

Rhizobacteria and arbuscular mycorrhizal fungi associated with poblano chili in the Sierra Nevada of Puebla, Mexico

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Abstract

In the lower parts of the Sierra Nevada, Puebla, Mexico, small farmers grow one of the most traditional chilis in Mexican cuisine, poblano chili. The variability in agricultural practices and the characteristics of the soil lead to various microenvironments of production, causing a variation in the populations of beneficial microorganisms. The objective of the present investigation was to quantify the microbial populations [total fungi (HT), total bacteria (BT), phosphate solubilizing bacteria (BSP), nitrogen-fixing bacteria (BFN), auxin-producing bacteria (BPA) and mycorrhizal fungi arbuscular (HMA)] and fruit production in plantations of poblano chili grown in the open field. By means of serial dilutions and seeding in specific media, HT, BT, BSP, BFN and BPA were quantified. The colonization by HMA was determined by the method of thinning and staining with trypan blue. The highest populations of BT and BPA were found in site seven (Huejotzingo), soil with high phosphorus content (428.8 mg kg⁻¹), HT, BSP and BFN were better in site one (San Matias Tlalancaleca), this site did not present the best soil characteristics, but higher altitude, which correlated positively with these microorganisms. Mycorrhizal colonization was better at site nine (Huejotzingo), in a soil with low phosphorus content (44.3 mg kg⁻¹) and moderately alkaline pH (7.6). The yield of fruits was higher in site 8 (Huejotzingo), which presented higher content of organic matter (1.48%) and total nitrogen (0.07%) in soil.

Keywords: *Capsicum annuum*, nitrogen fixing bacteria, phosphate solubilizing bacteria, auxin producing bacteria.

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Introduction

The poblano chili in the state of Puebla represents an ancient cultural tradition due to its gastronomic, economic and social importance, as it is associated with dishes such as “chiles en nogada” and “mole poblano” (Cyphers *et al.*, 2009). It is produced mainly in the Sierra Nevada, in municipalities such as San Matias Tlalancaleca, San Lorenzo Chiahutzingo, San Rafael Tlanalapa, Moyozingo, Huejotzingo and San Lucas (Huerta *et al.*, 2007). In 2007, the state of Puebla presented an area cultivated with Poblano chili of 600 ha and a production of 4 800 t (SDR Puebla, 2007), in this respect Rodríguez *et al.* (2007) mention that the production of poblano chili has decreased from 25 t ha⁻¹ to less than 10 t ha⁻¹ in the last 10 years.

The decrease in the production of poblano chili is associated to several factors, and highlight the "drying" and "drowning" caused by *Phytophthora capsici*, *Fusarium* spp., *Rhizoctonia solani*, *Pythium* spp., *Alternaria* spp. the wilt caused by the nematode *Nacobbus aberrans*, causing losses of up to 100% of the crop, either in the greenhouse, in the field or in storage (Rodríguez *et al.*, 2007, Bautista-Calles *et al.*, 2010). In addition, factors such as viral diseases and physical damage to crops caused by insects (aphids, mites and thrips) are added (Huerta *et al.*, 2007), as well as the availability of nutrients and water (Mena-Violante *et al.*, 2006). According to Huerta *et al.* (2007) the poblano chili of the Sierra Nevada is better suited to soils with sandy-silty texture than in clay soils, so excess moisture should be avoided.

According to the FAO classification, the soils that predominate in the study area are regosols, cambisols, feozems and fluvisols (INEGI, 2017), unlike the first two, the soils of the feozem and fluvisol types have high and moderate levels of organic matter (FAO, 2006, Galicia *et al.*, 2015). High levels of organic matter induce greater soil fertility, due to high nutrient contents and organic carbon; The latter, as an energy source, allows a greater diversity of bacteria, fungi, nematodes, earthworms, insects and arthropods that interact synergistically in the ecosystem (Mader *et al.*, 2002; Oehl *et al.*, 2002). But when organic matter is low, there is low organic carbon, low fertility and greater attack by phytopathogens when using crops as their source of food, according to Ghorbani *et al.* (2010), nitrogen fertilization in soils with a low amount of organic matter, favors the development of pathogens such as *Rhizoctonia* sp., *Fusarium* sp., *Sclerotium* sp., etc.

The soil is the physical substrate for the life of terrestrial animals (including humans) and a means for the growth of plants, dampens water flows, decomposes and releases nutrients and allows the regulation of emissions of greenhouse gases (Stott and Taylor, 2016). The microorganisms that live in the soil are intimately associated with all these functions (Aislabie and Deslippe, 2013). The microbiotic are found in higher density near the roots of plants, where there is greater availability of exudate organic compounds (Massensini *et al.*, 2014), the quality of these exudates allows the recruitment of certain microorganisms that will have a positive, neutral effect or negative on the plant, potentially determining the composition of plant communities (Wolfe and Klironomos, 2005).

The most studied groups of organisms are plant growth promoting rhizobacteria (RPCV) and arbuscular mycorrhizal fungi (HMA), due to the benefits they provide to plants. The RPCV provide nutrients to plants by fixing and solubilizing elements such as atmospheric nitrogen,

phosphorus and potassium; inhibit the development of phytopathogens and synthesize growth regulators such as auxins, gibberellins and cytokinins (Hariprasad and Niranjana 2009, Sandhya *et al.*, 2010). The HMA favor the absorption of water and nutrients, and increase the tolerance to stress caused by biotic and abiotic factors (Perner *et al.*, 2007, Sawers *et al.*, 2008). The objective of the research was to quantify the populations of RPCV and HMA in plantations of poblano chili cultivated in the open field and their possible effects on crop yield.

Materials and methods

In September 2012, rhizospheric chili poblano soil was collected in nine sites of the Sierra Nevada region in the state of Puebla, Mexico. The sampling included poblano chili fields from three municipalities: San Matias Tlalancaleca with three sites, whose altitudes were from 2 414 to 2 467 meters above sea level, San Lorenzo Chiautzingo with two sites at altitudes of 2 404 and 2 425 meters above sea level, and Huejotzingo with four sites of altitudes ranging from 2 284 to 2 313 meters above sea level (Table 1 and Figure 1).

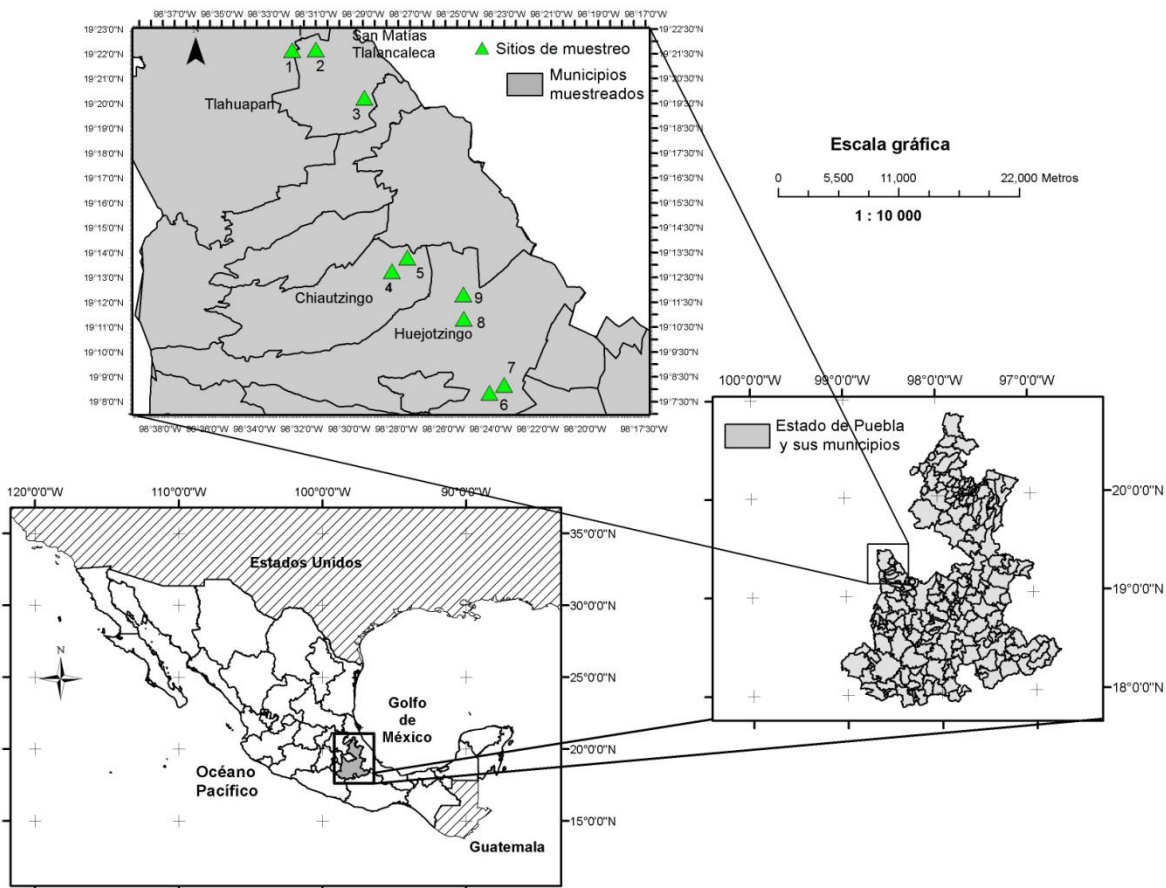


Figure 1. Geographic location of the nine sites where rhizospheric soil samples of poblano chili were collected in the Sierra Nevada, Puebla State, Mexico.

Four samples were collected per site, which included soil and plants of poblano chili selected visually for their best appearance (health and vigor), they were kept in a cooler for their transfer to the microbiology laboratory of the Montecillo Campus of the Postgraduate School, Montecillo, State from Mexico. In the sampling sites the pH values (soil, water 1:2) fluctuated between 4.9 to 7.6. The organic matter (MO) values were from 0.27 to 1.48%, the total nitrogen (NT) from 0.01 to .07%, the phosphorus (P) from 41.1 to 428.8 mg kg⁻¹ and of potassium (K) from 242 to 854 mg kg⁻¹. Soil texture was classified as sandy loam at sites one, three, four and five, sandy loam for site two and loamy sand for sites six, seven, eight and nine (Table 1).

Table 1. Soil characteristics and location of study sites in the Sierra Nevada region of Puebla, Mexico.

Sites	Localization	Altitude (m)	pH	MO	NT	P	K	Textural
San Matías Tlalancaleca								
1	19°22.313' N y 98°32.032' W	2467	5.5	0.27	0.01	184.7	412	FA
2	19°22.257' N y 98°31.924' W	2453	5.8	0.54	0.03	224	324	FAA
3	19°20.665' N y 98°29.209' W	2414	6.8	1.48	0.07	314.5	854	FA
San Lorenzo Chiautzingo								
4	19°13.440' N y 98°28.903' W	2425	6.9	0.94	0.05	153.7	546	FA
5	19°13.052' N y 98°28.261' W	2404	4.9	0.27	0.01	187.1	242	FA
Huejotzingo								
6	19°08.504' N y 98°24.700' W	2313	5.8	0.4	0.02	41.5	266	AF
7	19°08.968' N y 98°24.430' W	2292	6.7	0.94	0.05	428.8	508	AF
8	19°11.985' N y 98°25.711' W	2286	7.3	1.48	0.07	50.1	430	AF
9	19°12.295' N y 98°25.510' W	2284	7.6	0.67	0.03	44.3	410	AF

MO= organic matter (%); NT= total nitrogen (%); P= phosphorus (mg kg⁻¹); K= potassium (mg kg⁻¹); FA= sandy loam; FAA= loam clay sandy; AF= loamy sand.

The collected samples were processed in a laminar flow hood under aseptic conditions. The 10 g of sample was weighed and placed in bottles with 90 mL of sterile distilled water (first dilution) and from it serial decimals dilutions were made (up to 10⁻⁴). In Petri dishes, 0.1 mL of each dilution was seeded and the aliquot was distributed on the surface of the solid medium with the help of an L-shaped glass rod. Specific culture media were used: nutritive agar for total bacteria (BT), Pikovskaya, to detect phosphate solubilizing bacteria (BSP), Rennie, for nitrogen fixers (BFN), Luria-Bertani (LB), for auxin-producing bacteria (BPA) and PDA medium (potato dextrose agar) for total fungi. The colonies were quantified after three days of incubation at 28 °C.

The number of fruits was quantified in poblano chili plants at the time of collection in the field, the roots of the plants collected from poblano chili were evaluated for colonization by HMA following the method of thinning and staining with trypan blue (Phillips and Hayman, 1970), total colonization (PCT) was estimated in roots of 1 cm in length expressed in percentages (Biermann and Linderman, 1981). A part of the rhizospheric soil was used for the extraction and quantification of HMA spores by means of the wet sieving and decanting technique (Gerdemann and Nicolson, 1963), expressed in 100 g of dry soil (100 g). The data obtained were analyzed with the SAS statistical package for Windows (SAS Institute Inc. 2002), performing an analysis of variance and means comparison test (Tukey, $\alpha= 0.05$).

Results and discussion

The populations of microorganisms found at each site were statistically different (Tukey $\alpha=0.05$) (Table 2). Site seven, whose sample was collected in the municipality of Huejotzingo, presented the highest populations of BT (53×10^5 UFC g^{-1} soil) and BPA (92×10^4 UFC g^{-1} soil), while the site one of San Matías Tlalancaleca had the highest populations of total fungi (82×10^2 UFC g^{-1} soil), BSP (46×10^4 UFC g^{-1} soil) and BFN (36×10^4 UFC g^{-1} soil). The lowest populations were found in sites one for BT (10×10^5 UFC g^{-1} soil), nine for HT (3×10^2 UFC g^{-1} soil), three for BSP (1×10^4 UFC g^{-1} soil), five and eight for BPA (4×10^4 UFC g^{-1} soil) and five and six for BFN (10×10^4 UFC g^{-1} soil) (Table 2).

Table 2. Colony forming units (UFC) quantified in rhizospheric soil of poblano chili collected in nine sites of the Sierra Nevada de Puebla, Mexico.

Sites	BT	BFN	BSP	BPA	HT
	10^5 UFC g^{-1} suelo		10^4 UFC g^{-1} soil		10^2 UFC g^{-1} soil
San Matías Tlalancaleca					
1	10.3 \pm 2.2a	36 \pm 13.2a	46 \pm 13.7a	7 \pm 1.7bc	82 \pm 33.2a
2	11.2 \pm 3.8a	18 \pm 6.2ab	36 \pm 22.5ab	6 \pm 1.1bc	56 \pm 12.8ab
3	16 \pm 5.3a	11 \pm 1.5b	1 \pm 0.05c	22 \pm 2.8b	26 \pm 2.3bc
San Lorenzo Chiautzingo					
4	26.1 \pm 1.6a	12 \pm 2ab	8 \pm 1.5ab	10 \pm 2.2bc	7 \pm 2.5c
5	20.4 \pm 3.7a	10 \pm 1.3b	20 \pm 9.5ab	4 \pm 0.8c	10 \pm 2.5c
Huejotzingo					
6	27 \pm 6.7a	10 \pm 1.3b	26 \pm 8.6ab	5 \pm 1.8c	7 \pm 2.7c
7	53.2 \pm 27.4a	15 \pm 2.7ab	6 \pm 2.4b	92 \pm 42a	19 \pm 4.9bc
8	14.5 \pm 1.2a	11 \pm 1.2b	9 \pm 1.3ab	4 \pm 0.8c	6 \pm 0.9c
9	16.4 \pm 2a	14 \pm 1ab	9 \pm 0.7ab	11 \pm 1.9bc	3 \pm 0.7c

BT= total bacteria; HT= total fungi; BSP= phosphate solubilizing bacteria; BPA= auxin-producing bacteria and BFN= nitrogen-fixing bacteria. Different letters within the same column present significant statistical differences (Tukey, $\alpha=0.05$, a> b). Means n= 8, \pm standard error.

The densities of HT, BFN and BSP showed positive and statistically significant correlation ($\alpha=0.05$) with the altitude of the different sites studied (Table 3), which indicates that the densities of these rhizobacteria were increased in the higher altitude sites. In addition, altitude was negatively correlated with pH, organic matter and nitrogen content, and positively with the phosphorus content in soil, factors that determined the high densities of microorganisms at sites seven and one. The characteristics of the soil influenced the microbial populations evaluated, due to the fact that a negative and significant correlation ($\alpha=0.05$) was observed between the HT population and the pH of the soil; negative and significant correlation ($\alpha=0.01$) between BSP and pH, MO, NT and K and positive and significant correlation ($\alpha=0.01$) with BPA and P content (Table 3).

Table 3. Correlations between the number of microorganisms and soil characteristics in poblano chili collected in nine sites of the Sierra Nevada de Puebla, Mexico.

	Altitude	pH	MO	NT	P	K	Fruits
BT	-0.191	0.047	0.025	0.059	0.231	0.028	0.016
HT	0.47**	-0.289*	-0.212	-0.206	0.22	0.007	0.108
BFN	0.248*	-0.134	-0.196	-0.202	0.067	-0.031	0.109
BSP	0.238*	-0.337**	-0.36**	-0.356**	-0.093	-0.298*	-0.086
BPA	-0.2	0.134	0.131	0.168	0.457**	0.181	0.102
CT	-0.693**	0.538**	0.066	-0.074	-0.246*	-0.039	0.248*
V	-0.157	-0.163	-0.186	-0.149	0.004	-0.196	-0.136
E	-0.325**	-0.077	-0.18	-0.116	0.364**	-0.266*	-0.455**
Altitude	1	-0.55**	-0.274*	-0.258*	0.255*	0.126	-0.142
Fruits	-0.142	0.65**	0.56**	0.561**	-0.11	0.46**	1

BT= total bacteria; HT= total fungi; BFN= nitrogen-fixing bacteria; BSP= phosphate solubilizing bacteria; BPA= auxin-producing bacteria; CT= total colonization; V= vesicles; E= spores; MO= organic matter (%); NT= total nitrogen (%); P= phosphorus (mg kg⁻¹); K= potassium (mg kg⁻¹). *Significant correlation $\alpha=0.05$ and ** $\alpha=0.01$.

There are few studies on altitude and their influence on the distribution of soil microorganisms are contradictory, and have been related to factors of climate, soil, vegetation and biotic factors (Hofmann *et al.*, 2016). For example, the diversity of *Phylum acidobacteria* decreases at higher altitudes and is attributed mainly to soil pH (Bryant *et al.*, 2008). The bacterial abundance found by Hofmann *et al.* (2016) decreased with altitude, and attributed to the influence that altitude has on the temperature and soil organic matter. Weyens *et al.* (2009), mention that soil microorganisms are influenced by characteristics such as humidity, texture, pH, organic matter, nutritional content, temperature and salinity; they can also be influenced by factors such as the plant species, age and nutritional status of the plant (Adeboye *et al.*, 2006).

Soil ecosystems are highly complex due to the great diversity of microbial species that they harbor, which can be harmful or beneficial for plants. The beneficial microorganisms, such as those evaluated in this study, are classified as RPCV due to the functions they perform, for example the biological fixation of N₂ (BFN) and the solubilization of phosphorus (BSP) to make them available to plants in the form of ammonium and inorganic phosphates (Herridge *et al.*, 2008, Restrepo-Franco *et al.*, 2015); In addition, they promote the production of auxins (BPA) that directly influence the development of plants through cell elongation and division, tissue differentiation and apical dominance (Duca *et al.*, 2014). Additionally, RPCV allow the biological control of pathogens (Sandhya *et al.*, 2010), the degradation of organic matter, the adaptation of plants to contaminated soils, drought conditions and extreme pH values (Saraf *et al.*, 2011) and the synthesis of growth regulators such as ethylene, auxins and gibberellins (Kim *et al.*, 2010).

The roots of the chili plants form symbiotic associations with the arbuscular mycorrhizal fungi (Davies Jr *et al.*, 1992), which is confirmed in this study, since the poblano chili was colonized by the HMA between 5 and 68%, existing significant statistical differences between the nine sites sampled (Tukey $\alpha= 0.05$). The highest colonization (68%) was located in site nine, which corresponds to the collection of soil made in the municipality of Huejotzingo, the lowest value was found in site five, municipality of San Lorenzo Chiautzingo (Table 4). The high percentage of colonization of site nine, possibly related to the low content of P (44.3 mg kg^{-1}) found at the site, as opposed to site five which was much higher (187.1 mg kg^{-1}). According to Javaid (2009), HMA efficiently colonize plants when soils have low phosphorus content.

Table 4. Colonization by HMA in poblano chili plants collected in nine sites of the Sierra Nevada de Puebla, Mexico.

Sites	Total colonization (%)	Vesicles (%)	Spores 100 g ss	Fruits plant ⁻¹
San Matías Tlalancaleca				
1	8 ±3cd	1 ±0.5b	195 ±12d	6 ±0.3ab
2	12 ±4cd	1.5 ±0.9ab	477 ±55bc	5 ±0.6ab
3	16 ±7cd	0.3 ±0.3b	235 ±60d	6 ±0.9ab
San Lorenzo Chiautzingo				
4	23 ±12bcd	0.7 ±0.7b	486 ±60bc	5 ±0.4ab
5	5 ±2d	0.3 ±0.3b	654 ±107b	0 ±0c
Huejotzingo				
6	44 ±2b	5.2 ±3.1a	447 ±34c	4 ±0.8b
7	43 ±5b	2.8 ±1.5ab	896 ±98a	4 ±0.8b
8	27 ±7bc	0.4 ±0.4b	355 ±24cd	7 ±0.9a
9	68 ±10a	0 ±0c	483 ±71bc	5 ±0.8ab

Different letters within the same column, present significant statistical differences. Means $n= 4 \pm$ standard error (Tukey, $\alpha= 0.05$, $a > b$).

The presence of vesicles was relatively low, being the site six with the highest presence of these mycorrhizal structures with 5.2%, while site nine did not present vesicles. The highest number of spores in soil was found in site seven with 896 spores in 100 g, while in site one the smallest amount was found with 195 spores. The CT showed positive and significant correlation ($\alpha= 0.01$) with the pH and the number of spores with the P of the soil (Table 3), while the altitude influenced the CT and the spores present of HMA when presenting negative and significant correlation ($\alpha= 0.01$), indicating that at higher altitude there is less mycorrhizal colonization. The P presented negative correlation with the CT and positive with the number of spores, which indicates that the lower the P content, the higher the CT and as the P increases, the number of spores will increase.

Factors such as the altitudinal gradient, physical (texture, structure, porosity) and chemical characteristics of the soil (pH, organic matter, nutrients) (Coutinho *et al.*, 2015) and human activities (Dumbrell *et al.*, 2010) influence the diversity, colonization and number of spores present of HMA. The management of crops with fertilizers or herbicides affects the mycorrhizal symbiosis (Pasaribu *et al.*, 2011), causing a decrease in the diversity and abundance of spores (Oehl *et al.*, 2004). In the region where poblano chili is grown in the state of Puebla, no herbicides are used, so the variation in microbial populations is due to other factors.

The HMA-plant symbiosis is estimated to occur between 70-90% of terrestrial plant species, in agricultural ecosystems, as in this study, or in natural ecosystems (Smith and Reed, 2008; Zhu *et al.*, 2010). The HMA are obligate symbionts and acquire carbon from their host plants to complete their life cycle (Bago *et al.*, 2000); in return, the fungus provides several benefits for the plant, for example, increased absorption of nutrients mainly P, N and K (Perner *et al.*, 2007), Zn, Cu, Fe, S, Ca (Allen, 2009), Mg and B (Subramanian *et al.*, 2006; Altomare and Tringovska, 2011) and tolerance to stress caused by biotic and abiotic factors (Sawers *et al.*, 2008).

The quantified amount of fruits per plant presented significant statistical differences (Tukey $\alpha=0.05$), being the best site eight with seven fruits produced by plants on average, followed by sites one and three with six fruits, respectively. Site five did not present healthy fruits due to the presence of fungal diseases in the crop, which did not allow the phenological stage of fruiting and fruit development to be reached. The CT by HMA presented positive and significant correlation ($\alpha=0.05$) with the production of fruits per plant, indicative that the higher the CT, the higher the fruit yield; however, the soil characteristics also influenced this variable, since the plants with the highest fruit produced were found in those sites with better characteristics of pH, MO, NT and K, obtaining a significant correlation ($\alpha=0.01$) of these variables with the fruit produced by the plant (Table 3).

The fruit yield, found in each of the sites, was affected by soil characteristics (pH, MO, NT and K), which in turn were affected by the altitude of the sites. The number of fruits obtained was not very high because the sampling was done before the plant completed its reproductive cycle, however, in all the sites the phenological stage in which the poblano chili crops were found was very similar, which allowed to evaluate to a large extent the production of fruits that was had per plant and to compare between sites.

Conclusions

The present study highlights the importance of poblano chili culture and its association with soil microorganisms, mainly those that are beneficial for the soil-plant ecosystem. The altitudinal gradient of the sites was a determining factor in the chemical properties of the soil and in the distribution of the bacterial communities evaluated in this study.

The problems faced by poblano chili in the Sierra Nevada can be neutralized by decreasing the use of chemical products, which would imply less soil erosion and the maintenance of bacterial colonies, which could favor crop yield. However, additional studies are required to select these beneficial microorganisms that, when inoculated as biofertilizers, promote the growth of the plant and its adaptation to the field, when these are transplanted.

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