NATIVE STRAINS OF *Trichoderma* FROM NORTHERN CHILE: ADAPTIVE TOLERANCE IN BORIC SALINE SOILS

Ricardo Salvatierra-Martínez, Germán Sepúlveda-Chavera, Wilson Huanca-Mamani and Manuel Rodríguez-Molina

SUMMARY

Commercial strains of Trichoderma *show erratic results on soil diseases in northern Chile. Tolerance and antagonistic capacity were assessed in 10 isolates of native* Trichoderma *spp. and a commercial bioformulate of exogenous strains under saline-boric conditions. The following treatments were used in in vitro tolerance tests: APD amended with 8, 15 and 20g·l^{-1} NaCl and the same three doses of NaCl + 15mg·l^{-1} boron. Cation content of the three most tolerant isolates was measured. The antagonism and growth* in vitro *of* Trichoderma vs F. oxysporum *in APD with 8g·l^{-1} NaCl were evaluated. In addition, a test in tomato plants inoculated with* F. oxysporum *and* Trichoderma*, and irrigated with 8g·l^{-1} NaCl and 15ppm boron was made.* In vitro *ANOVA, Tukey test and t (student) test were used on the growth of* F. oxysporum*. Kruskall-Wallis and Mann-Witney U tests were used in the case of plants; all with 95% confidence. Native strains were the most tolerant (p≤0.05); higher levels of Na^{+} in the mycelium were associated with salinity tolerance. Saline solutions and boron further reduced growth and sporulation.* F. oxysporum *showed higher growth in a saline medium (p≤0.05). Native* Trichoderma *can protect plants from* Fusarium *infection in saline-boric environment and an antagonistic inter isolated activity may exist. In the north of Chile strains that are highly tolerant to saline-boric conditions exist. The native strains are the best alternative for the control of* Fusarium *in saline and arid zones.*

Introduction

In Azapa and Lluta valleys, Arica, Chile, agricultural systems are closely related to salinity and high levels of boron, where exogenous commercial strains of *Trichoderma* spp. show erratic results. Lluta river water has EC values between 1.0 and 4.0dS·m^{-1}, and the average boron concentration is 20g·l^{-1} during the year. In the Valley of Azapa, not only is water quality superior, but also EC is higher than 1.0dS·m^{-1}, and the concentration of boron is close to 1mg·l^{-1} (Albornoz *et al*., 2007).

Fungi of the genus *Trichoderma* are the most studied biocontrol agents (BCAs) (Kubicek and

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CEPAS NATIVAS DE *Trichoderma* DEL NORTE DE CHILE: TOLERANCIA ADAPTATIVA EN SUELOS SALINO BÓRICOS

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RESUMEN

En los valles de Arica cepas comerciales de Trichoderma *muestran resultados erráticos. Se evaluó,* in vitro*, la tolerancia de 10 cepas nativas y de una formulación comercial del hongo, a condiciones salino-bóricas, simulando las condiciones naturales de los valles del extremo norte de Chile. Los tratamientos fueron: APD, APD enmendado con 8, 15 y 20g·l^{-1} NaCl, y los tratamientos indicados más 15mg·l^{-1} boro. También se determinó el antagonismo de* Trichoderma *contra* F. oxysporum *en pruebas de cultivos duales en APD con 8g·l^{-1} NaCl y sin enmendar. Se hizo un experimento en plantas de tomate inoculadas con* F. oxysporum *y* Trichoderma*, regadas con 8g·l^{-1} NaCl y 15ppm de boro. Los datos generados con los ensayos* in vitro *se sometieron a ANOVA y al test de Tukey. Los datos del crecimiento de* F. oxysporum *se analizaron con la prueba t (student). Los datos del test en plantas se analizaron con Kruskall-Wallis y U de Mann-Witney, todos con una confianza del 95%. Los aislamientos nativos fueron más tolerantes (p≤0,05) a las condiciones salino-bóricas, y se asoció la tolerancia a la salinidad con niveles más altos de Na$^+$ en el micelio. Las soluciones salino-bóricas redujeron el crecimiento y la esporulación de las cepas comerciales de* Trichoderma*, mientras que el crecimiento de* F. oxysporum *no se afectó (p≤0,05). Para las mismas condiciones, las cepas nativas de* Trichoderma *no alteraron su crecimiento y presentaron significativa actividad biocontroladora sobre el fitopatógeno. En el norte de Chile existen cepas nativas altamente tolerantes a condiciones salino-bóricas, representando una alternativa efectiva para el control de* F. oxysporum *en zonas áridas.*

ESTIRPES NATIVAS DE *Trichoderma* DO NORTE DO CHILE: TOLERÂNCIA ADAPTATIVA EM SOLOS SALINOS BÓRICOS

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RESUMO

Nos vales de Arica, norte de Chile, estirpes comerciais de Trichoderma *apresentam resultados variáveis. Avaliou-se,* in vitro*, a tolerância de 10 estirpes nativas e uma formulação comercial do fungo* Trichoderma *simulando condições naturais dos vales do norte do Chile. Os tratamentos foram: APD, APD + 8, 15 e 20g·l^{-1} NaCl, e os tratamentos indicados mais 15mg·l^{-1} boron. O antagonismo* Trichoderma *contra* F. oxysporum *em teste de cultura dupla em APD com 8g·l^{-1} NaCl, e sem alteração também foi determinada. Um experimento foi realizado em plantas de tomate inoculadas com* F. oxysporum *e* Trichoderma*, irrigadas com 8g·l^{-1} NaCl e 15ppm boron. Os dados gerados pelos testes* in vitro*, foram submetidos a análise de variância e o teste de Tukey. Dados de crescimento de* F. oxysporum *foram analisados com o teste t (student). Os dados de ensaio em plantas foram analisados com Kruskal-Wallis e Mann-Whitney U, todos com uma confiança de 95%. Os isolados nativos foram mais tolerantes (p≤0,05) em condições bóricas salinas e tolerância à salinidade, com níveis mais elevados de Na$^+$ no micélio foi associado. As soluções salinas bóricas apresentaram crescimento reduzido e pouca esporulação de linhagens comerciais de* Trichoderma*. Enquanto o crescimento de* F. oxysporum *não foi afetada (p≤0,05). Para as mesmas condições, as estirpes de* Trichoderma *nativas não alteraram o seu crescimento e mostraram atividade de biocontrole significativo sobre o agente patogénico. No norte do Chile estirpes nativas de* Trichoderma *são altamente tolerantes a condições salino bóricas e são uma alternativa eficaz para o controle de* F. oxysporum*.*

Hartman, 2000; Harman *et al*., 2012; Hermosa *et al*., 2012). They show rapid growth and are found in agricultural soils, grasslands, forests, swamps and soils in all climatic zones (Danielson and Davey, 1973; Roiger *et al*., 1991). The mechanisms of action of *Trichoderma* are: antagonism, competition with pathogens and other microorganisms, lytic enzyme action, symbiosis, antibiosis, and fungistasis (Sivan and Chet, 1993; Grosh *et al*., 2006; Vinale *et al*., 2006). Mycoparasitism is another strategy of these fungi to colonize and dominate the substrates (Dix and Webster, 1995) and induction of systemic acquired response (SAR) in plants is a central mechanism by which *Trichoderma* diseases decrease (Harman *et al*., 2012; Hermosa *et al*., 2012).

There is little information on salinity and boron tolerance, or the effect of boron in the biocontrol ability of *Trichoderma*. Regragi and Lahluo (2005) note that different degrees of salinity affect the effectiveness of *T. harzianum* in *Verticillium* control in tomatoes and this affects the growth and sporulation of the biocontrol agent, though the effect of boron is still unknown. Salinity adaptation shows to be a response to stress, expressed through the generation of low molecular weight proteins (Mohamed and Haggag, 2006) and accumulation of elements such as Na+ in fungi. These are homeostatic responses that take place in the cytoplasm and represent biological strategies of halophyte organisms to tolerate salinity (Anthony, 1998).

The aims of this study were a) to evaluate the adaptive potential of *Trichoderma* spp. isolated in the coastal valleys of Arica compared with exogenous strains of a commercial bioformulate, in environments with excess salts and boron; and b) to assess gradients of Na^+, Ca^{2+}, Mg^{2+} and K^+ under salinity conditions.

Materials and Methods

Bioformulate and native isolates of Trichoderma

A commercial formulation was used, consisting of three species of *Trichoderma*: *T. harzianum* Rifai strain Queule,

T. parceanamosum Bissett Trailes strain and *T. virens* (JH Mill, Giddens & AA Foster) Arx Sherwood strain. It was prepared in sterile distilled water (SDW) at a concentration of 3.3×10^9 conidia/ml. Native isolates of *Trichoderma* spp. were obtained from serial dilutions of soil, from different sectors of Azapa and Lluta valleys (Table I) and plated on potato dextrose agar (PDA).

Pure cultures of Trichoderma *isolates*

The surface of the colonies of native *Trichoderma* in the Petri dish was scraped with SDW and 0.1% Tween 20. The suspension obtained was sieved with sterile gauze, obtaining only conidia. The conidial concentration was adjusted to 1 conidium/ml with an hematocymeter. Samples of 1mlL were spread on a slide with a PDA film and placed in a Petri dish. After 24h, and by placing it in a Petri dish to generate a PDA monosporic colony, it was observed under magnification that conidia germinated. After 72h of culture, the mycelial colony was taken and placed on slants with PDA for storage at 4°C.

Isolation and identification of F. oxysporum *(Fox)*

Colonies of *Fusarium oxysporum* were obtained from pieces of roots of tomato plants with wilt symptoms arranged in a moist chamber. Pathogenicity tests were conducted using four strains of the plant pathogen that were maintained in PDA. The plants were inoculated with the virulent strain according to an ordinal scale (1 to 5) developed by Santos (1997). This strain was used in the *in vitro* and *in vivo* assays. Additionally, using specific uni primers sets, sp13, sp23 and sprl developed by Hirano and Arie (2006) the *special form* and race were determined in accordance with Çolakt and Mehemed (2013).

TABLE I
ORIGIN OF *Trichoderma* ISOLATES

Trichoderma isolates	Origin
Putre	Putre
Llu2	Lluta Valley
Llu3	Lluta Valley
Llu4	Lluta Valley
Llu5	Lluta Valley
Llu6	Lluta Valley
Llu7	Lluta Valley
Aza1	Azapa Valley
Aza2	Azapa Valley
Aza3	Azapa Valley

Tolerance test on mycelial growth

A PDA medium amended with 8, 15 and $20g\cdot l^{-1}$ of NaCl and 15ppm of boron was prepared. These culture media were placed in Petri dishes and, once solidified, a disk of 4mm diameter, containing fungus growing colonies from 96h culture, was placed. The effect of NaCl and NaCl+B on the growth of *Trichoderma* was tested in parallel to the same test *F. oxysporun*, but only a dose of $8g\cdot l^{-1}$ was used. Evaluations were carried out 96h later by measuring the percent inhibition of radial growth (PIRG) with the formula:

$$PIRG= R1\text{-}R2/R1 \times 100$$

where R1: radial growth of the strain in a medium without amendment, and R2: radius of growth of each strain grown in an amended medium.

Antagonism in vitro: dual culture

A disk of 4mm in diameter carrying 96h old fungi growing colonies was placed at opposite ends of Petri dishes containing a culture medium with $8g\cdot l^{-1}$ NaCl and unamended agar. PIRG in *F. oxysporum* was measured after 4 days and *Trichoderma* mycoparasitic activity after 7 days, considering the scale shown in Table IV (Ezziyyani *et al*., 2004).

Content of Na^+, Mg^{2+} and K^+ in the mycelium

The three most tolerant, the less tolerant and the bio-based isolates were independently cultured at 25°C for 7 days in PDA amended with NaCl ($20g\cdot l^{-1}$). The developed colonies were obtained in ADE and washed with 35°C to remove traces of the culture medium; 0.5g of biomass of each one was obtained. The content of Na^+ and K^+ in the biomass of *Trichoderma* was determined by flame spectrophotometry (Varian 220AA, 1998, Australia), while the content of Mg^{2+} and Ca^{2+} was determined by molecular absorption spectrophotometry (Jenway Flame Industrial PFP7 Photomete, 2006, UK).

TABLE II
ASSESSMENT OF *F. Oxysporum* (FORL OR FOL RACES) BASED ON THE RESULTS OF PCR WITH SPECIFIC PRIMER SETS*

	Primer Sets			
	Uni	sprl3	Sp23	splr
FORL	+	-	-	+
FOL race 1	+	+	-	-
FOL race 2	+	-	+	-
FOL race 3	+	+	+	-

*Adapted by Çolakt and Mehemed, 2013).

Test Trichoderma-*tomato-Fox*

Tomato seedlings of the local cultivar 'Poncho Negro', tolerant to salinity (Caniguante *et al*., 2009) of 15 days were inoculated with FORL (*F. oxysporum* f. sp. *radici-lycopersici*) by submerging the roots in a suspension of 2×10^6 conidia/ml and afterwards transplanted into 250ml plastic containers with sterile substrate composed by sand: peat (1:1). After 2h, 50ml of a suspension of 3.3×10^6 conidia/ml of *Trichoderma* in sterile distillate water with $8g\cdot l^{-1}$ NaCl + $15mg\cdot l^{-1}$ boron was applied in each container. This was repeated after 48h. Before testing, the plants were irrigated 4 times in 6 days with water amended with $8g\cdot l^{-1}$ NaCl + $15g\cdot l^{-1}$ boron; unamended water was subsequently used. Four treatments and 15 plants per treatment were used (Table V). After 24 days, the biomass and the presence of symptoms produced by FORL were evaluated with the ordinal scale (1 to 5) of Santos (1997) used in the pathogenicity test.

Statistical analyses

In tolerance tests, a completely randomized 11×3 (*Trichoderma*×dose), experimental design with factorial

TABLE III
SCALE TO EVALUATE THE MYCOPARASITIC ACTIVITY OF *Trichoderma*, ACCORDING TO THE EXTENT OF INVASION OF THE SURFACE, AND SPORULATION OVER OTHER FUNGI *

Grade	Mycoparasitic activity
0	No invasion of the surface of the pathogenic fungus colony
1	Invasion of ¼ of the surface of the pathogenic fungus colony
2	Invasion of ½ of the surface of the pathogenic fungus colony
3	Invasion of the total area of the pathogenic fungus colony
4	Invasion total colony surface fungal pathogen sporulation on it

*Elijah and Arcos, 1984; amended by Ezziyyani *et al*., 2004.

TABLE IV
TREATMENTS USED IN THE TESTING OF BIOCONTROL ON TOMATO PLANTS cv. 'PONCHO NEGRO'

Treatments	Inoculation Fox	*Trichoderma* protection
Control (+)	X	
Llu 4, Llu6, Llu7	X	X
Native isolates *	X	X
Control (-)		

*A mixture of the ten native isolates was used.

TABLE V
INHIBITION OF MYCELIAL GROWTH AND CONIDIAL GERMINATION OF *Trichoderma* AND ANTAGONISM ON *F. oxysporum* IN PDA WITH DIFFERENT CONCENTRATIONS OF NaCl AND BORON

	Trichoderma spp.		*Trichoderma* spp. *vs F. oxysporum*			
	Inhibition of mycelial growth	Inhibition of conidial germination		Inhibition of mycelial growth**		
	20g·l^{-1} NaCl	20g·l^{-1} NaCl 15g·l^{-1} B	20g·l^{-1} NaCl	20g·l^{-1} NaCl 15g·l^{-1} B	WE*	8g·l^{-1} NaCl
Putre	27.74 bc	51.23 ab	No data	No data	52	36 bc
Llut2	11.48 bc	72.83 cde			56	52 ab
Llut3	11.44 bc	58.32 bcd			52	52 ab
Llut4	22.21 bc	56.7 bc			56	53.3 a
Llut5	0.0 a	47.57 ab			58.7	50.7 ab
Llut6	0.0 a	35.75 a			57.3	50.6 ab
Llut7	11.32 bc	71.27 cd			54.7	46 ab
Aza1	52.73 de	73.73 de			56	44 ab
Aza2	15.33 bc	51.61 ab			40	40 ab
Aza3	18.1 bc	59.27 bcd			46.66	36 bc
TNV	61.17 e	88.4 e			40	22 c

Data are the average of 3 replicates. Different letters between columns indicate significant statistical differences according to the Tukey test ($p \leq 0.05$).
* No statistical differences, WE: PDA without amend. **Data are percentage growth inhibition of *F. oxysporum*.

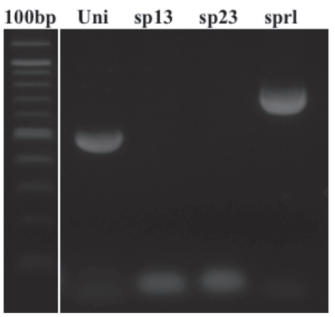


Figure 1. The universal primer for *F. oxysporum* (unif and unir) amplified a band of 672bp for all isolates confirming *F. oxysporum* identity. In the isolate only sprl set amplified a fragment of 947bp to that corresponding to *F. oxysporum* f. sp. *radici-lycopersici* (FORL)

arrangement and with 3 replicates was used. The results were analyzed to determine the interaction of factors: isolates, NaCl and NaCl + B.

ANOVA was used for the dose of 20g·l^{-1} NaCl and 20g·l^{-1} NaCl + B, and the antagonist assay culture statistical groups were separated using the Tukey test. The t (student) test was applied to the results of growth inhibition of *F. oxysporum*. Kruskal-Wallis and U Mann-Withney tests were used in the biocontrol assay in plants. All analyses were performed at 95% confidence with Statgraphics Plus 5.1 software (StatPoint Technologies, Inc., Warrenton, VA, USA).

Results and Discussion

NaCl and boron effect in the Trichoderma *spp. Development*

Morphology of Trichoderma in NaCl and B. The colony morphology of the *Trichoderma* on PDA without NaCl or boron shows mycelial hyaline growth, cottony aspect, with green or yellow peripheral sporogenous areas, depending on the isolate. Changes associated with their sensitivity to a saline medium were observed in the morphology of colonies. According to Regragi and Lahluo (2005), in a saline medium the sporogenous zone appeared dense in the center of the colony, depending on the amount of NaCl. Furthermore, boron affects growth and sporulation. In the bioformulate, the medium with 15mg·l^{-1} boron completely inhibited sporulation. Regragi and Lahluo (2005) also showed that salt has an effect on the growth and sporulation of *Trichoderma*; however, unlike what was reported by these authors (a reduction of 95% in the sporulation with 8g·l^{-1} NaCl) in this study non-sporulation was observed only in the isolates of the bioformulate when these were subjected to 20g·$^{-1}$ NaCl + 15ppm boron.

Mycelial growth. Factorial analyses showed significant differences ($p \leq 0.05$) in response to NaCl and NaCl + 15mg·l^{-1} boron (Figures 2a and b), which is explained by the interaction isolate/concentration. In native isolates only AZA1 responded significantly to increasing NaCl concentration as the bioformulates that were sensitive to a dose of 8g·l^{-1}. Llu5 and Llu6 isolates showed no direct relationship between colony development and increasing doses; thus suggesting that they were indifferent to increasing concentrations of NaCl (8-20g·l^{-1}). The other isolates showed a response only when the dose was increased from 15 to 20g·l^{-1}.

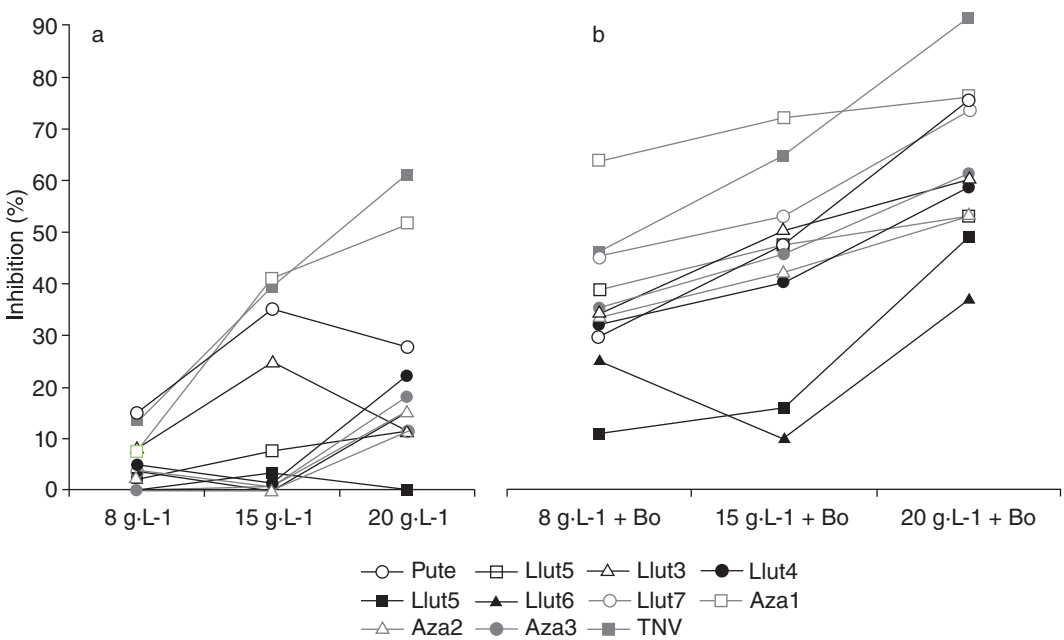


Figure 2. a: Interaction of *Trichoderma* and doses of NaCl, b: interaction of *Trichoderma* and doses of NaCl + 15mg·l^{-1} boron.

In the response of isolates and the bioformulate to NaCl + 15mg·l^{-1} boron it was observed that the addition of boron increased *Trichoderma* inhibition with respect to a medium with only NaCl addition. The Lluta isolates showed less inhibition, and Llu5 and Llu6 were the most tolerant again. The bioformulate was the most affected (Figure 2b).

The ANOVA test on 20g·l^{-1} NaCl shows statistical differences ($p \leq 0.05$). Subsequently, the Tukey test ($p \leq 0.05$) grouped Llu5 and Llu6 isolates as the less sensitive ($p \leq 0.05$). These isolates differ because they showed no inhibition of mycelial growth with 20g·l^{-1} NaCl. All Lluta isolates are statistically more tolerant ($p \leq 0.05$) than bio-based formulation strains; demonstrating a relationship between the salinity tolerance of *Trichoderma* and that of the area where they were isolated: Azapa isolates (area where there are less salts) were less tolerant, and the inhibition therefore was statistically equal to that from bio-based formulation strains. Results show that *Trichoderma* has a differential tolerance to salinity, which is related to the medium in which they were isolated. However, with the addition of boron to a saline medium, the response is inconsistent because some Azapa isolates showed higher tolerance than that shown by some Lluta isolates. This is a result that needs further study (Table V).

Salinity is one of the detrimental factors showing the highest impact on plant production (Abd-Alla and Omar, 1998) and in the coastal valleys of the region of Arica and Parinacota, northern Chile, it is a daily reality for horticultural producers. The mycelial growth of 9 out of 10 native isolates was not affected by 8g·l^{-1} NaCl, equivalent to an EC of 12dS·m^{-1}. Hence, the isolates with high growth rate, such as Llu5 and Llu6, have a great ability to colonize the rhizosphere in saline environments of most agro-ecosystems.

In vitro test

Antagonism. In dual cultures all *Trichoderma* show a hgher velocity of growth than that presented by FORL. No statistical differences ($p \leq 0.05$) were shown between native isolates and bio-based formulations; however, in a saline medium with 8g·l^{-1} NaCl, statistical differences were observed ($p \leq 0.05$) and the native isolates showed more inhibition than that presented by bio-based formulates strains on the mycelial growth of FORL. This is consistent with de tolerance result and shows the adaptation of native isolates to saline environment and the effect of salinity in antagonistic capacity reported by Regragi and Lahluo (2005; Table IV).

On the one hand, NaCl induced higher growth of FORL (Figure 3) and on the other hand, it reduced the antagonistic capacity of *Trichoderma* (Table IV). This interaction may explain the erratic results of exogenous strains of *Trichoderma* to control *F. oxysporum* in agro-ecosystems of northern Chile, where high salt plus high levels of boron are found.

Mycoparasitism. Ezziyyani *et al.* (2004) scale showed that 7 isolates of *Trichoderma* colonized colonies of FORL in seven days and, of these, 4 did so in a saline medium. Strains with better adaptation (Llu5 and Llu6) inhibited the growth of FORL but did not parasitize it. Similarly, strains of the commercial product showed no parasitism at the time of evaluation (Figure 4).

Mg^{+2}, K^{+}, Na^{+} y Ca^{+2} in Trichoderma *mycelia*

The most salt tolerant mycelium, that of the isolates Llu5, Llu6 and Llu4, shows higher level of Na^{+} (Figure 5). The levels of Na^{+} were higher than those found in mutant salt-tolerant strains by Mohamed and Haggag (2006), who reported levels from 2000 to 2500mg Na^{+} per kg of dry weight of mycelia. On the other hand, traces of Ca^{+2} were not found, which differs from the report by the same authors (10 to 50ppm). For K^{+} and Mg^{+2} levels, only TNV (bio-based formulates) have levels similar to those reported by Mohamed and Haggag (2006).

As reported by Khan *et al* (2001) and Mohamed and Haggag (2006), the content of Na^{+} in the mycelium increased under salinity conditions, whereas Ca^{2+}, Mg^{2+} and K^{+} decreased. However, results suggest that this adjustment capacity is higher in native strains studied compared with the results shown by Mohamed and Haggag (2006), or observed with the commercial product. Further studies are needed to understand the mechanisms that allow the evaluated strains display tolerance and the ability to accumulate such high levels of Na^{+} in the mycelium.

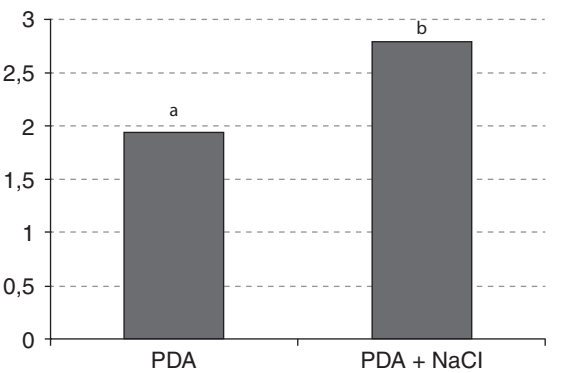
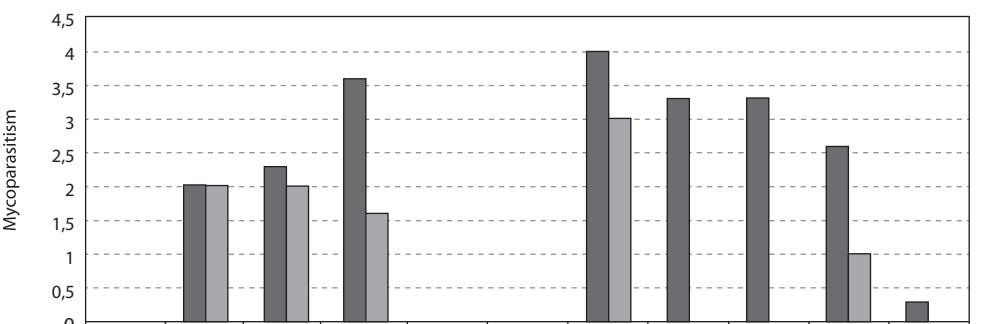


Figure 3. Mycelial growth of FORL at 4 days on PDA and PDA amended with 8g·l^{-1}. NaCl. Each bar corresponds to the average of 33 observations. Letters according to t (student) test.



	Putre	Llu2	Llu3	Llu4	Llu5	Llu6	Llu7	Aza1	Aza2	Aza3	TNV
APD	0	2	2,3	3,6	0	0	4	3,3	3,3	2,6	0,3
APD + 8g·L-1	0	2	2	1,6	0	0	3	0	0	1	0

Figure 4. *In vitro* mycoparasitism of *Trichoderma* on *F. oxysporum*. Each bar represents the average of 12 observations.

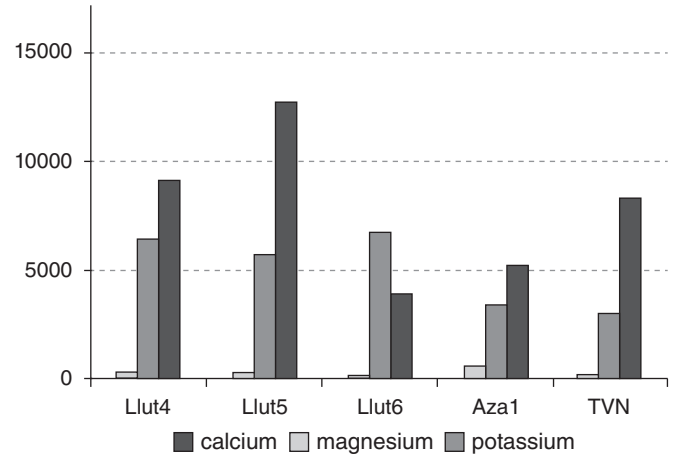


Figure 5. Cation content in the biomass of *Trichoderma* spp. developed in saline media (20g·l^{-1} NaCl).

Biocontrol in tomato plants

Since the goal of the study included obtaining information on the biocontrol ability of native isolates of *Trichoderma* in saline-boric environments, such as those in northern Chile, it was decided to leave out treatments with commercial bioformulates, given their inability to develop in these environments demonstrated by the *in vitro* tests. Through this, information regarding the possibility of using all strains together or just bioformulate strains with better performance in saline-boric conditions is also generated. According to the Kruska Wallis test, there were significant differences ($p \leq 0.05$) among treatments for severity index and dry weight, and the Mann Whitney U test applied to dry weight data showed no difference ($p \leq 0.05$) between the positive control (+) and the treatment where plants were protected with all Native isolates together; different from when only three strains are used. This shows the antagonistic effect among some strains of *Trichoderma* and gives light to the need to select these according to their compatibility. Though there were significant differences in severity, isolates showed the ability to considerably reduce the incidence and severity of the disease.

On the other hand, it is noteworthy that at 21 days, while symptoms occurred in the aerial part and vascular system of plants, no death occurred, which was explained by the detrimental effect of boron on the growth of FORL, determined in an unpublished test.

This is the first study in which the response of *Trichoderma* isolates in boric saline environments is analyzed, and in which they were compared to commercial bio-based formulations. It is demonstrated that isolates obtained in the coastal valleys of Arica are more tolerant to salinity and boron than a commercial bio-based formulation of *Trichoderma* spp. obtained under other soil conditions; however, complete correlation is not achieved because some isolates of Azapa Valley or Putre were more tolerant than others of Lluta Valley when boron was added to saline media. This is a result that needs further investigation and shows that tolerance to boron needs a mechanism unrelated to the ion buildup, as was observed for salinity. In addition to their ability to protect tomato plants from FORL infections, these isolates are highly tolerant to boric saline soil conditions.

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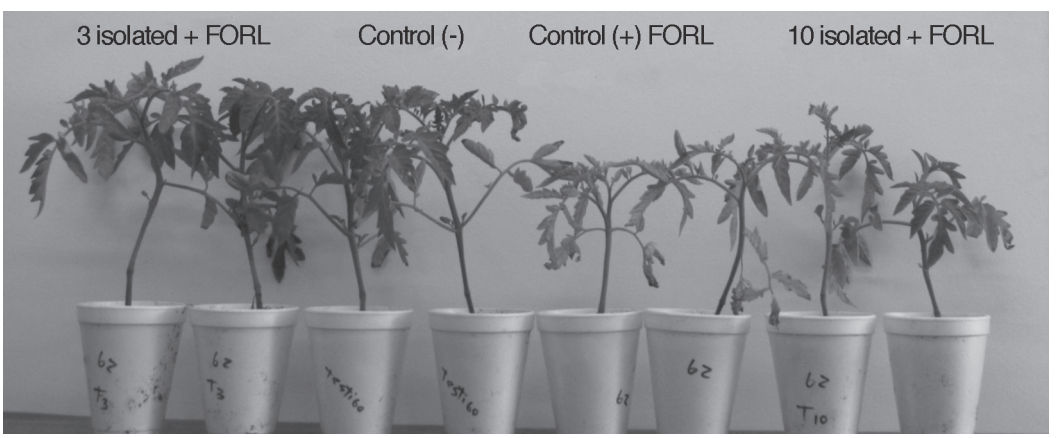

Figure 6. Tomato plants inoculated with FORL, protected with native isolates of *Trichoderma* and irrigated with saline-boric solutions

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