatment compared to control with 3.94 value (Figure 6d), which means a direct positive effect of *Bradyrhizobium*. For other side, the ANOVA analysis and SNK.test (p <0.05) showed that the Chem.trt treatment was superior (123 pods/plant) to the treatment with *Bradyrhizobium* (110 pods/plant) and to the control (101 pods/plant). Regarding the number of grains per pod, the bioinoculated treatment C.Br+Ba (4.01) was superior to the others, being statistically significant, only with the control treatment. The yield per hectare showed the same tendency, the Chem.trt treatment (2291.41 kg/ha) was statistically superior to the both C.Br+Ba treatments (1846.44 kg/ha) and the Control (1631.34 kg/ha) (Table 3).

inoculated plants showed the best quantitative characteristics in the lateral inflorescence, as well as for the number of pods, weight of the pods and the number of locules increase to 165.1 %, 116.9 % and 14 % in comparison with the control treatment. In addition, our results were similar what Mollinedo et al. (2018) found that the inoculated strains promoted a greater number of pods with an average of 12 pods per plant and the control with 6 pods per plant. Also that these strains are highly promising to increase the number of pods per plant, which is positively related to the yield. Regarding the performance Deaker et al. (2004), pointed out that the effect of the inoculations also increased the yield more than 25 %. Additionally, Monroy-Guerrero et al. (2022) using Andenes variety found that the *Bradyrhizobium* plus *Bacillus* treatment had a

**Table 3.** Effect of treatments on yield and its components

| Treatments | No. pods per plant | Number of locules | Bean grain /pod | Weight/100 seeds (g) | Yield kg/ha |
|---|---|---|---|---|---|
| C.Br+Ba (Organic) | 110.98 ab | 4:43 a | 4.01 a | 27.66 a | 1846.44 b |
| Chem.trt (Chemical) | 123.29 a | 4.15 b | 3.85 ab | 23.66 a | 2291.41 a |
| Control | 101.07 b | 3.94 b | 3.61 b | 28.52 a | 1631.34 b |

Different letters mean statistically significant differences (p <0.05) in Duncan 's test.

The bioinoculated treatment promotes the development of locules per pod, which directly affects the number of grains per pod and then its potential yield. It should be noted that the efficiency of this symbiotic relationship depends on biotic and abiotic factors, such as nitrogen deficiency in soils (Aasfar et al., 2021), optimum soil temperature and humidity, among others, which would explain the low variability between the treatments studied (Jamiołkowska et al., 2018).

The bioinoculated treatment (C.Br+Ba) with a value of 4.43 significantly surpass both treatment Chem.trt and Control, with values 4.15 and 3.94 respectively. There are few publications on the effect of symbiotic bacteria such as *Bradyrhizobium*, which actively participates in biological nitrogen fixation (BNF) in legumes (Taco-Taype & Zúñiga-Dávila, 2020). In this context, in a recent study conducted by Monroy-Guerrero et al. (2022), pointed out that the yield of 465.5 Kg/ha compared to the control that had 171.8 Kg/ha. In the present investigation, the yields which were found for the three treatments exceed the national average yield, which is 1398 Kg/ha (Sierra y Selva Exportadora, 2021).

Regarding correlations, it was observed that the number of locules promoted as a effect of the *Bradyrhizobium*, has a robust positive correlation directly with the bean grains/pod. However, a very weak negative correlation was observed with a value of -0.619* for the weight 100 of seeds with respect to yield; itself, the characteristic 100-seeds weight with values between -0.095 > r > -0.225 with respect to the number of grain-pod, number of locule/pod and grain weight per plant (Figure 7). In this regard, Taipe (2021), when evaluating the effect of inoculation with *Bradyrhizobium* found the best values in the number of pods of the lateral branches, for the inoculated plants (8.12 pods) and for the non-inoculated plants (7.87 pods), however, it was

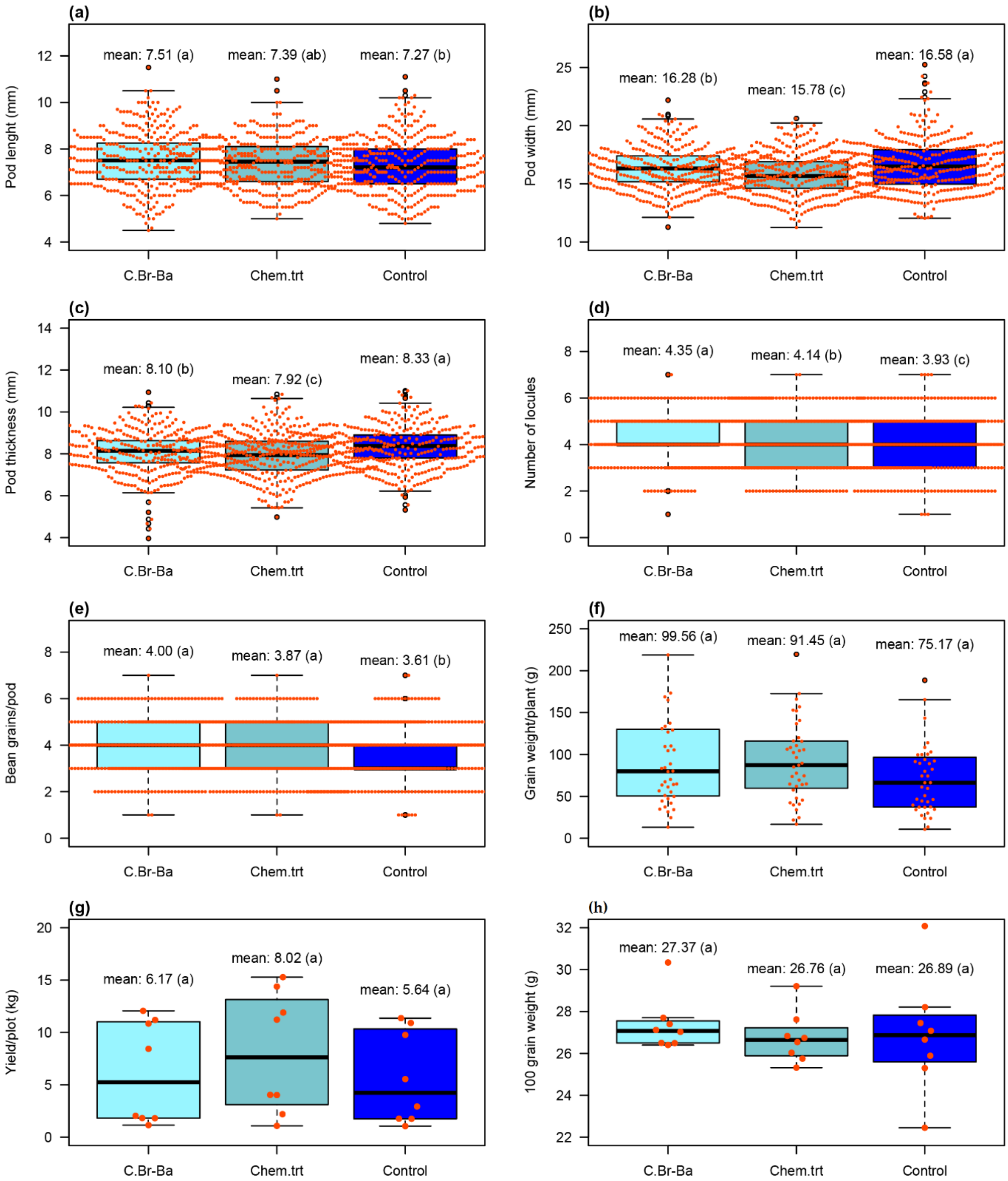


**Figure 6.** Comparison of means with Student–Newman–Keuls test (SNK.test) and boxplot and beeswarn for the variables: pod length (mm) (a), pod width (mm) (b), pod thickness (c), number of locules (d), bean grain/pod (e), grain weight per plant (f), Yield per plot (g) and 100 grain weight (h) at 240 DAS.

non-significant statistical differences. So, in this way Bradyrhizobium contributes to improve grain yield due to the atmospheric nitrogen fixation, which will be in greater quantity in an inoculated plant. In the same context, Mollinedo et al. (2018), affirm that the number of pods was positively related to the yield.

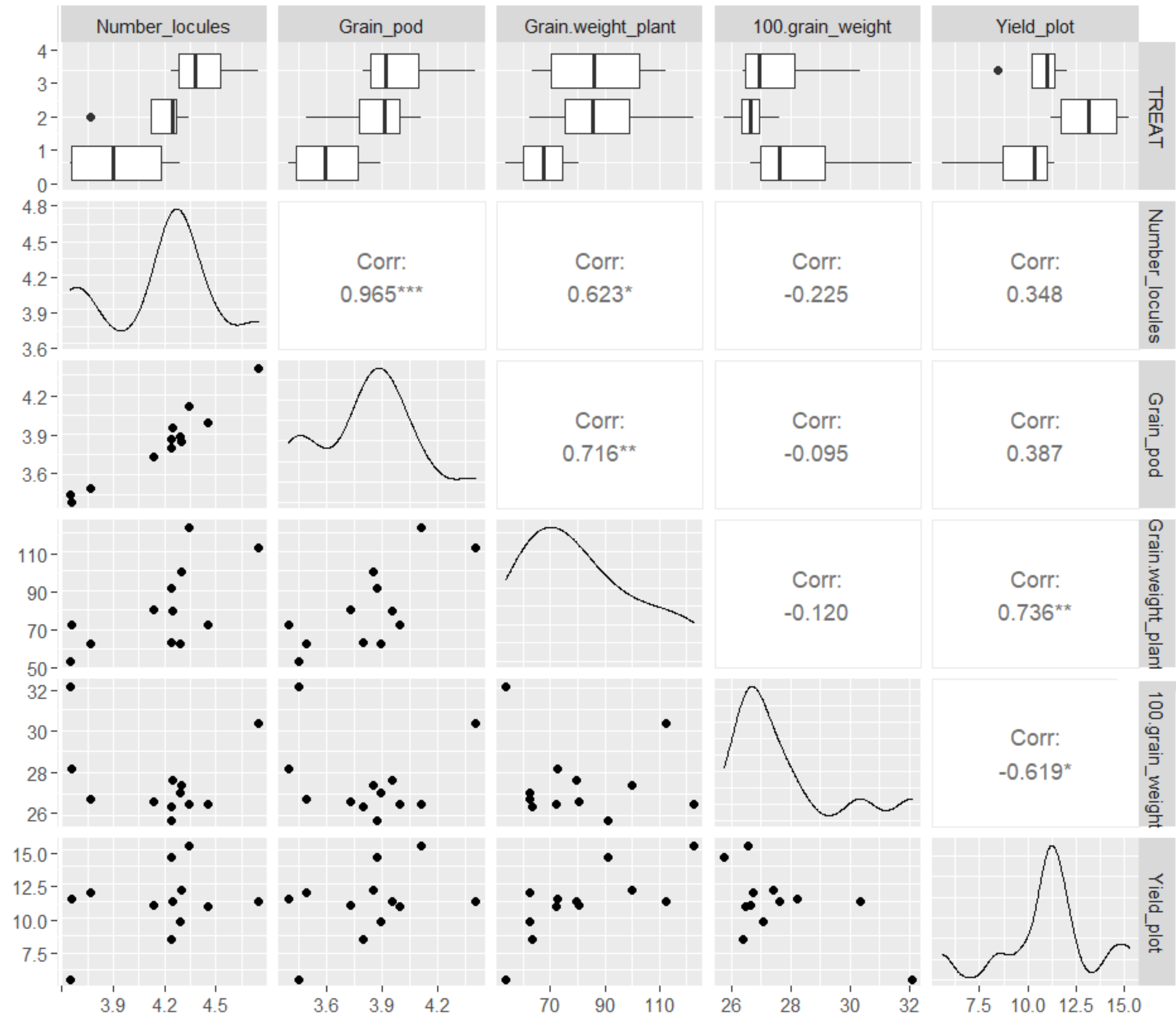


**Figure 7.** Multiple correlation analysis with correlogram (Pearson-*r*) for yield and components

According to the principal component analysis (PCA), the two first PC explained 75.5 % of the total variance (Figure 8). The PC1 was mainly explained with grains per pod, number of pods per plant and number of locules per plant and the PC2 was explained by the variables yield and weight of 100 grains. The correlation was inversely proportional between these last two variables (r = -0.619*) and its yield components showed a tendency to higher yields for the Chem.trt treatment (Table 3). Taco-Taype & Zúñiga-Dávila (2020) found a similarity for other characteristics, the first component y second component explained 43 % and 27 % of the total variation of *L. mutabilis*, which were related to the growth, the aerial length, the foliar cover and the number of secondary nodules for PC1 and the second component (PC2) was realted to the dry weight aerial and the dry weight of the root.

## Nutrient Content of grain

The nutrient content on the grain of the Altagracia variety did not show almost any difference for both treatment (C.Br+Ba and Chem.trt) and the control (Table 4).

**Table 4.** Nutritive value (%) of the seed of the "Altagracia Selection" variety.

| **Chemical composition** (100 g of original material) | **Treatments** | | |
|---|---|---|---|
| | **C.Br+Ba** | **Chem.trt** | **Control** |
| Proteins (Factor:6.25) | 41.1 | 41.0 | 40.8 |
| Fiber | 7.6 | 6.1 | 6.8 |
| carbohydrates | 32.9 | 32.3 | 32.4 |
| Fat | 14.2 | 14.3 | 13.8 |
| ashes | 3.1 | 6.1 | 3.1 |

Source: PLGO – UNALM

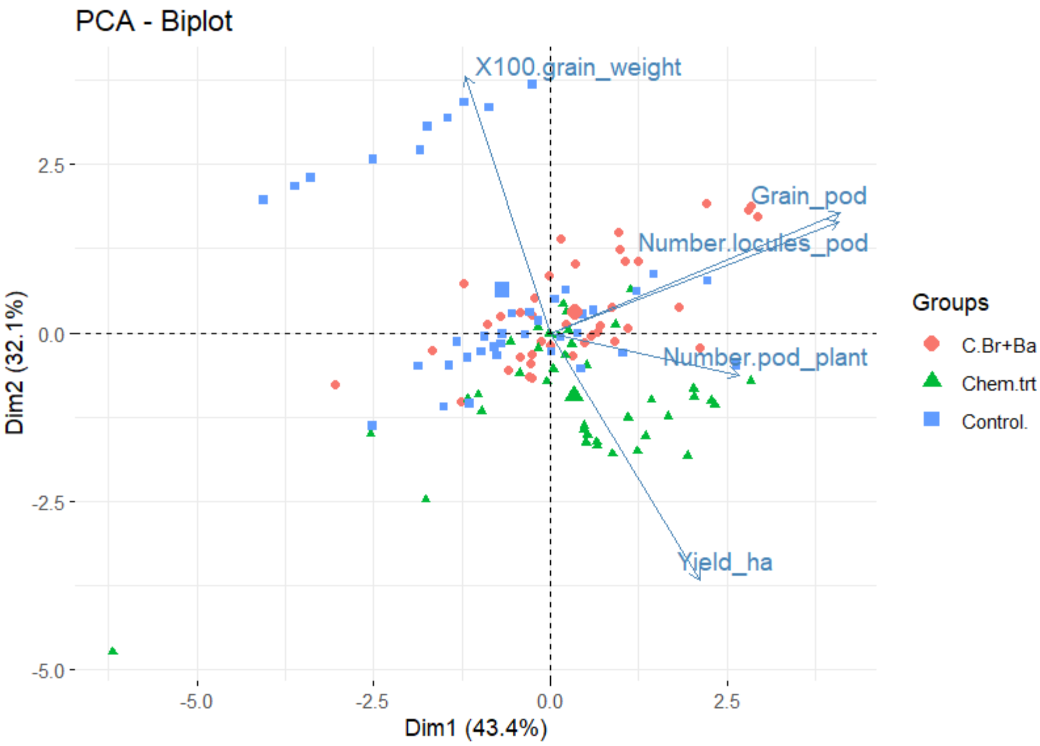


**Figure 8.** Principal component analysis (PCA) for yield and its components in the C.Br+Ba, Chem. trt and Control treatments in the Altagracia variety of tarwi.

Likewise, it was similar results for the macro and microelements composition, the only different results was found in Copper concentration for C.Br+Ba (14 ppm) and equal for both Chem.trt and Control ( 8 ppm) Iron and in Boron that were different values for each one (Table 5, Figure 9 and 10). However, Richardson et al., (2009) reported that inoculation with rhizobacteria increases the uptake of macro and micronutrients such as Ca, K, Fe, Cu, Mn and Zn. Those bacteria make these elements available in the soil by producing organic acids or secreting chelating compounds.

Therefore, using more than one strain, such as *Bradyrhizobium* bioinoculated with *Bacillus*, could allow plants to achieve balanced nutrition (Bashan et al., 2004) for optimal growth and production (Bautista et al., 2010). This research showed that the treatment with BR+BA in overall way did not increase as expected the nutritional quality of the grain. However, the application of these biotechnologies reduces agrochemicals and production costs and are environment friendly.

**Table 5.** Composition of minerals (macro and microelements) in the seed of "Altagracia variety" and each treatments (C.Br+Ba, Chem. trt and Control).

| Chemical composition macro and microelements | Treatment | | |
|---|---|---|---|
| | C.Br+Ba | Chem.trt | Control |
| Nitrogen (%) | 7.56 | 7.31 | 7.52 |
| Phosphorus (%) | 0.53 | 0.51 | 0.49 |
| Potassium (%) | 0.85 | 0.72 | 0.80 |
| Magnesium (%) | 0.20 | 0.19 | 0.18 |
| Calcium (%) | 0.09 | 0.11 | 0.12 |
| Manganese (ppm | 72.00 | 72.00 | 69.00 |
| Copper (ppm) | 14.00 | 8.00 | 8.00 |
| Iron (ppm) | 37.00 | 34.00 | 33.00 |
| Zinc (ppm) | 41.00 | 42.00 | 39.00 |
| Boron (ppm) | 11.00 | 7.00 | 19.00 |

## Conclusions

Bacterial inoculations have a positive influence on morphoagronomic and physiological behavior of tarwi var. Altagracia cultivated in the locality of Paltash in Marcará, Ancash; which increased the yield, however the increase of seed

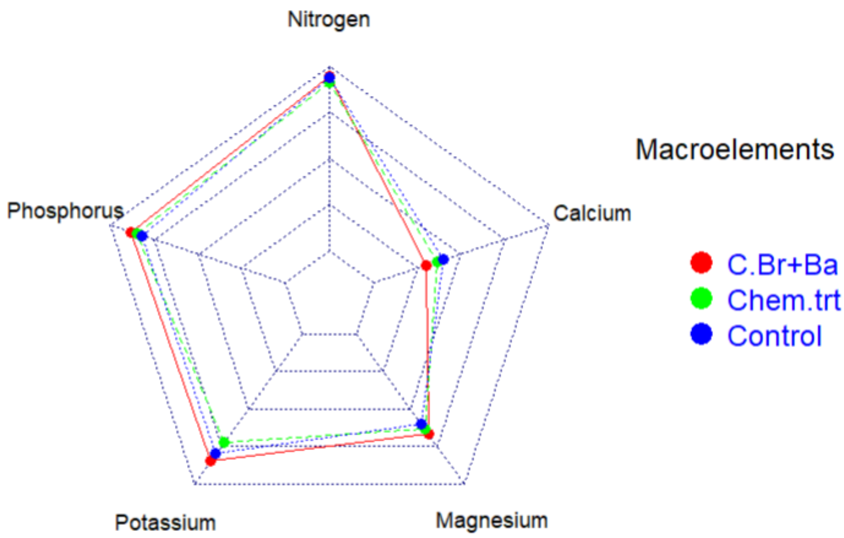


**Figure 9.** Macroelements concentration of the "Altagracia variety" by treatment.

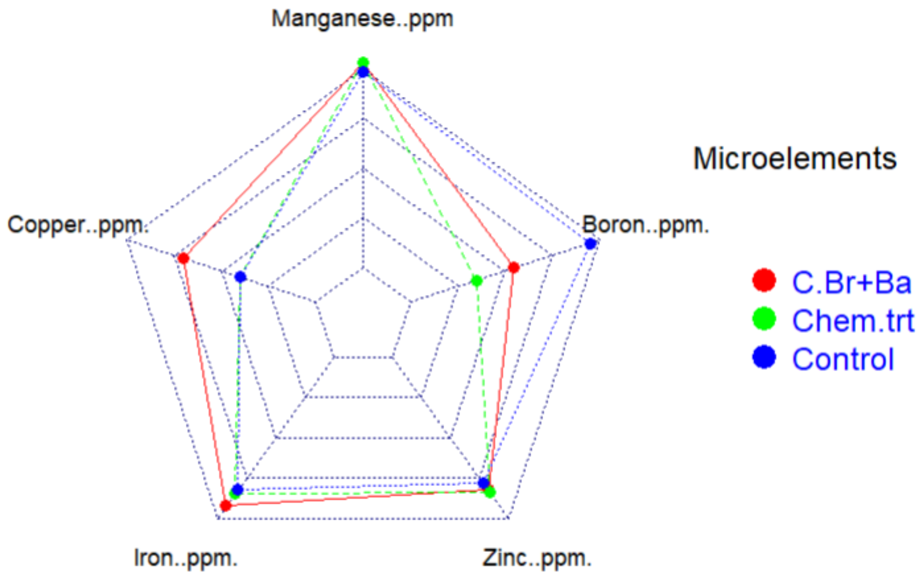


**Figure 10.** Microelements concentrations of the "Altagracia variety" by treatment.

nutrient content was not clear. *Bradyrhizobium* strains ensure nitrogen fixation and *Bacillus* sp. performs phytosanitary control of plants due to its antifungal properties. These results related to the Plant Growth Promoting Rhizobacteria (PGPR) is important because farmers could use as a part of integrated crop management and develop a sustainable crop production.

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## Authors contributions

FCM: Conceptualization of the work, experimental design and revision of the manuscript. DZD: Discussion of results DSN, support and supervision of the study. AHJ: Fieldwork supervision, manuscript review. EM: Support and supervision in field work, manuscript revision. DSN: Experimental design, statistical analysis of results. RQT, DSN: field work carried out, review of statistical analysis of results DSN.

VLH: Logistics management, execution of field work.

### Conflict of interest / Competition interests:

The signing authors of this research work declare that they have no potential conflict of personal or economic interest with other people or organizations that could unduly influence this manuscript.

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