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# Production of potato seed tubers under the effect of *Trichoderma* sp. and rhizobacteria in greenhouse conditions

*Produção de tubérculos de batata-semente sob efeito de Trichoderma sp. e rizobactérias em casa de vegetação*

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**ABSTRACT**

Potato (*Solanum tuberosum* L.) is one of the main crops in the Andean region and due to environmental aspects, the use of biocontrol agents is considered a safe way to produce potato seed tubers.The objective of the study was to evaluate potato inoculation with *Trichoderma* sp. as a matrix and rhizobacteria *Bacillus simplex* and *Azotobacter* sp. on the growth of potato seedlings from *in vitro* culture, for the production of seed tubers in a greenhouse. The inoculation of microorganisms was carried out in pots, using five potato genotypes for processing. The inoculation treatments were: control, *Trichoderma* sp., *Trichoderma* sp. + *Azotobacter* sp., *Trichoderma* sp. + *Bacillus simplex*, *Trichoderma* sp. + *B. simplex* + *Azotobacter* sp. The potato genotypes were cv. Unica (CIP392797.22), cv. Bicentenaria, the advanced clones CIP 396311.1, CIP 399101.1, and the experimental clone UH-09 from the Universidad Nacional José Faustino Sánchez Carrión. A completely randomized design was used, under a factorial arrangement and comparisons between treatments were made at p<0.05. All inoculant treatments exceeded the control in number and weight of tubers per plant as well as in tuber size. Inoculations of *Trichoderma* sp. alone or with *Azotobacter* sp. increased plant height, number of leaves per plant and vegetative uniformity; inoculations with the *Trichoderma* sp. + *B. simplex* + *Azotobacter* sp. consortium, improved the dry weight of the foliage, number of shoots per plant and vegetative vigor. There were significant interactions between potato genotypes and inoculant treatments for plant uniformity, vegetative vigor, and the foliage's dry weight. Coinoculation with *Trichoderma* sp. and some bacterial strains promote the growth of *in vitro* potato seedlings, increasing the size and weight of the seed tubers and plant biomass, indicating an interrelation between fungi and bacteria that influence the production of potatoes in a greenhouse.

**KEYWORDS:** *Trichoderma; Azotobacter; Bacillus*; potato for processing; potato seed tubers.

**RESUMO**

A batata (*Solanum tuberosum* L.) é uma das principais culturas da região andina e devido aos aspectos ambientais, o uso de agentes de biocontrole é considerado uma forma segura de produzir tubérculos de batata-semente. O objetivo do trabalho foi avaliar a inoculação de batata com *Trichoderma* sp. como matriz e rizobactérias *Bacillus simplex* e *Azotobacte*r sp. sobre o crescimento de mudas de batata provenientes de cultivo *in vitro*, para produção de tubérculos-semente em casa de vegetação. A inoculação dos microrganismos foi realizada em vasos, utilizando-se cinco genótipos de batata para processamento. Os tratamentos de inoculação foram: testemunha, *Trichoderma* sp., *Trichoderma* sp. + *Azotobacter* sp., *Trichoderma* sp. + *B.simplex*, *Trichoderma* sp. + *B.simplex* + *Azotobacte*r sp. Os genótipos de batata foram cv. Única (CIP392797.22), cv. Bicentenaria, os clones avançados CIP396311.1, CIP399101.1, e o clone experimental UH-9 da Universidade Nacional José Faustino Sánchez Carrión. O delineamento foi inteiramente casualizado, em esquema fatorial e as comparações entre os tratamentos foram feitas com p<0,05. Todos os tratamentos com inoculantes excederam o controle em número e peso de tubérculos por planta, bem como em tamanho de tubérculo. Inoculações de *Trichoderma* sp. sozinho ou com *Azotobacter* sp. aumento da altura das plantas, número de folhas por planta e uniformidade vegetativa; inoculações com o *Trichoderma* sp. + *B.simplex* + *Azotobacter* sp. consorcio, melhorou a massa seca da folhagem, número de brotações por planta e vigor vegetativo. Houve interações significativas entre genótipos de batata e tratamentos inoculantes para uniformidade e vigor vegetativo, e para a massa seca da folhagem. Coinoculação com *Trichoderma* sp. e algumas cepas bacterianas promovem o crescimento de mudas de batata *in vitro*, aumentando o tamanho e o peso dos tubérculos-semente e da biomassa vegetal, indicando que existe inter-relação entre fungos e bactérias que influenciam a produção de batata em casa de vegetação.

**PALAVRAS-CHAVE:** *Trichoderma; Azotobacter; Bacillus,* batata para processamento; tubérculos de batata-semente.

## INTRODUCTION

The potato (*Solanum tuberosum* L.) is one of the most important food crops in the world and Latin America, but its cultivation is affected by numerous challenges, such as pests, diseases and a high fertilizer requirement that has been associated with some environmental problems (ALOO et al. 2019). Due to these environmental aspects, using biocontrol agents is considered a safe way to produce potatoes within the sustainable agriculture approach, in which eco-friendly methods are sought to promote plant growth and improve crop productivity (EKIN 2019).

It is known that microorganisms in agricultural soil exert a profound influence on soil fertility status, in particular availability of nutrients, as well as in the suppression of plant diseases and regulating plant tolerance to stress factors (KENNEDY & SMITH 1995, YANG et al. 2018). There is evidence that soil biodiversity confers stability to stress and disturbance conditions (BRUSSAARD et al. 2007), but the mechanisms are still not fully understood.

A history of mutualism between potato plants and soil bacteria supports the hypothesis that Andean soils harbour plant growth promoting rhizobacteria (PGPR) (GHYSELINCK et al. 2013). Plant growth-promoting rhizobacteria (PGPR) are mostly non-pathogenic microbes which exert direct benefits on plants while rhizosphere bacteria indirectly help plants by ameliorating the biotic and/or abiotic stress or induction of defense response in plants. Regulation of these direct or indirect effects takes place via highly specialized communication systems induced at multiple levels of interactions (BUKHAT et al. 2020).

PGPR strains can enhance plant growth through the production of various plant growth-promoting substances (MHATRE et al. 2019). Many plants-associated microbes are mutualistic symbionts of crop plants which improve their fitness and productivity by nutrient mobilization, nitrogen fixation, phytohormone production, and producing a variety of antipathogenic chemicals (PAL et al. 2021).

*Trichoderma* is a genus of filamentous fungi widely studied and used as a biocontrol agent in agriculture on pathogenic fungi due to its ability to parasitize them, among other mechanisms of action (POVEDA 2021). *Trichoderma* sp. is beginning to be used in reasonably large quantities in plant agriculture, both for disease control and yield increases. The studies of mycoparasitism also have demonstrated that these fungi produce a rich mixture of antifungal enzymes, including chitinases and glucanases (HARMAN 2006). Many different strains of the genus *Trichoderma*, in addition to a direct activity against phytopathogens, are well-known producers of secondary metabolites and compounds that substantially affect the metabolism of the host plant. Harzianic acid is a *Trichoderma* secondary metabolite, showing antifungal and plant growth promotion activities (VINALE et al. 2013)

Application of *Trichoderma koningii* and *Bacillus megaterium*, alone or in combination, seven days earlier than soil infestation with *F. oxysporum* and/or the mixed population of *Meloidogyne* spp., significantly reduced Fusarium wilt disease incidence and nematode infection on potato and improved plant growth components; the combination of the two biocontrol agents was more effective in controlling the plant disease and improving plant growth components than either of the two organisms used singly (EL-SHENNAWY et al. 2016). In addition, a select microbial product composed of a consortium of *B. subtilis* and *T. harzianum* effectively suppressed common scab disease. They increased tuber yield by establishing a high relative abundance of beneficial bacteria in the rhizosphere (WANG et al. 2019).

The results of SUSIANA et al. (2018) showed that the application of *T. viride* could reduce the intensity of leaf blight disease (*Phytophthora infestans*) and increase the growth of the potato plant; *T. viride* application could improve the systemic resistance of potato plants. A significant reduction of *Globodera pallida* infection in potato roots was observed when the soil was amended with *T. harzianum*. *G. pallida* cysts recovered from soil and *G. pallida* reproduction rate were significantly reduced by 49% and 60%, respectively, compared to the non-amended soil (CONTINA et al. 2017). Volatile organic compounds (VOCs) emitted from *Trichoderma* strains T41 and T45 inhibited the mycelial growth of *P. infestans* grown on a laboratory medium by 80 and 81.4%, respectively, and on potato tubers by 93.1 and 94.1%, respectively (ELSHERBINY et al. 2020). The ways in which *Trichoderma* spores can be applied to crops include pre-planting applications to seed or propagation material, incorporation in the soil during seeding or transplant, watering by irrigation or application as a root drench or dip (POVEDA 2021).

OSWALD et al. (2010) demonstrated in trials under controlled conditions with *Bacillus* sp., the mechanisms that cause the best growth of potato plants are among others early tuberization, the rapid development of leaf area, and probably, a higher rate of photosynthesis. CALVO et al. (2010) conducted a prospection of bacteria extracted from the rhizosphere of native potatoes that grow in their natural habitat in the Andean region and the results suggested that they are a rich source of antagonists for phytopathogenic fungi, mainly the isolates belonging to the genus *Bacillus* spp. Thus, they concluded that the potato rhizosphere is a source of bacteria to be used in the future as inoculants to improve the agronomic performance of potatoes.

*Bacillus simplex* and other species trialled in the field in their respective countries of origin, i.e., Bolivia, Peru, and Ecuador, showed a significant increase in the yield of potato; *B. simplex* with organic manure alone all resulted in higher tuber yields than the control treatment with organic manure and statistically similar tuber yields to the control treatment amended with chemical fertilizer (VELIVELLI et al. 2014). *Pseudomonas brassicacearum* and *B. simplex*, exhibit antagonistic activities against the Pectobacterium potato pathogens (KHAYI et al. 2015).

Azotobacter has been a biofertilizer for over a century (JNAWALI et al. 2015). Azotobacter fixes nitrogen aerobically, elaborates plant hormones, solubilizes phosphates, suppresses phytopathogens, or reduces their deleterious effect. Application of wild-type Azotobacter results in better yield of potato and other crops (DAS 2019).

Inoculation with PGPR significantly improved the plant height, straw yield, and the number of tubers per plant; potato tuber characteristics (starch content, vitamin-C, relative water content) were also significantly improved (MUSHTAQ et al. 2021). In addition, plant growth-promoting determinants, namely indole acetic acid (IAA), siderophore and phosphate solubilization, and potato plant growth phenotypes indicated *Pseudomonas* spp. and *Bacillus* spp. seem potential biocontrol agents for field application (ISLAM et al. 2021).

Inoculating tuber propagules with microbial strains, CONTRERAS-LIZA et al. (2019) demonstrated in the greenhouse and on-farm experiments that it is possible to improve potato performance compared to non-inoculated plots, increasing potato sustainability. As a result, the industry has begun to exploit some of these microorganisms mainly as phytosanitary products or as biofertilizers; and therefore, there is still a growing interest in microbial products due to an increase in demand for alternatives to current pesticides and fertilizers (SESSITSCH & MITTER 2015). On the other hand, it has been determined that the good quality of the planting material increases the viability, germinative energy and growth of the potato seed tuber, the resistance of the plants to diseases and environmental factors (KOSTENKO et al. 2020).

In most developing countries, small-scale farmers continue to plant seed tubers acquired through the informal seed systems (THOMAS-SHARMA et al. 2016), and in Peru, less than 1% of potato tuber seed used by farmers comes from formal seed systems (HIDALGO et al. 2009). In this context, small-scale farmers need new approaches to increasing the use of quality potato seed tubers. Unfortunately, there is no information on the use of these microorganisms in the production process of potato seed tubers, but it is known that many diseases are transmissible through potato seed tubers (FIERS et al. 2012). For this reason, the study's objective was to evaluate potato inoculation with microorganisms *Trichoderma* sp. as a matrix and agriculturally important bacteria *Bacillus simplex* and *Azotobacter* sp. on the growth of potato seedlings from *in vitro* culture, for the production of seed tubers in greenhouse conditions.

## MATERIAL AND METHODS

### Location and environmental conditions

The experiment was carried out in pots under a greenhouse insect net at Universidad Nacional José Faustino Sánchez Carrión, Huacho, Perú. Geographically it is located at coordinates 11°07′26″S, 77°36′32″W, at an altitude of 68 m.a.s.l. Mean air temperatures were 25 °C (range 23-28 °C) and 91% relative humidity.

The pot substrate was a mix of 45 % washed sand, 45 % earthworm humus and 10 % rice straw. The earthworm humus (*Eisenia foetida*) and rice straw were purchased from local suppliers; earthworm humus content was 30% organic matter, 1% nitrogen, 2% phosphorus, pH 6.8 and C/N=10. The sand for the substrate was collected locally and washed several times before use in the mix. The organic matter content of the substrate for the pots was 3.04%, pH 8.1, 2.96 dS/m EC, 2.90% $CaCO_3$, 182 ppm P and 1080 ppm K, according to soil analysis.

### Experimental material

Plant material: *In vitro* seedlings were provided by the International Potato Center (CIP), recovered in Jiffy-7 and then transplanted into 4-liter pots in the greenhouse for inoculation treatments. For comparative purposes, two potato cultivars and three advanced clones for processing were used to evaluate the inoculation treatments (Table 1). The seedlings were transplanted into pots after 40 days of *in vitro* culture when they reached 5 to 6 cm in height.

Table 1. Potato genotypes used in the experiment.

| Potato genotype | N° Accession | Type | Origen | Number of plantlets |
|---|---|---|---|---|
| Faustina | CIP 399101.1 | Advanced clone | CIP Perú | 20 |
| Yasmine | CIP396311.1 | Advanced clone | CIP Perú | 20 |
| Única | CIP392797.22 | Cultivar | CIP Perú | 20 |
| Bicentenaria | UH 24 | Cultivar | UNJFSC[1] | 20 |
| Huaurina | UH 09 | Advanced clone | UNJFSC[1] | 20 |

[1]Universidad Nacional José Faustino Sánchez Carrión, Huacho, Perú.

Microorganisms preparation: For the design of the experiment, inoculation was carried out with two bacterial strains *Azotobacter* sp. and *Bacillus simplex* (Table 2), obtained from the potato rhizosphere according to the bacteria isolation protocol followed by CALVO et al. (2010). The preparation of the inocula of bacterial strains was realized in a commercial nutrient broth (Britania), incubating each strain separately at 30 °C for 24 h until having a concentration of $10^8$ CFU/mL; 100 mL of the inoculum was taken and diluted in 900 mL of distilled water. On the other hand, *Trichoderma* sp. was obtained from the commercial formulation Trichops(R), and used as a matrix in the substrate.

Table 2. Description of inoculation treatments used in the experiment.

| Treatments | | Description of treatments |
|---|---|---|
| $T_0$ | NPK (control) | No inoculation, fertilization NPK 200-180-120 |
| $T_1$ | *Trichoderma* | Inoculation at planting with Trichoderma[1] |
| $T_2$ | *Trichoderma + B.simplex* | Inoculation at planting with Trichoderma + *B. simplex*[2] |
| $T_3$ | *Trichoderma +Azotobacter* | Inoculation at planting with Trichoderma + *Azotobacter* sp.[2] |
| $T_4$ | *Trichoderma + B.simplex + Azotobacter* | Inoculation at planting with Trichoderma + *B*. simplex + *Azotobacter* |

[1]Commercial strain of *Trichoderma* spp. Trichops (R). [2]Strains isolated from potato rhizosphere by Universidad Nacional José Faustino Sánchez Carrión, Huacho, Perú.

Treatment application: Inoculation with *Trichoderma* sp. was mixed with the potting substrate in a dose of 10 g per plant of commercial formulation Trichops, while *Bacillus simplex and Azotobacter* sp. inoculations were realized in a distilled water solution (10%) applied to potato seedlings after planting time and then 45 days after, as described in Table 2. Control pots (not inoculated) were fertilized with a commercial dose of nitrogen, phosphorus and potassium (NPK) using the balanced formula 200-180-120.

**Variables**

The characteristics measured during the experiment for agronomic performance under greenhouse conditions 45 days after planting were plant height (cm), number of leaves per plant, number of stems per plant, plant uniformity and vigor. Plant uniformity and vigor were evaluated on a scale of 1 to 9, where "1" corresponds to less vigorous plants and "9" to the very vigorous (BONIERBALE et al. 2010); this moment corresponded to the hilling of the potato plants in the pots.

At the harvest (110 days after planting), the traits evaluated were: tuber weight (g) per plant, tuber number per plant, tuber diameter (g) and dry weight of foliage (g); this moment corresponded to the presence of senescent leaves in the potato genotypes.

**Experimental Design and data analysis**

Experimental design used was a completely randomized design with four replicates in a 5x5 factorial arrangement (potato genotypes and inoculant treatments). The obtained results were subjected to proper ANOVA (analysis of variance) and the statistical test Scott-Knott (SK) was used to compare the mean values of the treatments at $p<0.05$; mean values are presented in tables. The test proposed by Scott-Knott is a procedure of means grouping, considered an effective option to perform multiple comparisons without ambiguity in controlled conditions (BHERING et al. 2008). Data was processed in statistical software Infostat v. 2019 (BALZARINI et al. 2014).

## RESULTS

**Main effects of microorganisms applied on potato plants in a greenhouse**

Significant increases were observed in potato growth, tuber production, and biomass after inoculation with both PGPR (Bacillus and Azotobacter) and Trichoderma-PGPR mixed culture. For agronomic traits (Table 3), the inoculation with *Trichoderma* sp. and *Azotobacter* sp. or *Trichoderma* sp. alone, obtained the highest plant height; it is necessary to state that plants were measured for plant height at the planting time, with no statistical differences.

Table 3. Effect of inoculant treatments on agronomic characteristics 45 days after planting in the greenhouse.

| Treatments | Plant height, cm | Number of leaves per plant | Plant Uniformity | Plant Vigor$^{++}$ | Number of stems per plant$^{++}$ |
|---|---|---|---|---|---|
| $T_0$ NPK (control) | 20.02 $^{b}$ | 6.45 $^{a}$ | 4.05 $^{a}$ | 1.89 $^{a}$ | 1.43 $^{b}$ |
| $T_1$ *Trichoderma* | 24.25 $^{a}$ | 7.50 $^{a}$ | 3.97 $^{a}$ | 1.80 $^{a}$ | 1.58 $^{b}$ |
| $T_2$ *Trichoderma + Bacillus simplex* | 22.02 $^{b}$ | 7.30 $^{a}$ | 3.45 $^{b}$ | 1.59 $^{b}$ | 1.77 $^{a}$ |
| $T_3$ *Trichoderma +Azotobacter* | 24.88 $^{a}$ | 7.80 $^{b}$ | 4.15 $^{a}$ | 1.58 $^{b}$ | 1.61 $^{b}$ |
| $T_4$ *Trichoderma + B.simplex+ Azotobacter* | 22.44 $^{b}$ | 6.90 $^{b}$ | 2.75 $^{c}$ | 1.50 $^{b}$ | 1.82 $^{a}$ |

$^{++}$Square root transformation. Means with the same letter in columns are not significant (p<0.05).

For the number of leaves per plant, the consortium *Trichoderma* sp. + *B. simplex* + *Azotobacte*r sp. were the best treatment as well as *Trichoderma* sp. +*Azotobacter* sp. In the case of the number of stems per plant the consortium *Trichoderma* sp. + *B. simplex* + *Azotobacter* sp. or *Trichoderma* sp. + *B. simplex* showed the highest number of leaves.

For plant uniformity and plant vigor, the responses were similar, i.e. the consortium *Trichoderma* sp. + *B. simplex* + *Azotobacter* sp. was the best treatment obtaining the highest score for uniformity or vigor, being superior to inoculation of *Trichoderma* sp. alone or the control (NPK). Combinations of strains *Trichoderma* sp. + *B.simplex* and *Trichoderma* sp. + *Azotobacte*r sp. also outperformed *Trichoderma* sp. treatment alone or control.

In Table 4, it can be shown that a higher tuber weight, tuber diameter and the number of tubers per plant were obtained significantly for the inoculation with the consortia of microorganisms or *Trichoderma* sp. alone, compared to the control.

Table 4. Effect of inoculant treatments on the yield characteristics 110 days after planting in the greenhouse.

| Treatments | Dry weight of foliage, g | Number of tubers per plant$^{++}$ | Tuber diameter, mm | Tuber weight per plant, g |
|---|---|---|---|---|
| $T_0$ NPK (control) | 8.90 $^{c}$ | 2.45 $^{b}$ | 30.67 $^{b}$ | 157.49 $^{b}$ |
| $T_1$ *Trichoderma* | 9.79 $^{b}$ | 2.97 $^{a}$ | 33.53 $^{a}$ | 254.60 $^{a}$ |
| $T_2$ *Trichoderma + Bacillus simplex* | 10.69 $^{a}$ | 2.96 $^{a}$ | 32.77 $^{a}$ | 233.36 $^{a}$ |
| $T_3$ *Trichoderma + Azotobacter* | 10.17 $^{b}$ | 2.85 $^{a}$ | 33.28 $^{a}$ | 234.74 $^{a}$ |
| $T_4$ *Trichoderma + B. simplex + Azotobacter* | 11.32 $^{a}$ | 2.94 $^{a}$ | 32.68 $^{a}$ | 250.99 $^{a}$ |

$^{++}$*Square root data transformation.* Means with the same letter in columns are not significant (p<0.05)

*Trichoderma* sp. + *B. simplex* + *Azotobacter* sp. influenced positively the dry weight of potato foliage as well as *Trichoderma* sp. + *B. simplex*; there were no significant differences between *Trichoderma* sp. or *Trichoderma* sp. + *Azotobacte*r sp. for the dry weight of foliage (Table 4).

**Interactions between potato genotypes and inoculation treatments**

From the evaluation of the factorial effects of potato genotypes and inoculation treatments in the greenhouse, there were significant interactions for plant vigor, plant uniformity and dry weight of potato foliage. Such differences can be explained as a result of crossed effects of the inoculation with *Trichoderma* sp. and PGPRs or *Trichoderma* sp. alone, with potato cultivars or advanced clones in controlled conditions.

Vigor: the control surpassed the inoculant treatments for cv. Bicentenaria and for the advanced clone UH-09. For the advanced clone Faustina there were no differences between treatments with respect to the control for vegetative vigor. For the cv. Unica the inoculation with treatment T1 surpassed in vegetative vigor the rest of the treatments (Figure 1).

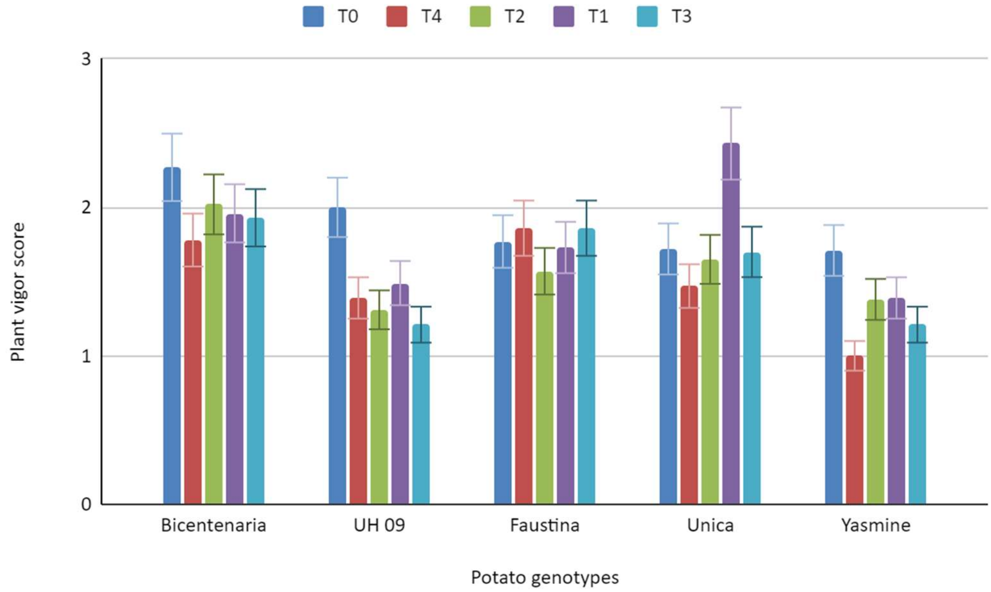


Figure 1. Effect of microbial inoculants on plant vigor (score) in five potato genotypes. Data was transformed with a square root. Plant vigor was evaluated on a scale of 1 to 9, where "1" corresponds to less vigorous plants and "9" to very vigorous. T0=control, T1= *Trichoderma* sp., T2= *Trichoderma* + *Bacillus simplex*, T3= *Trichoderma* + *Azotobacter* sp., T4= *Trichoderma* + *B. simplex* + *Azotobacter* sp.

Uniformity: For the cv. Bicentenaria and in the advanced clone UH-09, the treatments T1, T2, T3 and the control surpassed the treatment T4. For the cv. Unica there were no significant differences between treatments. For the advanced clone Faustina, treatments T1, T3 treatments and control outperformed T2 and T4 treatments. For the advanced clone Yasmine, the T3 treatment outperformed the control and T1, and these in turn outperformed T2 and T4 treatments (Figure 2).

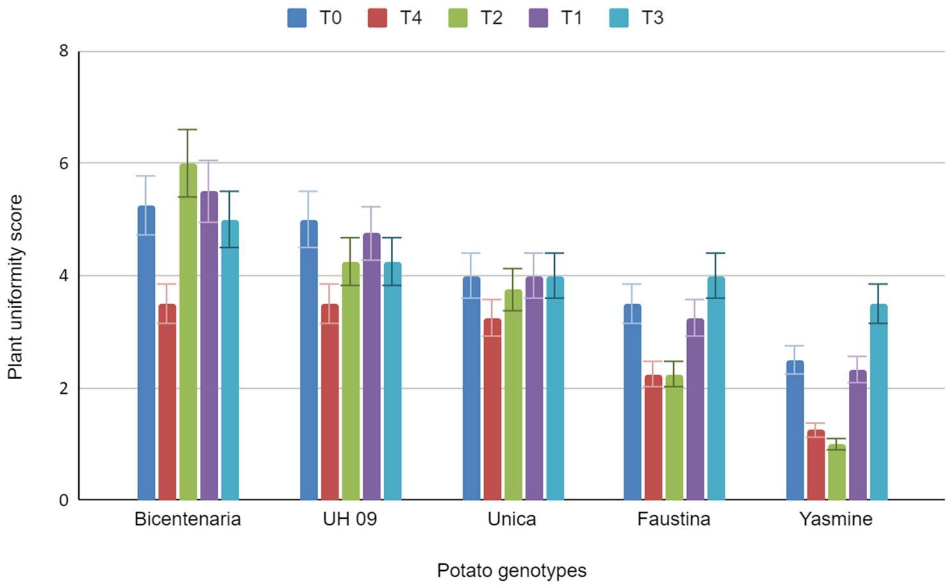


Figure 2. Effect of microbial inoculants on plant uniformity (score) in five potato genotypes. Plant uniformity was evaluated on a scale of 1 to 9, where "1" corresponds to less vigorous plants and "9" to the very vigorous. T0=control, T1= *Trichoderma* sp., T2= *Trichoderma* + *Bacillus simplex*, T3= *Trichoderma* + *Azotobacter* sp., T4= *Trichoderma* + *B. simplex* + *Azotobacter* sp.

Foliage dry weight: For cv. Bicentenaria and the advanced clone Faustina, all the inoculant treatments presented higher dry weight of foliage than the control. For the advanced clone UH-09, the treatments T1, T2 and T4 surpassed the control and treatment T3. The cv. Unica and the advanced clone Yasmine did not show significant differences in the dry weight of the foliage due to the effect of any treatment (Figure 3).

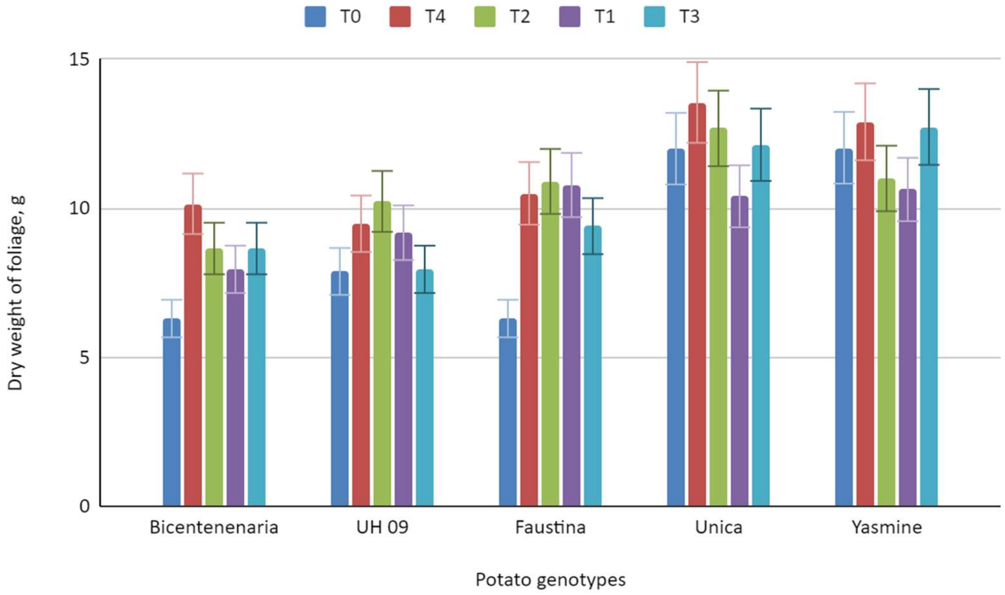


Figure 3. Effect of microbial inoculants on the dry weight of foliage (g) of five potato genotypes. T0=control, T1= *Trichoderma* sp., T2= *Trichoderma* + *Bacillus simplex*, T3= *Trichoderma* + *Azotobacter* sp., T4= *Trichoderma* + *B. simplex* + *Azotobacter* sp.

**DISCUSSION**

Inoculation with *Trichoderma* sp. and PGPR significantly improved the potato plant height and this could be a result of the production of various plant growth-promoting substances (MHATRE et al. 2019), in particular, *Azotobacter* which fix nitrogen aerobically and elaborate plant hormones (DAS 2019). Some inoculation consortia also increased the number of leaves per plant and the number of stems per plant, specifically T2 (*Trichoderma* sp. + *B. simplex*) as pointed out by OSWALD et al. (2010) who demonstrated that the mechanisms that cause the best growth of potato plants are probably a higher rate of photosynthesis. In line with this, DE PALMA et al. (2021) found that *Trichoderma* had significant stimulation effects on *Solanum lycopersicon* and after stress, allowed recovery of growth and photosynthesis to the levels of unstressed plants, suggesting some kind of physiological effect on plant growth.

In the present research, the data indicated an increase in the tuber yield and plant biomass (dry weight of foliage) as a result of the use of microbial inoculants, as other evidence from the literature suggests (DUFFY & CASSELLS 2000, DOUDS et al. 2007, CALVO et al. 2010). The contribution of this research is that under controlled conditions a higher tuber weight, tuber diameter and the number of tubers per plant were obtained significantly for the inoculation with any consortia of microorganisms or *Trichoderma* sp. alone, compared to the control. Similar results for the consortium *T. harzianum* and *B. subtilis* were found by WANG et al. (2019) by effectively increasing tuber yield, the growth of the potato plant or improving the systemic resistance of potato plants (SUSIANA et al. 2018).

The microbial diversity associated with potato plants is very broad, and the maintenance of the natural microbial population in the form of inoculants can help reduce the effects derived from monoculture, strengthening the potato production system (SESSITSCH & MITTER 2015). Moreover, soil microbial communities can help in the restoration of degraded soils, mainly regarding fertility, the capacity for nutrient retention and the availability of elements necessary for growing potatoes (TURNBULL et al. 2014). Agroecological management consists of the partial substitution of the conventional chemical inputs by microorganism-based products that can lead to an improvement of the environmental performance of the crop, which will be of interest in potato production (MAIN & FRANCO 2016); for the present research, combined effects of multiple inoculation treatments were greater than those of individual treatments or they were generally additive.

By this moment, there is sufficient scientific evidence confirmed with this research that through the symbiosis between potato plants and microorganisms, it is possible to reduce the use of high energy consumption inputs with negative environmental impact, such as fertilizers and agrochemicals, mitigating their harmful effects on the ecosystem (BRUSSAARD et al. 2007). The processes derived from the interaction between plants and microorganisms include the biological fixation of atmospheric nitrogen, solubilization and absorption of phosphorus, induced systemic resistance and plant immunity, among other physiological aspects (PAL et al. 2021).

Small-scale farmers devoted to seed tuber production can improve their production process by the use of some of the strategies suggested by this research. Small-scale potato producers can take advantage of the *Trichoderma* and PGPR consortia application, especially those that integrate *Trichoderma* + *Bacillus* or *Trichoderma* + *Azotobacter*, to improve the number, caliber and weight of tubers per plant in the potato seed system.

## CONCLUSION

Inoculation with *Trichoderma* sp. and some PGPR strains in consortia promoted the growth of *in vitro* potato seedlings, and significantly improved the potato plant height, increasing the size, caliber and weight of the seed tubers and plant biomass. In addition, there were significant interactions between potato genotypes and inoculant treatments for plant uniformity and vegetative vigor scores, as well as for the dry weight of the foliage in a greenhouse environment. According to the research results, potato seed producers could improve tuber production with the use of consortiums of *Trichoderma* sp. together with *Azotobacter* sp. and *Bacillus simplex* to increase seed tubers' weight, number and caliber under greenhouse conditions.

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