

Remediation of salt and boron-affected soil by addition of organic matter: an investigation into improving tomato plant productivity

Remediación a la sal y boro que afecta al suelo, mediante la adición de materia orgánica: una investigación para mejorar la productividad de plantas de tomate

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ABSTRACT

To mitigate the effect of salt stress and excess B in soil and consequently increase crop yield and improve the behaviour of cv. "Poncho Negro" there are low-cost strategies such as the application of organic amendments. This study aimed at remediating excess salt (150 mM NaCl) and B (20 mg L⁻¹ B) in soil using organic matter treatment (4% and 6%). Stress was supplied by irrigation. The rate of net CO₂ assimilation, B, Na⁺, K and Ca²⁺ content, soluble sugars and proline were measured. All growth parameters measured were higher with 6% organic matter (OM) in soil than with 4% OM, including salinity treatments and excessive B, while Na⁺ and B concentrations were reduced in leaves and roots. The photosynthesis rate was maintained at the level of control plants with 6% OM applied in soil in treatments with salinity alone and with excess B. In the salinity alone treatment there was a marked increase in the content of compatible solutes, but with the application of 6% OM their content was reduced by more than 100%. The remediation of salt and boron-affected soil by addition of organic matter (6%) is an effective way to improve the behaviour of tomato.

Key words: organic matter, boric acid, salinity, *Solanum lycopersicum* L.

RESUMEN

Para mitigar el efecto del estrés de sal y exceso B en el suelo y, consecuentemente, aumentar y mejorar el comportamiento de cv. de tomate "Poncho Negro", como una estrategia de bajo costo como es la aplicación de enmiendas orgánicas. Este estudio tuvo como objetivo remediar el exceso de sal (NaCl 150 mM) y B (20 mg L⁻¹ B) en el suelo mediante el tratamiento de aplicación de materia orgánica (4% y 6% M.O.). El estrés fue suministrado por el riego. La tasa de asimilación neta de CO₂, contenido de B, Na⁺, K⁺ y Ca²⁺, los azúcares solubles y prolina fueron medidos. Todos los parámetros de crecimiento medidos fueron mayores con 6% O.M. en el suelo que con 4% de M.O., incluyendo los tratamientos de salinidad y excesivo B, mientras que las concentraciones de Na⁺ y B se redujeron en hojas y raíces. La tasa de fotosíntesis se mantuvo a nivel de las plantas de control con 6% de M.O. aplicada en el suelo en los tratamientos con salinidad sola y con exceso de B. En el tratamiento de solo salinidad hubo un marcado incremento en el contenido de solutos compatibles, pero con la aplicación de 6% de M.O. su contenido se redujo en más de el 100%. La remediación en el suelo afectado por exceso de sal y B en la adición de materia orgánica (6%) es una forma efectiva para mejorar el comportamiento del tomate.

Palabras clave: materia orgánica, ácido bórico, salinidad, *Solanum lycopersicum* L.

Introduction

Salinity is a major adverse environmental constraint for plant productivity, limiting the utilization of about 800 million ha of agricultural land globally (Yang *et al.*, 2011). Salinization of agricultural soils is a worldwide concern, especially in irrigated land. Saline soil is characterized by the presence of toxic levels of sodium and its chlorides and sulphates (Rajaravindran and Natarajan, 2012).

Salt induces osmotic stress by limiting absorption of water from soil, and ionic stress results from high concentrations of potentially toxic salt ions within plant cells. Plants have evolved a variety of protective mechanisms that allow survival and growth under these unfavourable environmental conditions, including the accumulation of ions and osmolytes such as proline (Maggio *et al.*, 2010; Rajaravindran and Natarajan, 2012). Boron (B) is likely the micronutrient whose concentration within

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vascular plants must be kept within the narrowest range to achieve optimal growth; for this reason excess B in soils is more difficult to manage than its deficiency (Herrera-Rodríguez *et al.*, 2010). Boron toxicity is an agricultural problem that reduces crop yield (Bonilla and González-Fontes, 2011). Irrigation with saline groundwater containing high B concentrations occurs worldwide, with notable examples found in several areas; Israel, USA, Canada and Chile (Díaz *et al.*, 2011). The mechanics of the relationship between B and salinity are not yet elucidated. Nevertheless, the palliative effect of B under saline conditions may be due to an improvement in the functionality of aquaporin, which prevents salt-induced nutrient imbalance, interaction between B and Ca^{2+} with respect to cell wall stability and lower Cl^- uptake (Bastías *et al.*, 2004a; Bonilla and González-Fontes, 2011). Salinity and B toxicity often occur simultaneously and have interactive effects on plant responses. An alleviating effect of the combined stress on toxic ion concentrations has been confirmed, which did not prevent additive growth reductions (Masood *et al.*, 2012). Excess B could alter the structure of primary metabolic compounds through binding to ATP, NADH and NADHP, and high internal B concentrations may affect plant development negatively by binding to the ribose of RNA (Reid *et al.*, 2004).

A need exists for low-cost, efficient treatment strategies to reduce the salt toxicity of soils and to improve soil properties, such as the application of organic amendments (Shaaban *et al.*, 2013). The physical, chemical and biological properties of salt-affected soil are improved by application of organic matter (OM), leading to enhanced plant growth and development by accelerating the exchange of cations in soil solids and leaching of salts from the root zone (Clark *et al.*, 2007), hence preventing salt injuries and allowing roots to grow more uniformly. The decaying organic matter increases soil CO_2 concentration, releases H^+ , enhances CaCO_3 dissolution and liberates more calcium for sodium exchange (Ghaffor *et al.*, 2008). Therefore the application of OM for soil remediation is important for sustainable land use and crop productivity (Wong *et al.*, 2009; Cha-um and Kirdmanee, 2011). Boron uptake by plants is controlled by the B level in soil solution rather than the total B content in soil. The affinity of OM for B can affect B uptake by plants because of changing B concentration in the soil solution, which has a

prominent effect in reducing B uptake by plants. Boron uptake by plants is expected to decrease as the OM content in the soil increases (Yermiyahu *et al.*, 2001).

Tomato is one of the most important fleshy fruit vegetables with high economic value in the world. Its global production has been increasing and is now over 145 million tons according to the Food and Agriculture Organization of the United Nations. The tomato production area is expected to expand; however, the sensitivity of tomato to environmental stress restricts the production area (Uozumi *et al.*, 2012). Salt stress has been shown to decrease dry matter production, nitrogen uptake, low dry matter, fruit weight and relative water content (Al-Rawahy *et al.*, 1990; Tuna *et al.*, 2007). One study also observed significant reduction in the photosynthetic pigments and total soluble protein (Doganler *et al.*, 2010).

In this study we use the tomato (*Solanum lycopersicum* L.) variety called “Poncho Negro” grown by farmers of the Lluta valley (northern Chile), which represents an important source of genetic resources of agricultural interest, because it has managed to thrive at levels of salinity and excess B that other varieties of tomato cannot tolerate (Bastías *et al.*, 2010). Under salinity these plants increased their turgor, possibly due to a decrease in leaf water potential through the reduction in the leaf osmotic potential by accumulation of soluble sugars and proline (Contreras *et al.*, 2011); the interaction of B and salinity reduced the movement of Na^+ to the leaves by accumulating more leaf B (Díaz *et al.*, 2011).

In order to find a solution to the effect of salt stress and excess B in soil and consequently increase crop yield, we evaluated the ability of OM to retain excess salts and B and hence to improve the behaviour of the Poncho Negro variety.

Materials and Methods

Plant material and culture conditions

The experiment was conducted in an environmentally controlled greenhouse with day/night temperatures of 28/18 °C, day/night relative humidity of 45/85% and a 16-h photoperiod. Seeds of *Solanum lycopersicum* L. cv. “Poncho Negro” were planted after germination into individual pots (1.8 L) containing medium mixture and arranged in

a randomized complete block experimental design. The soil was collected from an uncultivated field in the Lluta Valley of Chile and the OM used was mature compost. The total amounts of OM added in the mixture were 4% and 6%. Soil mixture characteristics obtained from the saturated paste are shown in Table 1. Pots were irrigated every 2 d in order to maintain soil water at field capacity. Chemical fertilizer (Ultrasol crecimiento; 25:10:10) was applied along with Hoagland's solution. The solution was prepared using de-ionized water and the pH, osmotic potential, and conductivity were monitored each week. During the first fifteen days after germination plants were irrigated with the nutrient solution. Subsequently, when the third leaf was fully expanded the plants were exposed to salinity and excess B for 30 d. The control treatments consisted of basic nutrient solution without salt (NaCl-0) or excess boron (B-0) and 4% or 6% OM. This basic nutrient solution was supplemented with 150 mM NaCl. An excess of B in the form of H_3BO_3 was added to nutrient solutions to obtain concentrations of 20 mg L⁻¹ of B. Physiological measurements and plant harvest were performed on the 30th day of treatment. Measurements and harvest were performed in the middle of the light period. The photon flux density was 400-500 $\mu E m^{-2} s^{-1}$.

Growth parameters

At the end of the experimental period, the following growth parameters were determined:

Table 1. Chemical analysis of soil of the different treatments performed at remediation procedures.

Parameter	2% O.M.	4% O.M.	6% O.M.
pH	5.66	6.49	5.92
Ce mS/cm a 25 °C	41.10	15.66	12.38
N-disp mg/Kg	45.17	151.26	899.15
P-disp mg/Kg	22.97	134.78	27.07
K-disp mg/Kg	1,224.00	1,354.00	1,300.00
O.M. %	2.22	4.09	5.96
Calcium (Ca ²⁺) mg/L	1,991.00	1,008.00	2,596.00
Magnesium (Mg ²⁺) mg/L	279.89	429.00	74.78
Sodium (Na ⁺) mg/L	6,541.00	1,627.17	491.00
Potassium (K ⁺) mg/L	215.50	65.29	53.68
Chloride (Cl ⁻) mg/L	15,939.00	4,137.50	2,804.50
Sulphate (SO ₄ ⁻²) mg/L	1,671.70	2,556.60	2,066.04
Soluble boron (B) mg/L	17.27	24.81	22.89

* The results were performed on saturated extract.

plant dry weight; total (g), stems, leaves and root; and root/shoot ratio.

Net CO₂ assimilation rate

The gas exchange parameter net CO₂ assimilation rate were measured on fully expanded leaves from 11:00 to 13:00 h, using a portable photosynthesis system LI-6400 (Licor Inc., Lincoln, NE, USA) with an infrared gas analyser (IRGA). The measurements were carried out at relative humidity 45-60% and temperature 20-27 °C in the greenhouse.

Proline content

The proline content of salt-stressed and control plants was determined using the method of Bates *et al.* (1973). Proline was extracted from leaf samples (20 mg FW) according to Weimberg (1987), with minor modifications. For proline measurement, reaction with ninhydrin was carried out and the absorbance was read at 520 nm, using toluene as a blank. The proline concentration was determined as $\mu mol g DW^{-1}$, using a standard curve.

Soluble sugar content

Soluble sugars were extracted with 80% ethanol from 20 mg FW and determined colorimetrically at 625 nm using the anthrone method (Irigoyen *et al.*, 1992).

Ion analysis

Leaves were separated from stems and roots, rinsed for 15 s with deionized water, dried at 60 °C and ground. The dried tissue was weighed, ground and stored in a desiccator. The plant tissues were dry-ashed in a muffle furnace at 500 °C for 6 h, digested with sulphuric acid and peroxide, and their K⁺, Ca²⁺, and Na⁺ contents determined with a flame photometer (model PFP7, Jenway, Stone, UK). Boron was determined in the dry ash using the Azomethine-H method (Matt *et al.*, 1975).

Data analysis

All experiments described were repeated three times independently with six replications each time. A completely randomized design was used. The

experimental data were analysed by ANOVA and the differences were compared by employing the Duncan test with a significance of $p < 0.05$ using the SPSS software (SPSS version 15.0, 2006).

Results

At the end of the treatment salinity alone significantly reduced the whole plant, leaf, stem and root biomass by about 57% with 4% OM applied to the soil compared to the control, while 6% OM reduced these values by almost 40%. All measured parameters were sustained better with 6% OM in the soil than with 4% OM (Table 2). Plants treated with B+NaCl reduced biomass less than plants treated with NaCl alone, both with 4% and 6% OM in soil. In contrast, with the application of B under NaCl-O conditions and 6% OM in soil, total plant dry weight was maintained to a greater degree (60%) than in plants grown with 4% OM and salinity. The root-to-shoot ratio was affected by treatment with salinity, but this ratio was reduced less with 6% OM (Table 2). In plants treated with B+NaCl and soil 6% OM the ratio was significantly greater than with 4% OM.

The net assimilation rate (A), a sensitive parameter, showed no significant differences between control and plants treated with B and salinity with 6% OM in soil; in the plants treated with B+NaCl A decreased by about 18% of OM. In contrast, plants subjected to salinity and excess B in 4% OM reduced A drastically (Figure 1a). Chlorophyll content in plants also decreased with excess B and salinity with both OM treatments,

although the net photosynthetic rate was not affected (Figure 1b).

The soluble sugar content in the plants grown without salinity and with OM treatment in soil showed similar values; in contrast, with salinity only and 4% OM soluble sugar content increased significantly compared to the control. This behaviour was not observed in soil with 6% OM. However, the soluble sugar content in plants treated B+NaCl with both OM treatments was markedly lower (Figure 1c).

The proline content presented similar behaviour to soluble sugar content; plants with salinity only and 4% OM significantly increased proline content by up to 4-fold compared to the control. The proline content in plants treated with B+NaCl in both OM treatments was higher than the control. It is important to note that with 6% OM the proline level was lower under salinity alone, an effect that should be expected (Figure 1d).

The Na^+ concentration in leaves (Table 3) was increased 5-fold by OM application in both NaCl and NaCl +B treatments; values were more or less similar in these treatments. The Na^+ content in roots was increased 3-fold in both NaCl and NaCl +B treatments by OM application; the application of 6% OM to soil reduced Na^+ content in roots by almost 33%. Similar behaviour was observed in stems (data not shown), but the Na^+ reduction was more drastic (72%). The highest B content was in leaves under B conditions; with 6% OM in soil the B content decreased in all treatments by almost 22%. The B content was lower in roots than in leaves, but B content was reduced about 55% with the application of 6% OM to the soil in saline

Table 2. Effects of organic matter (O.M.) in individual and combined treatments of 150 mM NaCl and B excess (20 mg L^{-1}) on leaf dry weight (g^{-1} plant), stem dry weight (g^{-1} plant), root dry weight (g^{-1} plant) and root-shoot ratio in tomato plants. Values represent the mean of six samples \pm SE. Values in a column with the same letter are not significantly different according to the LSD at $P < 0.05$ using Duncan's multiple-range test.

Treatment	Remediation	$(\text{g}^{-1}$ plant dry weight)				
		Leaves	Stems	Roots	Total dry wt	Relation root/shoot
Control	4% O.M.	5.47 d	2.55 d	1.16 c	9.18 d	0.14 c
	6% O.M.	6.95 e	3.23 e	2.74 e	12.92 e	0.27 d
B20	4% O.M.	5.40 d	2.63 d	0.86 b	8.89 d	0.11 ab
	6% O.M.	8.10 f	3.54 f	1.58 d	13.22 e	0.14 c
150 mM NaCl	4% O.M.	2.61 a	1.08 a	0.38 a	4.06 a	0.10 ab
	6% O.M.	4.45 c	2.14 c	0.86 b	7.45 c	0.13 bc
B20+150 mM NaCl	4% O.M.	3.53 b	1.80 b	0.47 a	5.72 b	0.09 a
	6% O.M.	3.72 b	2.49 d	0.92 b	7.01 c	0.15 c

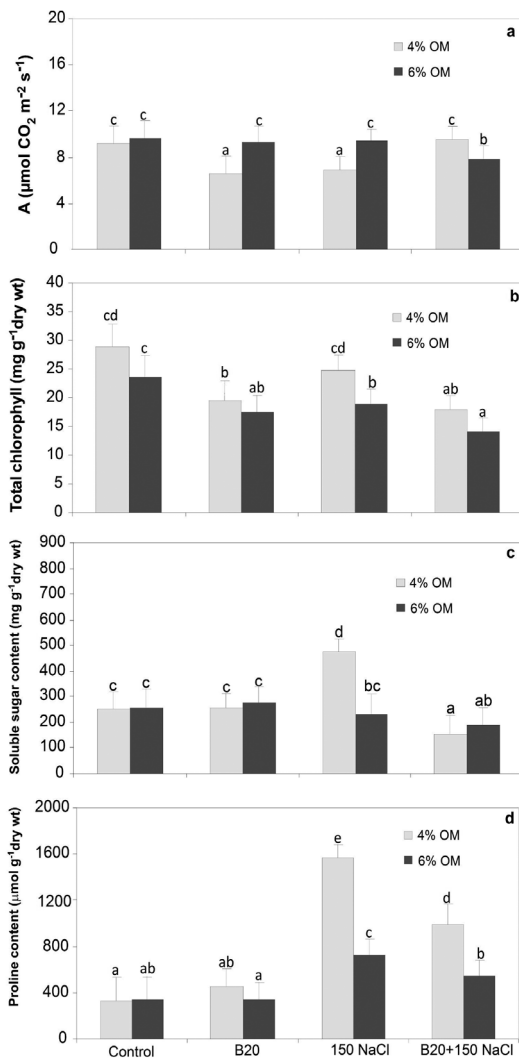


Figure 1. Effects of organic matter (OM) in individual and combined treatments of 150 mM NaCl and B excess (20 mg L⁻¹) in tomato plants: (a) net photosynthetic rate; (b) total chlorophyll; (c) soluble sugar content and (d) proline content. Values represent the mean of six samples \pm SE. Bars with the same letter are not significantly different according to the LSD at $P < 0.05$ using Duncan's multiple-range test.

condition. The K⁺ and Ca²⁺ concentrations were 2-3 times higher in leaves than in roots, showing a tendency to increase with the application of 6% OM. In contrast, in roots with 6% OM there was a decrease in the concentrations of these ions.

Discussion

When the plant growth medium is saline, replenishment of organic matter is important in

improving the sorption capacity and soil structure. The positive effect of organic substrates on the growth of numerous plants is well documented (Marosz, 2012). Positive correlation between soil organic matter content and B adsorption has also been found (Hue *et al.*, 1988). Application of organic matter in this experiment increased the sorption capacity of the growth medium; these results are consistent with ours (Table 3), in which Na⁺ and B decreased markedly in roots with 6% OM in the soil, and there was also a marked decrease in the concentration of B in leaves. The results presented here are consistent with those reported by Yermiyahu *et al.*, (2001), which indicate that OM plays an important role in controlling B concentration in the soil solution, and that it has a prominent effect in reducing B uptake by plants. The Na⁺ in roots was also decreased by the application of 6% OM; this prevents root from salt injuries and roots can grow as shown in Table 2 (Clark *et al.*, 2007). These results are also in agreement with Yang *et al.* (2004) and Shaaban *et al.* (2013). This may be associated with the enhancement of biological activity in the crop rhizosphere by some physiologically active substance in the OM. Wu and Wang (2012) reported that OM also decreased the sodium absorption ratio (SAR), indicating the migration of exchangeable Na⁺ from the top soil layer and accumulation in lower depths with application of OM. The K⁺ and Ca²⁺ concentrations in leaf were maintained or increased under OM treatments (Table 3). Our results are also in agreement with Ali and Abbas (2003) and Muhammad and Khattak (2011), that OM increases the amount of soluble Ca²⁺ for soil reclamation. Application of a combination of OM and mineral fertilizer may induce an increase in the humus content, nitrogen, and available phosphorus and potassium levels (Gondek and Filipek-Mazur, 2005). Moreover, a previous study showed that cv. "Poncho Negro" increases leaf K⁺, which increases or maintains leaf turgor under stress conditions (Díaz *et al.*, 2011). In addition, the ability to maintain K⁺ homeostasis during salt stress is considered a characteristic of better salt tolerance (Hauser and Horie, 2010).

As mentioned above, addition of OM can favour the growth of both the roots and aerial parts of the plant (Chen and Aviad, 1990; Verlinden *et al.*, 2009); this is confirmed by the results shown in Table 2, where total plant dry weight was sustained to a greater degree, maintaining levels of compatible

Table 3. Effects of organic matter (OM) in individual and combined treatments of 150 mM NaCl and B excess (20 mg L⁻¹) on concentrations of ions of leaf tissue (a) and root tissue (b) of the tomato plant.

Values represent the mean of six samples \pm SE. Values in a column with the same letter are not significantly different according to the LSD at $P < 0.05$ using Duncan's multiple-range test.

Remediation		Na*		B		K ⁺		Ca ²⁺	
				4% OM	6% OM	mg ⁻¹ dry weight			
<i>Leaves (a)</i>									
Treatment									
NaCl (0 mM)	B0	16.97 b	10.74 a	0.31 abc	0.24 ab	36.80 e	34.79 de	30.80 e	21.70 d
	B20	10.96 a	10.83 a	0.81 e	0.79 e	32.26 cd	62.50 f	14.12 a	18.03 b
NaCl (150 mM)	B0	47.16 d	44.96 c	0.33 bc	0.21 a	18.10 a	29.92 bc	21.30 cd	16.59 b
NaCl (150 mM)	B20	50.81 e	51.07 e	0.55 d	0.38 c	28.65 b	27.92 b	18.12 b	19.95 c
<i>Roots (b)</i>									
NaCl (0 mM)	B0	12.13 c	7.94 a	0.13 d	0.05 a	21.37 e	13.17 b	15.10 c	7.66 a
	B20	8.18 b	6.69 a	0.06 a	0.10 c	15.09 c	17.58 b	7.22 a	7.72 a
NaCl (150 mM)	B0	32.58 e	23.51 e	0.21 e	0.07 b	12.27 b	8.70 a	6.22 a	9.92 b
NaCl (150 mM)	B20	33.79 e	15.81 d	0.30 f	0.11 c	9.37 a	13.81 bc	16.70 c	11.15 b

solutes such as the content of soluble sugars and proline in soil in all treatments. The most serious cause of yield reduction in crops grown in saline paddy fields that have not undergone remediation are the subsequent effects of toxic ion accumulation in the plant cell, which causes pigment reduction, diminishes chlorophyll *a* and lowers *A* (Cha-um and Kirdmanee, 2011). However, in the present study *A* was effectively alleviated with 6% OM in soil in treatments with excess of B and with salt alone (Figure 1), similar to the control plants. The level of total chlorophyll pigments in Poncho Negro tomato decreased in saline conditions and B excess; this level was maintained better with OM. In *Hordeum maritimum* and rice cv. RD6 total chlorophyll pigment levels were also maintained better with the application of OM (Lakhdar *et al.*, 2008; Cha-um and Kirdmanee, 2011). Organic matter may contain salt ion-binding agents which detoxify the toxic ions, especially Na⁺ and Cl⁻, as indicated by low EC in treated soil (Tejada *et al.*, 2006; Zahid and Niazi, 2006).

Plants produce osmotically active solutes such as proline in response to salt stress that balance the water potential of the cytosol with the apoplast and vacuolar lumen (Szabados and Savoure, 2010). A recent study indicated a strong role of arbuscular mycorrhizal symbiosis in enhancing stress tolerance in chickpea by significantly modulating proline

metabolism and Na⁺ uptake (Garg and Baher, 2013). The functions of other compatible solutes such as glucose and sucrose include osmotic adjustment, carbon storage and radical scavenging (Parida *et al.*, 2002). Previous studies reported high levels of soluble sugar and proline accumulated in leaves of "Poncho Negro" tomato plants grown under salinity and excess B, but the increase was different depending on the salinity and B treatment (Contreras *et al.*, 2011; Díaz *et al.*, 2011). In the present study the application of OM in soil changed the behaviour of plants, maintaining similar levels of both solutes compared to the control. This could be attributed to the role organic matter plays in controlling B concentration in the soil solution, since it has a prominent effect in reducing B uptake by plants in saline or non-saline conditions (Yermiyahu *et al.*, 2001). Our results showed an increase of these compatible solutes in the treatment with only salinity with a lower percentage of organic matter (4%); however, with the higher percentage (6%) the levels of both solutes decreased markedly, similar to the levels observed in control plants. This behaviour is an interesting point that could explain the increase in biomass under saline conditions, as the plant spent the extra metabolic energy in the synthesis of proline and soluble sugars.

In summary, the present and previous studies show that the "Poncho Negro" variety is able

to adjust osmotically by increasing turgor, and no toxic effects of salinity and excess of B were observed (Contreras *et al.*, 2011; Díaz *et al.*, 2011). Masood *et al.*, (2012) concluded that the effects of salt are different at different levels of B supply. With an adequate B supply, salt increases soluble B concentrations, whereas under excessive B supply B concentrations are reduced. In the “Poncho Negro” tomato, B concentration increased both in leaf and root with excessive B (Table 3) under salinity. It is suggested that combined salt and B excess produced an alleviating effect, which did increase growth (Table 2). Maintenance of the net photosynthetic rate (A) in the salt-stressed and excess of B leaves was strongly related to increase in total dry weight, by the decrease in the concentration of B and Na⁺ in both leaf and as root, maintaining levels of compatible solutes such as the content of soluble sugars and proline, thus the application of 6% OM treatment in soil could effectively alleviate the problem of stress, resulting in behaviour improvement of “Poncho Negro” tomato. All these results show that cv. “Poncho Negro” from the Lluta valley (northern Chile), is

a germplasm with agronomic interest and which yields well in the field. This study demonstrates the potential use of 6% of OM in soil with saline and excess of B conditions for managing crops that are subjected to stresses, with the improvement of soil properties mitigating salinity or other specific ions with organic amendments.

Conclusion

We conclude that maintaining 6% OM in soil with saline and excess of B is an appropriate condition for managing crops that are subjected to these stresses, along with improvement of soil properties mitigating salinity or other specific ions with organic amendments.

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