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DISPONIBILIDAD DE FÓSFORO Y SU ABSORCIÓN EN TRIGO, EN SUELOS DE ORIGEN VOLCÁNICO INOCULADOS POR BACTERIAS SOLUBILIZADORAS DE FOSFATO
(PHOSPHORUS AVAILABILITY AND ITS UPTAKE IN WHEAT, IN VOLCANIC SOILS INOCULATED WITH PHOSPHATE SOLUBILIZING BACTERIA)

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TABLA DE CONTENIDOS

ÍNDICE DE FIGURAS Y TABLAS

DISPONIBILIDAD DE FÓSFORO Y SU ABSORCIÓN EN TRIGO, EN SUELOS DE ORIGEN VOLCÁNICO INOCULADOS POR BACTERIAS SOLUBILIZADORAS DE FOSFATO

Phosphorus availability and its uptake in wheat, in volcanic soils inoculated with phosphate solubilizing bacteria

Palabras adicionales: Solubilización de fosfato; rizósfera; disponibilidad de fósforo; suelos volcánicos; *Bacillus thuringiensis*.

RESUMEN

La utilización de bacterias solubilizadoras de fosfato (PSB) es una estrategia ecológica que permite aumentar la disponibilidad de fósforo (P) y la absorción de P por las plantas, lo cual es muy importante en suelos volcánicos con alta capacidad de fijación de P. El objetivo de este trabajo fue evaluar la disponibilidad de P y su absorción por las plantas de trigo en diferentes fases de desarrollo en suelos derivados de cenizas volcánicas (Andisol sitio I y Ultisol sitio II) inoculados con PSB (*Bacillus thuringiensis)*. El trabajo fue realizado en macetas, condiciones de invernadero, el diseño experimental utilizado fue completamente al azar, las plantas de trigo fueron inoculados y re-inoculados a los 20 y 46 días después de la siembra (DDS), respectivamente, con PSB del genero *B. Thuringiensis.* Se realizaron muestreos de suelo y planta pasado los 46, 66 y 87 días de la escala de Zadoks (Z). La inoculación con *B. thuringiensis* incrementó significativamente en un11% el contenido de P de la rizósfera (Z46, Sitio I), 34 y 67% la concentración de P de los tejidos aéreos (Z46, Sitio I y II), 26% P en el tejido radical (Z87, sitio II). También incrementó la actividad de la enzima fosfatasa ácida, la biomasa microbiana del suelo (sitio I y II) y la biomasa radical de las plantas (sitio II), sin lograr incremento de la biomasa de aérea de las plantas. Los resultados pueden permitir el desarrollo de una nueva estrategia agronómica para mejorar la mineralización de P proveniente del *B. thuringiensis* aplicado en el suelo.

ABSTRACT

The use of phosphate solubilizing bacteria (PSB) is an ecological strategy that allows increasing the availability of phosphorus (P) and its uptake by plants, which is very important in volcanic soils with high P fixing capacity. The objective of this study was to evaluate the availability of P and P uptake in wheat at different plant growth stages, in soils derived from volcanic ash (Andisol site I and Ultisol site II) inoculated with PSB (*Bacillus thuringiensis*). The experiment was conducted in pots under greenhouse conditions using a completely randomized design. Once wheat plants were inoculated and re-inoculated at 20 and 46 days after sowing (DAS) respectively, with *B. thuringiensis*. Soil and plant sampling was performed after 46, 66 and 87 days based on the Zadoks growth scale (Z). The inoculation resulted in an increase of 11% P for rhizosphere (Z46, Site I). Also, increased 34 and 67% P concentration in aerial tissues at Z46 growth stage in (site I and II), respectively, while an increase of 26% was observed in root tissues at Z87 in (site II). Similarly, the inoculation resulted in increases in acid phosphatase activity, soil microbial biomass and root biomass in plants, without achieving increase of the aerial biomass of the plants. The results may develop a novel agronomic strategy for improving the P mineralization from *B. thuringiensis* applied to soils.

CAPÍTULO 1.

INTRODUCCIÓN GENERAL

INTRODUCCIÓN

El fósforo (P) es uno de los macronutrientes esenciales para el crecimiento y desarrollo de las plantas y está involucrado en varios procesos metabólicos tales como: la división celular y desarrollo; transporte de energía; fotosíntesis y otros (Khan et al., 2014). Aunque el P es abundante en los suelos en ambas formas, orgánicas (Po) e inorgánicas (Pi), su deficiencia es una seria preocupación para la productividad, y que afecta a 42% de los suelos cultivados del mundo (Liu et al., 1994).

Los suelos derivados de cenizas volcánicas tienen una gran importancia en la economía de muchos países. En Chile los suelos volcánicos están presentes en la mayor parte de la actividad agrícola y forestal, cubriendo más de $5{,}3X10^{6}$ ha^{-1} y representan cerca del 50-60% de los suelos arables del país (Besoain, 1985). Según Shoji *et al*. (1993), los suelos derivados de cenizas volcánicas poseen propiedades que los distinguen de suelos formados de otros materiales como: alta retención de agua, carga variable, baja densidad aparente, alta friabilidad y estabilidad de los agregados y alta retención de P. Los mecanismos de retención de P en estos suelos están asociados a reacciones de adsorción con minerales del suelo como óxidos de hierro (Fe), aluminio (Al) y arcillas no cristalinas (alofan y imogolita), además de complejos con la materia orgánica (MO) (Dahlgren *et al.,* 2004; Takahashi y Dahlgren, 2016). Una característica común de estos suelos, es que son muy productivos. Sin embargo, presentan deficiencias importantes de algunos nutrientes como P, cuya disponibilidad disminuye con un aumento en el desarrollo del suelo, debido a la retención de este nutriente (Dahlgren *et al.,* 2004; Takahashi y Dahlgren, 2016). Aunque estos suelos aportan cantidades elevadas de P total, sólo una pequeña fracción se encuentra disponible para las plantas, limitando así el rendimiento de los cultivos. De esta forma, se entiende que se deba agregar cantidades altas de fertilizantes fosforados y de manera localizada, para cubrir los requerimientos de las plantas y obtener buenos rendimientos (Sadzawka y Carrasco, 1985). Por otro lado, las fuentes de fertilizantes y yacimientos de fosfatos (roca fosfórica) son limitadas y no serán suficientes en el futuro para la demanda agronómica (Abelson, 1999). Sin embargo, la utilización de bacterias solubilizadoras de P (PSB) podría ser una alternativa para aumentar el suministro de este nutriente en las plantas, dado que las continuas aplicaciones de P en cantidades que exceden lo absorbido por

los cultivos, tienen como resultado una inevitable acumulación de P insoluble en el suelo. En este contexto, la fijación de P, es quizás una de las mayores limitantes agronómicas en los suelos volcánicos (Borie y Rubio, 2003).

En los últimos años se ha constatado un mayor interés en la agricultura por el bajo uso de insumos, lo que consecuentemente ha proporcionado un creciente desarrollo y uso de inoculantes biológicos comerciales (bacterias o hongos) para aumentar la movilización de nutrientes. (Owen et al., 2015). Estos microorganismos también podrían aumentar la absorción de nutrientes en las plantas, lo que reduce la necesidad de fertilizantes y previene su acumulación en los suelos agrícolas. Una reducción en el uso de fertilizantes disminuye los efectos de la contaminación a través del agua de escorrentía y promueve un ahorro de recursos para los agricultores. Alguna de las alternativas que se están utilizando son los biofertilizantes y bioestimuladores microbianos, que constituyen un medio ecológicamente aceptable para reducir insumos externos y mejorar la cantidad y calidad de los recursos internos (Fang et al., 2013). Se han señalado incrementos en la biodisponibilidad de P en el suelo cuando existen aumentos paralelos en la actividad microbiana (Estrada et al., 2012). Esta liberación de P insolubles a formas disponible para las plantas se obtiene mediante procesos de: i) acidificación por liberación de protones (H^+) o producción de ácidos inorgánicos fácilmente disociables; ii) disolución de fosfatos mediada por enzimas a través de la liberación extracelular de enzimas específicas (fosfatasas); iii) quelación de los elementos responsables de la insolubilidad de los fosfatos presentes; iv) asimilación directa de fosfatos insolubles o reducción del Fe v) Y la producción de ácidos orgánicos (AO), que reaccionan con aniones fosfato fijados, lo que permite su solubilización de formas no disponibles de P, para convertirlas en asimilables por las plantas (Wickramatilake et al., 2010; Khan et al., 2014).

En general, cuando se aplica fertilizantes fosforados sintéticos a este tipo de suelos, un rango entre el 5 y el 25% del P aplicado es utilizado por las plantas, donde la otra porción (75-95%) no es absorbido por los cultivos en el primer año, siendo fijado o retenido en formas insolubles por los coloides del suelo, principalmente complejos activos de Al, Fe y Ca (Stevenson y Cole, 1999). Una porción de este P residual es obtenida y utilizada por cultivos siguientes, pero a menudo son requeridas nuevas aplicaciones de fertilizantes para mantener altas

tasas de producción. Así, al utilizar microorganismos seleccionados como bacterias solubilizadoras de P (PSB, de su sigla en inglés) se aporta a los cultivos P disponible a partir de formas escasamente solubles en el suelo y sustancias fisiológicamente activas que, al interactuar con la planta, desencadenan una mayor actividad del metabolismo vegetal (Chaturvedi, 2006). En los sistemas de agricultura sustentable, el manejo de microorganismos a través de la producción de biofertilizantes, reduce o elimina el uso de fertilizantes tradicionales, mejorando la productividad de los cultivos (Douds et al., 2007).

El trigo (*Triticum aestivum* L.) es el segundo cereal más producido en el mundo después del arroz (trnka et al., 2014). En Chile, el trigo es uno de los cultivos más importantes a nivel nacional, presentando gran importancia socioeconómica, por ser cultivado mayoritariamente por pequeños productores (Larraín y Olfos, 2012). La productividad nacional de trigo se sustenta en el empleo de variedades de alto potencial de rendimiento, que requieren un uso intensivo de insumos como fertilizantes, enmiendas calcáreas y pesticidas (Fundación Chile, 2005). Por otro lado, una superficie relevante de la producción de trigo se localiza en suelos de origen volcánico. La alta tasa de absorción de P en las primeras etapas del ciclo de vida del cultivo de trigo no es una expresión de lujo, sino que refleja un alto requerimiento de P en las plantas (Römer y Schilling., 1986). La demanda de P por las plantas del trigo se inicia desde la primera etapa de desarrollo vegetativo (establecimiento) de la planta, desarrollo reproductivo hasta la fase de madurez del cultivo (antesis y formación del grano), porque después el P es translocado en el interior de la planta, pasando de los tejidos viejos a los jóvenes, donde la semilla (grano) se convierte en el sumidero final de P (Batten et al., 1986., Rubya y Iqbal., 2016).

Conociendo la problemática del P en suelos volcánicos, la aplicación de bacterias solubilizadoras de P como *Bacillus thuringiensis*, contribuiría a aumentar la disponibilidad de este nutriente para las plantas, y reducir la necesidad de aplicación de P como fertilizante sintético, y el efecto medioambiental asociado a excesos de estas aplicaciones.

1.1. HIPOTESIS

Bacterias solubilizadoras de P (PSB) aumentan la disponibilidad de este nutriente para los cultivos en suelos de origen volcánico, caracterizados por su alta retención de P.

1.2. OBJETIVO GENERAL

Evaluar la disponibilidad de P y su absorción en trigo en diferentes estados de desarrollo, en suelos derivados de cenizas volcánicas en dos suelos de diferente orden taxonómico (Andisol y Ultisol), inoculados con bacterias solubilizadoras de P (*Bacillus thuringiensis).*

1.2.1. OBJETIVOS ESPECÍFICOS

i. Evaluar los efectos del *B. thuringiensis* en la disponibilidad P y el pH en dos suelos de diferente orden taxonómico (Andisol y Ultisol).
ii. Determinar el contenido de P absorbido por plantas de trigo creciendo en dos suelos de diferente orden taxonómico (Andisol y Ultisol) inoculado con *B. thuringiensis*.
iii. Evaluar la actividad y cantidad de los microorganismos en suelos inoculados con *B. thuringiensis*.
iv. Cuantificar el efecto de la aplicación del *B. thuringiensis* en dos suelos de diferente orden taxonómico (Andisol y Ultisol) el rendimiento de las plantas de trigo.

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CAPÍTULO 2.

Phosphorus availability and its uptake in wheat, in volcanic soils inoculated with phosphate solubilizing bacteria

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ABSTRACT

The use of phosphate solubilizing bacteria (PSB) is an ecological strategy that allows increasing the availability of phosphorus (P) in soil. The objective of this study was to evaluate the availability of P in soils derived from volcanic ash (Andisol, site I; and Ultisol, site II) inoculated with *Bacillus thuringiensis* and P uptake in wheat plant. The experiment was conducted in pots under greenhouse conditions using a completely randomized design. Wheat plants were inoculated and re-inoculated at 20 and 46 days after sowing (DAS), respectively, with *B. thuringiensis*, and soil and plant sampling was performed after 46, 66 and 87 days based on the Zadoks growth scale (Z). The inoculation resulted in an increase of 11% P for the rhizosphere (Z46, site I). Also, increased 34 and 67% P in aerial tissues at Z46 (site I and II), respectively, while an increase of 26% was observed in root tissues at Z87 (site II). Similarly, the inoculation resulted in increases in acid phosphatase activity, soil microbial biomass and root biomass in plants, without achieving increase of the aerial biomass of the plants. The results may develop a novel agronomic strategy for improving the P mineralization from *B. thuringiensis* applied to soils.

1. INTRODUCTION

Phosphorus (P) is one of the most important macronutrients for plant growth and development in fact, it forms part of the ATP molecules and plays a decisive role in the DNA chain [1]. However, availability of P is limited for most natural ecosystems and plant growth [2]. P is particularly important in Chilean volcanic soils with high phosphorus retention or fixation [3]. In general, when synthetic phosphorus fertilizers are applied to this type of soils, between 5 and 25% of the applied P is absorbed by plants, while the remaining (75-95%) is fixed or retained in insoluble forms by soil colloids, active complexes of Al, Fe, Ca and organic matter [4], and then used by subsequent crops. Thus, new fertilizer applications are required to maintain high production rates. Chilean volcanic soils present high contents of total P and organic matter, and high acidity. These type of soils, classified as Andisols, are the least extensive soil order [5] and occupy only 0.7% of the earth's land surface or just below 963,000 km^2. Nevertheless, in Chile, volcanic soils support the bulk of agricultural and forestry production, covering more than $5.3x10^6$ hectares and representing nearly 50-60% of the country's arable land [3].

Wheat (*Triticum aestivum L.*) is one of the most important crops in Chile and it is mainly produced by small farmers [6]. Most of Chilean wheat production is obtained from volcanic soils, while wheat productivity is based on the use of varieties with high yield potential, which requires the intensive use of inputs, such as fertilizers, calcareous amendments and pesticides [7]. The high P uptake rate in the early growth stages of wheat demands a high P amount from soil [8]. P uptake by wheat starts from the first stage of the plant vegetative development (establishment) of the plant, and continues through the reproductive development until the maturity stage of the crop (anthesis and grain filling). This occurs because P is transported inside the plant passing from old to young tissues, and finally, the grain becomes the final supply of P [9]. However, fertilizers applications can have a negative effect on the environment if they are not properly managed, i.e. increasing losses by leaching, runoff of nutrients, especially nitrogen (N) and P. Some of the reasons for these problems are low use efficiency of fertilizers and the continuous long-term use [10].

In this regard, soil microorganisms, such as bacteria, have a potential role in developing sustainable systems for plant growth [11]. The use of biofertilizers and microbial biostimulators is considered an environmentally acceptable practice to reduce external inputs and improve the quantity and quality of internal resources [12]. In fact, microorganisms such as phosphate solubilizing bacteria (PSB) can provide crops with available P from barely soluble forms in the soil and physiologically active substances that trigger a higher metabolic activity once they interact with the plant [10]. Therefore, the use of PSB would be an alternative to increase P availability to plants and the supply of this nutrient in plants [1]. On the other hand, continuous applications of P at rates exceeding plant uptake result in an accumulation of insoluble P in the soil [10].

Among the different strategies adopted by microorganisms to solubilize P, the involvement of low-molecular mass organic acids (OA) secreted by microorganisms has been a well-recognized and widely accepted theory as a primary means of P solubilization, and several studies have identified and quantified organic acids and define their role in the solubilization process [13,14]. Acidification of the microbial cells and their surroundings allows the release of P ions from the mineral P by replacing H^+ by Ca^{2+}. However, solubilization efficiency depends on the type of organic acids released in the medium and their concentration. In fact, the quality of the acid is more important than the total amount of acids produced by PSB [15]. Simultaneous production of different OAs by PSB strains may contribute to a greater solubilization potential of insoluble inorganic phosphates [14]. Additionally, some inorganic acids such as hydrochloric, nitric and sulfuric acids, produced by chemoautotrophs and the H^+ pump, contribute to soil P solubilization. Inorganic acids released as tricalcium phosphate by converting to di- and monobasic phosphates result of an enhanced availability of the P in the soil and to the plants [1].

Knowing the problem of the P in volcanic soil, the application of P solubilizing bacteria such as *B. thuringiensis*, would contribute to increase the availability of this nutrient for plants, and reduce the need for application of synthetic P fertilizers, and the environmental effect associated with excess of P applications. The objective of this study was to assess the availability of P in soils derived from volcanic ash in two sites with soils of different taxonomic order (Andisol and

Ultisol), inoculated with P solubilizing bacteria (*Bacillus thuringiensis*), and their uptake by wheat plants at different growth stages.

2. MATERIALS AND METHODS

2.1. Experimental site

The experiment was conducted in pots under greenhouse conditions at El Nogal Experimental Station of the Faculty of Agronomy, University of Concepción, Chillán, Biobío Region, Chile (36°35'43.2" S, 72°04'39" W, and 140 m a.s.l.

2.2. Site I

It corresponded to the order Andisol of the Santa Barbara series (*Typic Haploxerands*) [16] whose samples were obtained in El Carmen (19º23´35.04" S, 59º11´17" W, and 262 m a.s.l, Biobío Region (Ñuble Province). The soil had been used for a grassland system for more than 8 years. The chemical and physical characterization of soils was conducted prior to the establishment of the experiment, pH - H_2O, 7.0; Organic matter, 5.61 %; NO_3^--N, 2.8 mg kg^{-1}; NH_4^+-N, 0.90 mg kg^{-1}; available-P, 10.8 mg kg^{-1} and extractable K, 113.8 mg kg^{-1} [17]. Sand, 31.2; silt, 40.4 and clay, 28.4 (%) [18].

2.3. Site II

It corresponded to the order Ultisol of the Metrenco series (Palehumult) [19] whose samples were obtained in Metrenco (38º 51´3.82" S, 72º 27´24.25" W), Araucania Region. Soil had been submitted to intensive use, with rotation of wheat, oats and rapeseed. The chemical and physical characterization of soils was conducted prior to the establishment of the experiment, pH - H_2O, 5,6, Organic matter, 7,8 %; NO_3^--N, 11,3 mg kg^{-1}; NH_4^+-N, 0,40 mg kg^{-1}; available-P, 15,4 mg kg^{-1} and extractable K, 652,0 mg kg^{-1} [17]. Sand, 18,9; silt, 40,0 and clay, 41,1 (%) [18]. Samples from both sites (I and II) were taken 0-20 cm deep.

2.4. Experimental design

The experimental design applied was completely randomized and consisted of 4 treatments and 12 replicates. The following treatments were applied in both sites I and II: C= control; C+Bt = control inoculated with *B. thuringiensis;* CE = sterilized control; and CE+Bt= sterilized control inoculated with *B. thuringiensis*.

All management treatments received a corrective fertilization for macro and micronutrient deficiency, according to the nutrient demand for wheat and the soil analysis for an expected yield of 8 t ha^{-1}. Excluding the applications of P. Site II (Ultisol) received an application of 2 t ha^{-1} of limestone to correct soil acidity and 0.5 t ha^{-1} of calcium sulfate to correct calcium and sulfur limitations in both soils. Sterilization of soil in the respective treatments (CE and CE+Bt) was conducted using an autoclave at 121 °C for 1 hour for 2 times with 2 days interval (soil without intervention) between each sterilization [20]. In addition, destructive sampling of the pots was carried out for both soil and plant analyzes at 46, 66 and 87 days after sowing (DAS), according to the development scale proposed by Zadoks: Stem elongation (Z46), Anthesis (Z66) and Dough development stage (Z87) [21].

2.5. Pots

Pots with a capacity of 3.5 kg were filled with 2.5 kg of soil and seven wheat seeds (*Triticum aestivum* L) Var. Pantera INIA variety were sown. After plant emergence, four plants were inoculated with strains of *Bacillus thuringiensis* in a concentration of 10^6 UFC mL^{-1} (colony forming unit). The experiment was carried out under greenhouse conditions. Soil moisture in the pots was maintained at 70% of field capacity by using a moisture sensor (TDR) and distilled water. Irrigation was applied manually.

2.6. Origin of the strain and inoculum preparation

AG-82 strains (*B. thuringiensis*) were used which were obtained from the microbial collection of the Faculty of Agronomy, University of Concepción, Chillán, Chile. These were isolated from the rhizosphere of industrial chicory plants (*Cichorium intybus* L.), capable of solubilizing 117.32 mg L^{-1} P in vitro [22]. Strains were multiplied using the methodology described by Slack and Wheldon [23], which, consisted in reproducing in standard nutrient broth I (Merck) under constant agitation at 150 rpm and 25° C for two days (Lab Companion, model SI-600). In order to determine the concentration, the optical density was measured in a spectrophotometer at 600 nm and its equivalence in CFU mL^{-1} was calculated.

2.6.1. Inoculation

Plants were inoculated at 20 DAS with PSB (*B. thuringiensis*) at a concentration of 10^6 CFU mL^{-1}. For inoculation, the substrate was applied to the soil by adding 5 mL of the bacterial suspension around each plant. For the treatments with no use of PSB (control) a sucrose solution 10% (previously submitted to autoclave treatment) was applied 5 mL around each plant. At 46 DAS, a re-inoculation was performed to strengthen and ensure the presence of bacteria in the soil and sucrose (previously submitted to autoclave treatment) in the control treatments (C and CE).

2.7. Analysis of P in the plants and soil and pH levels

The P concentration in the tissues was determined by drying the samples at 65 °C and then grinding them to a total passage through a 1.0 mm sieve, then 1.0 g of the samples were weighed and subsequently calcined at 500°C for 5 hours, and the ashes were dissolved in HCl at 2 mol L^{-1}, then heated at 120°C for 40 minutes. They were then filtered and the P determined by colorimetry with nitro-vanado-molybdate at 720 nm [24]. Olsen-P was extracted with $NaCO_3$ 0.5 mol L^{-1}, pH 8.5 and also determined by colorimetric method at 820 nm. Measurements of the pH in H_2O suspension and potentiometric determinations (soil:water ratio of 1:2.5) were conducted according to the methods recommended for Chilean soils [17].

2.8. Enzymatic activity and soil microbial biomass

All samples (those taken at Z46, Z66 and Z87 growth stages) were stored in a cold room at -4ºC prior to measurements of enzymatic activity and soil microbial biomass.

Acid phosphatase activity in soil was determined using as substrate: p-nitrophenyl disodium phosphate (PNPP 0.115 M), 2 mL^{-1} of 0.5 M sodium acetate buffer at pH 6.5, using acetic acid and a volume of 0.5 mL of substrate of 0.5 g of sieved soil (<2 mm), and then incubated at 37°C for 30 min [25].

Microbial soil biomass activity was determined by hydrolysis of fluorescein diacetate (FDA). For which 1.0 g of wet soil was weighed in screw cap test tubes (samples were in triplicate and a blank was also included), then 9.9 mL of sodium phosphate buffer and 0.1 mL of FDA were added with subsequent stirring and

brought to the thermoregulated bath at 25°C for 1 hour, then samples were withdrawn and placed in an ice bath. 10 mL of acetone was added, shaken and filtered, and absorbance was read at 490nm in a spectrophotometer against a reagent blank [26].

2.9. Plant yield

Yield was evaluated by cutting aerial parts of the wheat plants tissue at Z46, Z66 and Z87 growth stages. Tissue samples were cut and separated from the root and washed with abundant P-free distilled water. Samples were stored in paper bags and weighed fresh in a high precision scale (Mettler-Toledo®, BB2440, Greifensee, Switzerland). Then dried in a forced-air drying oven (Memmert®, 854 Schwabach, Germany) at 65°C for 72 h to constant weight.

2.10. Statistical analysis

The results were tested for normality (Shapiro - Wilks), homogeneity of variances and one-way analysis of variance (ANOVA) was used to determine significant treatment effects at 5% significance level, using the Tukey test ($P < 0.05$) for the comparison of means using the statistical package SPSS Version 23.0 for MAC OS [27].

3. RESULTS

The effect of the inoculation with *B. thuringiensis* in the two sites under study (I and II) was expressed as a positive or negative effect on the chemical and microbiological properties of the rhizosphere, plant growth and P nutrition in the inoculated rhizosphere. Corresponding effects observed in the uninoculated rhizosphere are attributed to the plant itself.

3.1. Effect of B. thuringiensis inoculation on site I (Andisol) in different growth stages of wheat plants

3.1.1. Soil chemical properties

B. thuringiensis inoculation resulted in no significant differences in pH in all of the treatments compared to the controls. However, significant differences were found between sterilized and non-sterilized treatments during stem elongation (Z46)

according to Zadoks growth scale. The same occurred in the Anthesis phase (Z66) and in the Dough development (Z87) grow stages (Table 1).

Table 1. Effect of *B. thuringiensis* inoculation on pH, Olsen-P, acid phosphatase and soil microbial biomass in wheat plants at different growth stages according to Zadoks scale in an Andisol (Site I).

	pH H_2O	Olsen-P mg kg^{-1}	Acid phosphatase µmoles pNF g ss^{-1} h^{-1}	Microbial biomass µg F g ss^{-1}
Treatments		**Z46**		
C	5.7±0.05 b	10.61±0.23 a	0.59±0.01 a	19.33±0.69 b
C+Bt	5.7±0.00 b	11.15±0.10 a	0.34±0.01 b	36.32±1.27 a
CE	6.2±0.04 a	7.86±0.33 b	0.30±0.00 c	11.24±0.68 c
CE+Bt	6.3±0.02 a	7.86±0.51 b	0.12±0.01 d	35.74±0.64 a
Treatments		**Z66**		
CE	5.0±0.0 b	9.16±0.37 a	0.08±0.0 b	52.68±3.27 a
C+Bt	5.1±0.02 b	8.42±0.30 a	0.13±0.01 a	25.01±0.48 b
CE	6.2±0.06 a	6.35±0.30 b	0.01±0.0 c	55.62±3.14 a
CE+Bt	6.2±0.05 a	6.77±0.53 b	0.02±0.0 c	21.39±1.10 b
Treatments		**Z87**		
C	4.6± 0.02 b	7.89±0.29 a	0.10±0.0 b	12.53±1.30 b
C+Bt	4.5±0.02 b	7.85±0.06 a	0.17±0.01 a	26.83±3.09 a
CE	5.7±0.04 a	5.47±0.07 b	0.05±0.0 c	10.70±0.16 b
CE+Bt	5.8±0.05 a	5.21±0.32 b	0.08±0.0 b	7.34±0.61 b

Mean values with the same letter in the column and Zadoks scale are not significantly different according to Tukey test (p> 0.05). (C= control; C+Bt = Control inoculated with *B. thuringiensis*; CE= Sterilized control; CE+Bt= sterilized control inoculated with *B. thuringiensis*; pNF = p-nitrophenyl; F = fluorescein; Z = Zadoks scale in days after sowing; ± = standard error).

The Olsen-P remained similar between treatments and variable at different sampling time (Table 1). The concentration of soil P did not increase significantly with the inoculation in all treatments for the Z46, Z66 and Z87 growth stages, and considering the comparison with the sterilized and non-sterilized treatments, there were significant differences in the three growth stages (Table 1). These are generally low values according to the reference values for the interpretation of the soil analysis methods recommended for Chilean soils [17].

3.1.2. P in the plant

P concentration in plants (aerial tissues) showed a significant increase of 34% in the CE+Bt treatment at Z46 growth stage compared to the CE, and no significant differences were found between the inoculated soil and C (Figure 1).

The same occurred at stage Z66 as inoculation resulted in a 21% increase of P concentration in the C+Bt treatment compared to the C. However, the CE+Bt treatment decreased by 46% compared to the CE (figure 1). In addition, P concentration by the plants decreased 13% in C+Bt at stage Z87 compared to the C. No significant differences were observed between CE+Bt and the CE (Figure 1).

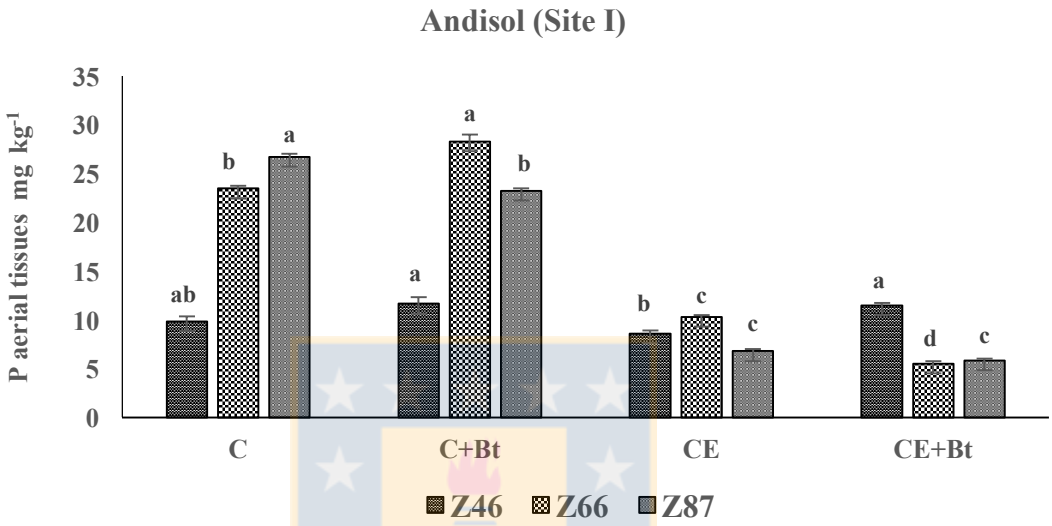

Figure 1. Effects of *Bacillus thuringiensis* inoculation on the P concentration in wheat plants (aerial part) at different growth stages of the Zadoks scale in an Andisol (Site I). Means values with the same letter are not significantly different according to Tukey test (p> 0.05). (C= control; C+Bt = Control inoculated with *B. thuringiensis*; CE= sterilized control; CE+Bt = sterilized control inoculated with *B. thuringiensis*; Z = days after sowing according to the Zadoks scale).

The inoculation significantly decreased the P concentration in the plant (radical tissue) at Z87. C+Bt and CE+Bt recorded decreased in 75% and 34% compared to the C and CE, respectively (Figure 2).

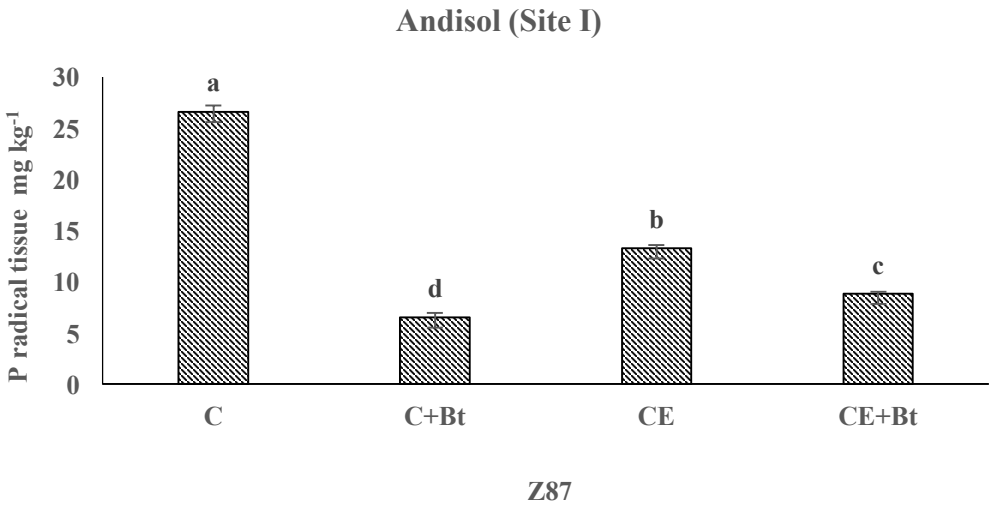

Figure 2. Effects of *B. thuringiensis* inoculation on P concentration in roots of wheat plants in the Z87 in an Andisol (Site I). Mean values with the same letter are not significantly different according to Tukey test (p> 0.05). (C= control; C+Bt = control I inoculated with *B. thuringiensis*; CE= sterilized control; CE+ Bt = sterilized control inoculated with *B. thuringiensis*; Z = days after sowing according to the Zadoks growth scale).

3.1.3. Soil biological properties

Acid phosphatase activity decreased 42% and 60% with the inoculation in C+Bt and CE+Bt treatments at Z46 stage compared to the C and CE (Table 1). A significant increase of 62% and 100% % was also observed in treatments C+Bt and CE+Bt at Z66 stage (Table 1), while values increased 70% and 60% with the inoculation in treatments C+Bt and CE+Bt at Z87 stage compared to the C and CE (Table 1). Regarding microbial biomass of the soil, the inoculation significantly increased of 88% and 218% in C+Bt and CE+Bt at Z46 growth stage compared to the C and CE (Table 1). At Z66 growth stage, it decreased 52% in C+Bt and 60% in CE+Bt compared to the C and CE, respectively (Table 1). At Z87 growth stage, microbial biomass increased significantly in C+Bt (114%) compared to the C, while it decreased by 31% in CE+Bt compared to the C, respectively (Table 1).

3.1.4. Plant biomass

Aerial biomass of the plants decreased significantly with the inoculation at all stages under study (Z46, Z66 and Z87) in C+Bt compared to the C. However, no significant differences were found between CE+Bt and the CE, respectively (Table 2).

Table 2. Aerial and root biomass influenced by inoculation with *B. thuringiensis* in wheat plants at different growth stages according to Zadoks scale in an Andisol (Site I).

Treatments	Z46		Z66		Z87	
	Biomass		Biomass		Biomass	
	aerial	root	aerial	root	aerial	root
	g plant^{-1}					
C	0.42±0.04 a	0.23±0.01 a	1.83±0.15 a	0.06±0.01 a	1.93±0.03 a	0.21±0.01 a
C+Bt	0.37± 0.01b	0.24±0.01 a	1.29±0.14 b	0.07±0.02 a	1.35±0.09 b	0.21±0.01 a
CE	0.07±0.01 c	0.03±0.0 b	0.24±0.03 c	0.01±0.0 b	0.18±0.02 c	0.14±0.0 b
CE+Bt	0.08±0.01 c	0.03±0.0 b	0.07±0.01 c	0.01±0.0 b	0.23±0.02 c	0.01±0.01 c

Mean values with the same letter in the column and Zadoks scale are not significantly different according to Tukey test ($p > 0.05$). (C= control; C+Bt = control inoculated with *B. thuringiensis*; CE= sterilized control; CE+Bt= sterilized control inoculated with *B. thuringiensis*; Z = Zadoks scale in days after sowing; ± standard error).

The inoculation did not increase the root biomass at Z46 and Z66 growth stages, showing no significant differences. However, differences were observed between the sterilized and the unsterilized treatments (Table 3). For Z87 growth stage, root biomass decreased significantly in CE+Bt compared to the CE (Table 2).

3.2. Effect of the B. thuringiensis inoculation in site II (Ultisol) in different growth stages of wheat plants

3.2.1. Soil chemical properties

Values of pH did not show significant differences with the inoculation at Z46 and Z86 growth stages. However, a significant increase of 0.33 units was observed in C+Bt at Z66 stage compared to the C (Table 3).

Table 3. Effects of *B. thuringiensis* inoculation on pH, Olsen-P, phosphatase and soil microbial biomass in wheat plants at different growth stages according to Zadoks scale in an Ultisol (Site II).

	pH H_2O	Olsen-P mg kg^{-1}	Acid phosphatase µmols pNF g ss^{-1} h^{-1}	Microbial biomass µg F g ss^{-1}
Treatments			**Z46**	
C	5.8±0.0 b	14.92±0.26 b	0.49±0.01 a	11.88±0.65 a
C+Bt	5.9±0.04 b	12.89±0.23 c	0.49±0.02 a	10.91±0.65 a
CE	6.3±0.04 a	15.16±0.47 b	0.14±0.01 b	11.47±0.76 a
CE+Bt	6.4±0.02 a	16.80±0.42 a	0.18±0.01 b	6.60±0.23 b
Treatments			**Z66**	
C	5.2±0.02 c	10.91±0.19 c	0.43±0.01 a	2.20±0.20 b
C+Bt	5.6±0.02 b	10.19±0.25 c	0.41±0.0 a	20.84±1.29 a
CE	6.5±0.02 a	13.19±0.33 a	0.08±0.0 b	4.31±0.46 b
CE+Bt	6.6±0.04 a	11.74±0.25 b	0.07±0.01 b	21.62±0.01 a
Treatments			**Z87**	
C	5.5±0.04 b	10.42±0.18 b	0.43± 0.01 a	17.05±0.21 b
C+Bt	5.5±0.05 b	9.62±0.27 b	0.36±0.02 b	17.92±0.84 b
CE	5.9±0.02 a	10.68±0.14 ab	0.07±0.0 c	12.19±0.63 c
CE+Bt	5.9±0.05 a	11.15±0.36 a	0.07±0.04 c	20.99±0.67 a

Mean values with the same letter in the column and Zadoks scale are not significantly different according to Tukey test ($p > 0.05$). (C= control; C+Bt = control inoculated with *B thuringiensis*; CE= sterilized control, CE+Bt = sterilized control inoculated with *B. thuringiensis*; pNF = p-nitrophenyl; F= fluorescein; Z = Zadoks scale in days after sowing; ± standard error).

Olsen-P increased 11% in CE+Bt compared to the C, but it decreased 14% in C+Bt at Z46 stage compared to the C (Table 3). A significant decrease of 18% was also observed in CE+Bt at Z66 growth stage compared to the CE (Table 3). At Z87 stage, no significant differences were observed with the inoculation between C+Bt and the C or CE+Bt and the CE. However, significant differences were observed between the sterilized and unsterilized treatments (Table 3). For example, these values are in general means according to the reference values for the interpretation of the soil analysis methods recommended for Chilean soils [17].

3.2.2. P in the plant

The P concentration in plants (aerial tissue) increased significantly by 20% and 67% with inoculation treatments C+Bt and CE+Bt, respectively at Z46 stage compared to the C and CE, for Z66 stage. However, for the Z87 stage, P in the plant significantly decreased 25% in CE+Bt treatment compared to the CE,

showing no differences in the unsterilized treatments. Instead, the P concentration in plants increased significantly 22% in the C+ Bt treatment compared to the C (Figure 3).

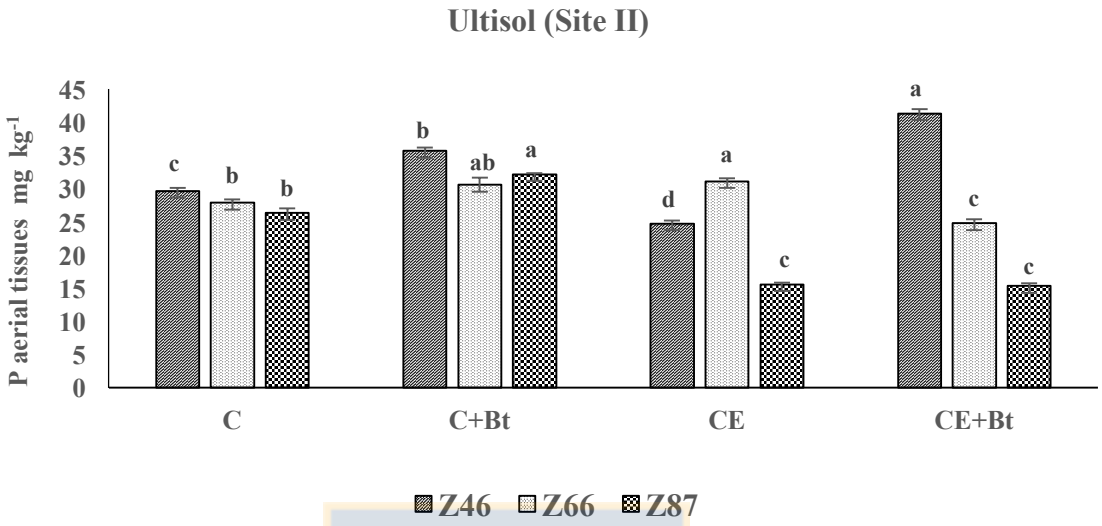

Figure 3. Effects of *B. thuringiensis* inoculation on the P concentration in wheat plants (aerial part) at different growth stages of the Zadoks scale in an Ultisol (site II). Mean values with the same are not significantly different according to Tukey test (p 0,05). (C= control; C+Bt = control inoculated with *B. thuringiensis*; CE= sterilized control; CE+Bt = Sterilized control inoculated with *B. thuringiensis*; Z = days after sowing according to the Zadoks scale).

The inoculation increased 26% the P concentration in the roots in treatment C+Bt compared to the C Z87 days after sowing. No differences were found in the rest of the treatments (Figure 4).

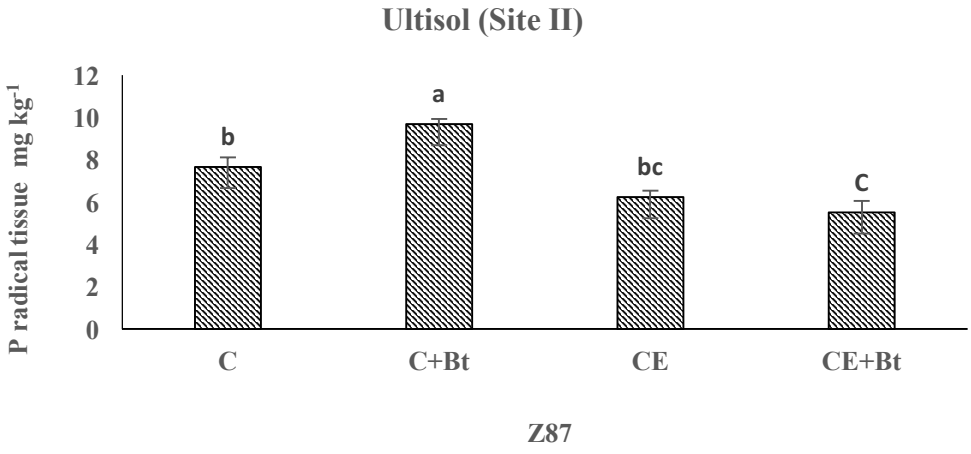

Figure 4. Effects of *B. thuringiensis* inoculation on P concentration in roots wheat plants in the Z87 in an Ultisol (site II). Mean values with the same letter are not significantly different according to Tukey test (p > 0,05). (C= control; C+Bt = control

inoculated with *B. thuringiensis*; CE= sterilized control; CE+Bt = Sterilized control and inoculation with *B. thuringiensis*; Z = days after sowing according to the Zadoks scale).

3.2.3. Soil biological properties

The activity of the acid phosphatase enzyme did not show significant differences with the inoculation at Z46 and Z66 stages, but differences were found between sterilized and unsterilized treatments (Tables 3). For Z87 stage, the C+Bt treatment had a significant decrease of 16% compared to the C (Table 3). On the other hand, the inoculation decreased the microbial biomass 42% in the CE+Bt treatment at Z46 stage compared to the CE. No significant differences were found in the other treatments and significantly increased 848 and 402% treatments C+Bt and CE+Bt compared to the C and CE at Z66 stage. For Z87 stage the CE+Bt treatment increased 72% compared to the CE (Table 3).

3.2.4. Plant biomass

Aerial biomass did not show significant differences between the inoculated treatments and the controls. However, differences were found between the sterilized and unsterilized treatments, with significant differences at Z46 and Z66 stages. However, at Z87 stage, aerial biomass decreased 30% in CE+Bt compared to the CE (Table 4).

Table 4. Effect of *B. thuringiensis* inoculation on aerial and root biomass in wheat plants at different growth stages according to Zadoks scale in an Ultisol (site II).

Treatments	Z46		Z66		Z87	
	Biomass		Biomass		Biomass	
	aerial	root	aerial	root	aerial	root
	g plant^{-1}					
C	1.76±0.21 a	0.42±0.21 b	3.11±0.29 a	0.68±0.01 a	3.50±0.32 a	0.30±0.01 b
C+Bt	1.76±0.65 a	0.59±0.12 a	3.14±0.27 a	0.63±0.01 a	3.99±0.15 a	0.35±0.01 a
CE	0.19±0.76 b	0.35±0.02 c	2.86±0.27 a	0.54±0.02 b	3.61±0.12 a	0.20±0.0 c
CE+Bt	0.10±0.23 b	0.43±0.01 b	2.42±0.09 a	0.48±0.01 b	2.54±0.19 b	0.06±0.0 d

Means with the same letter in the column and Zadoks growth scale are not significantly different according to Tukey test (p> 0.05). (C = control; C+Bt = controll inoculated with *B. thuringiensis*; CE= sterilized control; SE+BT = sterilized control inoculated with *B. thuringiensis*; Z = Zadoks scale in days after sowing; ± standard error).

Root biomass increased 40% in C+Bt and CE+Bt in 23% at stage Z46 compared to the C and CE, respectively (Table 4). At stage Z66, differences were found

when comparing the sterilized and unsterilized treatments (Table 4). Finally, at stage Z87, root biomass increased 17% in C+Bt compared to the C (Table 4).

4. DISCUSSION

Previous studies have reported positive effect that the use of PSB as inoculants increase P availability in the soil as well as P concentration in plants [1,13], affecting chemical and biological parameters of the soil, and agro-morphological parameters related to plant development [28,29].

4.1. Effects of B. thuringiensis in whaeat crop growing in site I (Andisol)

The fact that the inoculation had no significant effects on soil pH in all growth stages of wheat (Z46, Z66 and Z87; Table 1) agrees with the findings reported by other authors. Namli *et al*. [30] found no significant differences in the pH rhizosphere with the application of PSB, as in this study and site, while Chen *et al*. [31] reported that the application of PSB reduced soil pH under certain conditions due to the release of organic acids to solubilize the insoluble P in the soil layers. Other microbial metabolites are also responsible for reducing soil pH. Significant pH differences between sterilized and unsterilized treatments (Table 1) are consistent with the findings of Mahmood *et al*. [32]. This occurs because soil sterilization affects the physical, chemical and biological characteristics of the soil [33,34] so that effects vary depending on the type of soil. Increased pH values due to sterilization result from the denaturation of organic acids, and the release of base cations [35], also leading to an improved base saturation.

The literature has also described no increases in available P (Olsen-P) with inoculation [36,37,30], as shown in our results (Table 1). The non-significant increase of Olsen-P at Z46, Z66 and Z87 stages with respect to the control treatments may be due to a higher concentration of P observed by the inoculated plants, or also due to P immobilization by the microorganisms and P fixation or retention by the soil colloids. Ogut and Er [36] evaluated the availability of P in the soil at different growth stages of wheat using different bacterial strains and found no significant differences. Therefore, the ‘dynamics’ of available phosphorus in the soil during plant growth depends on many factors, including P mineralization, P immobilization, and P sorption and desorption.

Some studies have reported increases of P in plant tissues with inoculation [38,28]. The increase observed at Z46 and Z66 stages (Figure 1) indicates that inoculation increases P concentration in plants. Furthermore, it shows that there is a high rate of P concentration in the early growth stages of the life cycle of wheat [8], also indicating a higher availability of P in the soil as a result of the inoculation at those growth stages. The reduction of P concentration in the aerial and root tissues at Z66 (only in the case of sterilized treatment) and Z87 stages (Figure 1 and 2) may be attributed to the low availability of P in the soil caused by P immobilization by microorganisms, and other soil reactions that are involved in P availability. Wani *et al*. [38] concluded that the microbiologically solubilized P in the rhizosphere can be immobilized by soil microorganisms or by physicochemical reactions of the soil. Ogut and Er [36] found no evidence of the increase of P in plant tissues of inoculated wheat after 31, 61 and 92 days according to Zadoks growth scale. In addition, as roots are responsible for the absorption of essential nutrients, the low concentration of P in plants grown in the sterilized treatments may be caused or influenced by the poor root development observed in these treatments (root biomass; Table 2).

The increase in acid phosphatase activity observed at later growth stages in this study (Table 1), is particularly important for the Andisols, where P is limiting for vegetable development, as observed by previous studies [39,28]. This can be attributed to the increase in microbial biomass activity in the inoculated soil. According to Beech *et al*. [40], these enzymes, which are released outside the cell (exo-enzymes), are non-specific in nature and use organic P as a substrate to convert it into an inorganic form. This enzyme is crucial for the mobilizing P in soils with a high content of organic P [41]. Therefore, even small increases in phosphatase activity especially around the root may contribute to P mineralization for the benefit of plants. Gianfreda [42] reported that enzyme activities and soil respiration approach the condition of the microbial population and soil functioning as enzyme measurement in the soil also defines plant health. The increase in the microbial biomass of inoculated soil has also been described in the literature; e.g. Dinesh *et al*. [43] reported an increase in microbial biomass in soil inoculated with PGPR Plant Growth Promoting Rhizobacteria (PGPR). In addition, a study conducted by Wan and Wong [44] showed that the PSB population increased in the soil with the inoculation in the first 14 days, followed

by a significant decrease over time. Metabolic activities are responsible of a number of processes in the soil, such as organic matter mineralization and humification, which in turn affect other processes involving fundamental elements in the soil (C, N, P and S), as well as all the transformations involving the microbial biomass of the soil [42,45].

Inoculation did not affect significantly the aerial biomass of the plants at all the growth stages evaluated, at least in sterilized treatments. An increase after inoculation in unsterilized treatments was not observed either (Table 2). Furthermore, root biomass was not influenced significantly almost at all growth stages (with the exception of Z87 in the sterilized treatment), could be related to adequate soil available P dynamics in our greenhouse conditions. Wani *et al*. [38] observed that inoculation with *Bacillus* or *Pseudomonas* alone did not significantly affect the biomass of chickpea plants 45 and 90 days after sowing. Ogut and Er ([36], observed increases in those parameters in wheat plants inoculated with *Bacillus sp* and other strains. Our study revealed a significant high aerial biomass in the unsterilized control at Z87 state, as compared to the inoculated treatment. This occurs due to a higher P concentration in aerial and root tissues in this treatment. The relationship between the amount of P per hectare in the aerial part per unit of dry matter produced (kg ha^{-1}) is expressed through the concentration of P in the tissues. This concentration does depend on the formation requirements of carbon products that are dominated by the ontogeny of the crop [46]. In addition, there was also no increase in root biomass with the inoculation, but significant differences were found between the sterilized and unsterilized treatments. These differences are due to the effect of sterilization on the physicochemical and biological properties of the soil. In fact, soil sterilization usually results in reduced plant growth due to the toxic effect of Mn available in the soil released by the organic fraction and the elimination of microorganisms [47]. In addition, sterilization in some soils affects plant growth due to the elimination of microorganisms responsible for the mineralization processes of nutrients such as N, P and S. Mahmood *et al.* [32] observed a significant increase in the macronutrients (NH_4^+-N, NO_3^--N, P and pH of the soil) of wheat plants as effect to autoclave sterilization, as result of the release of nutrients from the microbial biomass death. These positive or negative effects

depend on the type of soil. In addition, the effects of sterilization on soil properties mainly depend on the technique used and the time of exposure of the soil.

4.2. Effects of B. thuringiensis in whaeat crop growing in Site II (Ultisol)

The pH increased 0.3 units with the inoculation at stage Z66 (Table 3), whose results agree with those indicated by Dinesh et al. [43] and Namli *et al.* [30]. This may be a strategy to increase the availability of P or other nutrients in the soil and provide a greater concentration for plants through changes in the pH rhizosphere induced by roots or microorganisms. The increase in P available with inoculation has also been reported in previous studies [44,48]. In turn, Ogut and Er [36] also observed an increase in the available P in the rhizosphere of wheat inoculated with *Bacillus sp* after 31 days of the Zadoks scale. Furthermore, Ul Hussan and Bano [49] also reported an increase of available P in the rhizosphere of wheat plants inoculated with *Bacillus cereaus*. These findings, shown that the increase of P in the rhizosphere can provided a better development of the plants.

The significant increase in P concentration (aerial tissue) in wheat plants with inoculation (Figure 3) was also observed by Schoebitz *et al*. [50] The increase of P concentration at Z46, Z66 and Z87 stages and root biomass at Z87 (Table 4) highlights the important role that PSB play in plant nutrition by increasing available P in soil and plant nutrition. A study conducted by Morales *et al*. [39] in an Ultisol soil showed that inoculation with *Penicillum albidum fungus* significantly affects P in both plant and roots. In addition, Turan *et al.* [28] reported increases in P concentration in plant tissues inoculated with *Bacillus åmagaterium*. The multidimensional functions of PGPRs also contribute to a greater uptake of nutrients by plants, leading to a higher content of phosphorus and other elements in the upper parts of plants [51].

The acid phosphatase activity did not increase with the inoculation (Table 3). In that regard Wu *et al.* [52] found no significant differences in this enzyme with the inoculation. This may be caused by the decrease in soil microbial biomass or soil organic P content, the latter being most consistent than the former Morales *et al.* [39] reported a significant increase of the acid phosphatase in an *Ultisol* soil inoculated with *Penicillum albidum fungus*. In fact, microorganisms and enzymes have key roles in all activities occurring in the rhizosphere [42]. Therefore, this parameter gives an approach to soil health. These microorganisms can mobilize

P from unavailable forms so that an increase of the microbial biomass can promote a greater availability of P and other nutrients to plants. All processes and functions occurring in the rhizosphere are controlled by the activities of plant roots, rhizosphere microorganisms and interactions between roots and microorganisms [42]. The increase of root biomass due to inoculation (e.g. at earlier stage, Z46, in all treatments; and also at Z87 in unsterilized ones in this study), has been reported in several studies [28,29,41]. Different growth promoting characteristics of the PGPR and PSB bacteria are related to a greater growth of the roots of plants [53]. The growth of the roots in response to inoculation of bacteria also leads to a better absorption of nutrients by plants, particularly P.

The contrasting effects caused by inoculation with *B. thuringiensis* in site I (Andisol) in relation site II (Ultisol) and the growing plants were as expected. Differences are mainly attributed to the physical, chemical, biological characteristics of soils and soil management practices. Andisols have particular soil characteristics: e.g. low bulk density, high content of organic C, high P retention. Phosphorus sorption to soil minerals (like allophane, imogolite and Fe- or Al- oxides associated with humic substances and Al present in the interlayers of expandable phyllosilicates in these soils [54,55] can protect them against microbial and enzymatic decomposition [56] and can difficult their response to inoculation. In general, the inoculation had both positive and negative effects on the P concentration in plants and on the availability of P in the soil (Olsen-P). According to Jorquera *et al*. [57], the bioavailability of P depends on the solubility and structure of its chemical forms in the soil-root environment, as well as on the susceptibility to microbial attack. The use of phosphobacteria as inoculants in agricultural/grazing systems in volcanic soils can help reduce applications of P fertilizer, resulting in lower environmental pollution and promoting sustainable agriculture in Chile [57].

5. CONCLUSIONS

Due to the results obtained in this work indicate that *B. thuringiensis* has potential to be used as an inoculant in wheat crop in Southern Chile, with the purpose of solubilizing soil P and improve P concentration in plants. However, for all the parameters measured, results were more consistent in the case of site II (Ultisol) than in site I (Andisol), probably due to the special characteristics of the latter soil type, and there is a need for more research, specially, in these type o soils, considering their relevance for agriculture in Chile.

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CAPÍTULO 3

CONCLUSIONES GENERALES Y PROYECCIÓN

CONCLUSIONES GENERALES

- La inoculación con *Bacillus thurigiensis* incrementó la disponibilidad de P en el suelo sitio I (Ultisol).
- La inoculación con *B. thurigiensis* incrementó la concentración de P por las plantas de trigo en las diferentes fases de desarrollo sitios I y II (Andisol y Ultisol).
- La inoculación con *B. thurigiensis* incrementó la actividad de la enzima fosfatasa acida y la biomasa microbiana del suelo en ambos suelos volcánicos.
- La inoculación con el *B. thurigiensis* incrementó la biomasa radical de las plantas en el sito II (Ultisol), lo que no se observó en la biomasa aérea de las plantas en todas las fases de evaluación en ambos suelos volcánicos.

PROYECCIÓN

- Es necesario desarrollar más conocimiento en esta área de investigación para poder comprender en su cabalidad el rol de la utilización de las bacterias solubilizadoras de fosfato como *Bacillus thurigiensis* y otros microrganismos en los suelos volcánicos.
- Seleccionar más cepas bacterianas para su uso individual y en consorcio desde cultivos locales y en suelos con retención de P. Además de la realización de experimentos en condiciones de campo para poder evaluar su rol en rendimiento de los cultivos.
- Evaluar los mecanismos de solubilización de P por los microrganismos solubilizadores de fosfato en estos tipos de suelos.
- Realizar estudios que complementen el uso de ^{32}P y de bacterias solubilizadoras de P, para determinar la procedencia del P absorbido por la planta en presencia de bacterias solubilizadoras de este elemento.
- Desarrollar métodos de aplicación más efectivos en campo y que aseguren la inoculación y sobrevivencia de los microrganismos.