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Saline-boron stress in northern Chile olive accessions: water relations, B and Cl contents and impact on plant growth

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Abstract

H. Escobar, N. Lara, Y. Zapata, C. Urbina, M. Rodriguez, and L. Figueroa. 2013. Saline-boron stress in northern Chile olive accessions: water relations, B and Cl contents and impact on plant growth. Cien. Inv. Agr. 40(3): 597-607. The objective of this study was to analyze the effect of saline-boron stress on the vegetative growth, dry leaf weight, water potential (Ψ_w), relative water content, and leaf and root B and Cl^- contents in 8 accessions of olive. Rooted one-year-old plants were cultivated for 132 days with 50% shading in 5-L pots containing sand substrate and watered with Jensen's nutrient solution. After eight days of uniform ferti-irrigation, the plants were exposed to saline-boron stress, which was administered in three successive stages to condition them to a final stress of 0.49 mM $B(OH)_3$ and 200 mM NaCl. The accessions were identified by their place of origin as Suca, Chiza, San Pedro I (SPI), Taltal, Azapa, San Pedro II (SPII), Frantoio and Lluta. The results showed that saline-boron stress decreased vegetative growth and dry matter in all accessions. The levels of Cl^- in leaves and roots increased significantly, although Suca and SPI experienced the least increases. B increased in leaves and roots but did not reach toxic levels. Water potential decreased except in the accession Taltal. RWC decreased in all accessions. Cv. Frantoio, known internationally for its high salt tolerance, was used as a reference for the observed responses in the other accessions.

Key words: Boron, chloride, olive, salinity, water relations.

Introduction

Salinity and excessive boron in the soils and irrigation water of northern Chile are important characteristics of the agriculture of this area, which is among the most arid zones of the planet. Boron is an essential element for plants and is found in high concentrations in arid and semiarid zones (Phillip and William, 1997; Therios, 2009; Chatzissavvidis and Therios, 2010). Soils may receive a high quantity of boron from irrigation (Figueroa *et al*., 1994), which may reach toxic levels that damage sensitive plants (Chauhan and Powar, 1978). One of the principal functions described for B in plants is sugar transport through

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membranes (Brown and Hu, 1996; Matoh, 1997), either by facilitation of transport by sugar-borate complexes or due to the modification of membrane permeability caused by B. Other functions have been linked to the lignification and differentiation of xylem (Gil, 1995). The entrance of B into cells may occur by passive transport, in which molecules of $B(OH)_4^-$ enter the cytoplasm and form cis-diols (Coke and Whittington, 1968; Will *et al.*, 2011), or as a response to a gradient concentration of B (Bingham *et al.,* 1970). Other authors have suggested that B transport may be active and mediated by 2,4 dinitrophenol (Wilders and Neales, 1971). Once B has entered a plant, it is transported to the leaves by transpiration flow (Brown and Hu, 1997). Boron thus accumulates preferentially in leaves, which are the first organs to manifest toxicity symptoms when more is absorbed than is needed by the plant. Before the symptoms of marginal foliar chlorosis manifest, there may be a decrease in growth, chlorophyll concentration, and leaf area, and less CO_2 may be fixed (Nable, 1988; Nable *et al.,* 1997; Reid *et al.,* 2004). Boron toxicity in olives may manifest as a terminal necrosis in older leaves at concentrations greater than 160 mg L^{-1} (Cantero, 1991). Chatzissavvidis and Therios (2010) reported that some Greek olive cultivars can continue to grow in the presence of up to 4 mg L^{-1} B without presenting symptoms of toxicity. In northern Chile, an area greatly affected by salinity-boron stress in soils and irrigation water, some olive trees have been found to grow when irrigated with water containing 0.6 to 16.6 mg L^{-1} B, 71.3 to 469.2 mg L^{-1} Na^+ and 33.3 to 1,609.4 mg L^{-1} Cl^- without apparent effects on growth (Figueroa *et al.*, 1994). Chartzoulakis (2005) indicated that olives must be irrigated with water containing no more than 1-2 mg L^{-1} B, 0.25 g L^{-1} Na^+ and 0.35 g L^{-1} Cl^-. Kchaou *et al.* (2010) indicated that some olive cultivars can experience reduced growth (by between 15.5% and 69%) when exposed to 200 mM NaCl.

Although the mechanism of the relationship between B and salinity is not yet clear, studies on the subject (Yermiyahu *et al.*, 2008; Camacho *et al.*, 2008) indicate that the combined toxicity of B and salinity is less toxic than would be expected if these stress factors were additive, showing that the relationship between excess boron and salinity is generally antagonistic (Martinez *et al.*, 2008).

However, it is important for farmers in northern Chile that any expansion of olive agriculture uses only soils that are less suitable for agriculture and affected by boron and salinity stress due to the limited amount of arable land and irrigation water available. In this study we tested 7 genotypes collected from northern Chile to evaluate the stress caused by excess B and NaCl on vegetative growth, dry leaf weight, relative water content (RWC), water potential (Ψ_w) and leaf and root B and Cl^- contents in a controlled environment using 1-year-old trees. The Frantoio cultivar, known internationally for its high salt tolerance (Chartzoulakis, 2005), was used as a reference for the observed responses in the other accessions tested.

Materials and methods

Growth conditions and experimental design

The study examined eight olives accessions that were stressed with salt and boron. The accessions used in this study were collected in northern Chile in arid and saline environments between the cities of Arica (18º28'30"S) and Vallenar (28º35'43"S). The plants were propagated vegetatively by rooting semi-woody cuttings in a hotbed containing Perlite as substrate. In the tests, we used 80 one-year-old plants. The plants were transplanted to 5-L pots containing coarse sand. After planting, these individuals were watered with Jensen's nutritive solution (Jensen and Collins, 1985) for 8 days before testing. Boron and NaCl were applied in three successive stages to condition the plants to the final stress, which was 200 mM NaCl and 0.49 mM $B(OH)_3$. Two solutions with lower concentrations (50 mM NaCl and 0.03 mM

$B(OH)_3$ and 100 mM NaCl and 0.08 mM $B(OH)_3$) were applied on days 23 and 34, respectively, before the final solution was applied. The plants were watered daily with 400 mL of solution per pot, and the pots were washed with purified water every third day to avoid concentrating the salts. These chemical compounds were added to Jensen's medium. The eight accessions were identified according to their place of origin as Chiza, San Pedro I (SPI), Taltal, Azapa, San Pedro II (SPII), Frantoio and Lluta. Five replicates were used for each accession, with one plant per replicate. The treatments were applied for 132 days. The parameters measured included vegetative growth, leaf dry weight, relative water content (RWC), water potential (Ψ_w) and the B and Cl^- contents of the leaves and radicle.

Measurement of vegetative growth

The vegetative growth of two shoots was measured per plant over a period of 60 days.

Dry weight of leaves. Two completely expanded leaves from the fourth apical node were collected from each plant. After recording the fresh weight, the leaves were dried in an oven at 60 ºC for 48 hours. Dry weight was expressed as:

$$Dry\ \ weight = \frac{dry\ \ weight * 100}{fresh\ \ weight}$$

Water potential (Ψ_w). Leaf Ψ_w was measured using a pressure bomb (PMS Model 600, USA) according to Scholander *et al.* (1965).

Relative water content (RWC). From leaves located between 10 cm and 20 cm from the apex, 10 leaf tissue disks were removed with a punch from each plant (50 disks per treatment). RWC was expressed as described in Weatherley (1970):

$$RWC = \frac{(fresh\ \ weight - dry\ \ weight) * 100}{(turgid\ \ weight - dry\ \ weight)}$$

Boron and Cl^- contents. Boron content was measured in leaves and roots using a molecular absorption spectrophotometer, and Cl^- content was determined in ash using the selective ion method (Sadzawka *et al.*, 2007).

Design and statistical analysis

The results were analyzed using 1-way ANOVA; the *a posteriori* LSD test of Fisher ($P \leq 0.05$) was used when ANOVA detected significant differences among the accessions. Plants of the same accessions, with and without stress, were compared based on the p-value obtained using the Student test.

Results

Vegetative growth

Vegetative growth was strongly reduced by saline-boron stress (Table 1). The Azapa accession exhibited the greatest growth, and the growths of the other accessions were similar. The growth depended significantly both on the effect caused by the saline-boron stress and on the individual response of each selection.

Dry leaf material

Dry leaf matter was reduced significantly in all accessions exposed to saline-boron stress (Table 1). The accessions exhibiting the greatest efficiency regarding the production of dry matter under saline-boron stress were Chiza, Suca, SPI, Taltal and SPII. The differences in dry leaf matter depended significantly on both the stress and the accession.

Chlorine in leaves and roots

The levels of Cl^- were significantly higher in the leaves of all accessions under saline-boron stress

Table 1. Vegetative growth and leaf dry weight in eight olive accessions due to the effect of saline-boron stress. Values represent the means of measurements on 5 plants of each accession and their standard deviations. Different letters represent significant differences between the means of different accessions (Fisher test, P≤0.05), and the P-value represents the difference between the means of the same accession (Student test, P≤0.05).

	Vegetative growth (mm plant^{-1})			Leaf dry weight (%)		
Accession	Control	Saline-boron	P - value	Control	Saline-boron	P - value
Suca	9.3 ± 0.4 f	1.5 ± 0.2 b	1×10^{-10}	40.7 ± 1.2 a	25.5 ± 1.5 a	9×10^{-8}
Chiza	9.8 ± 0.6 f	1.0 ± 0.2 bcd	5×10^{-6}	34.2 ± 2.8 bc	25.6 ± 1.3 a	2×10^{-4}
SP I	23.7 ± 3.0 e	1.4 ± 0.2 bc	8×10^{-5}	35.6 ± 1.9 bc	25.9 ± 1.5 a	1×10^{-5}
Taltal	106.6 ± 6.5 a	0.8 ± 0.3 cd	3×10^{-6}	33.4 ± 1.4 c	23.8 ± 2.1 ab	5×10^{-5}
Azapa	71.2 ± 3.7 b	8.8 ± 1.3 a	3×10^{-7}	34.5 ± 4.0 bc	19.6 ± 1.5 c	5×10^{-4}
SP II	61.5 ± 3.1 c	0.4 ± 0.1 d	1×10^{-6}	36.5 ± 2.5 b	24.3 ± 1.8 ab	2×10^{-5}
Frantoio	40.3 ± 1.5 d	1.3 ± 0.3 bc	5×10^{-7}	41.9 ± 1.4 a	23.1 ± 2.4 b	1×10^{-6}
Lluta	36.0 ± 3.4 d	0.6 ± 0.2 d	2×10^{-5}	34.5 ± 2.7 bc	19.4 ± 1.4 c	3×10^{-6}

(Table 2) compared to the respective controls. The Lluta, SPII and Frantoio accessions exhibited the greatest accumulations of Cl^- (2,791.2 ppm, 2,635.5 ppm, and 2,152.8 ppm, respectively), and SPI and Suca exhibited the lowest accumulations (1,072.7 ppm and 1,446.3 ppm, respectively). The Cl^- content in SPI and Suca were higher by 113% and 135% compared to the respective controls. The variation in leaf Cl^- levels depended both on the stress and on the accession. All accessions showed a considerable and significant increase in Cl^- content in their roots under saline-boron stress compared to the respective controls (Table 3); the greatest increases were in Taltal and SPII with 27,682.3 ppm and 26,473.3 ppm, respectively, whereas Suca and SPI accumulated the least amount of salt in their roots (8,854.5 ppm and 9,767.9 ppm, respectively). As with the leaves, the increase of Cl^- in the roots depended significantly both on saline-boron stress and the accession.

Boron in leaves and roots

The saline-boron stress applied during irrigation caused a significant increase in leaf boron content compared to that in non-stressed plants (Table 4). The amount of leaf boron was also significantly different among the accessions. The greatest increases in leaf accumulation occurred in SPI and SPII, at 33.6 and 33.0 mg L^{-1}, respectively. The lowest accumulations were found in Suca, Azapa,

Table 2. Cl^- contents of the leaves of eight olive accessions due to the effect of saline-boron stress. Values represent the means of measurements on 5 plants of each accession and their standard deviations. Different letters represent significant differences between the means of different accessions (Fisher test, P≤0.05), and the *p-value* represents the difference between the means of the same accession (Student test, P≤0.05).

	Cl^- in leaves (mg L^{-1})		
Accession	Control	Saline-boron	P -value
Suca	613.4 ± 63.4 b	1,446.3 ± 139.1 d	1×10^{-6}
Chiza	607.7 ± 55.1 b	1,647.5 ± 138.0 cd	2×10^{-7}
SP I	503.3 ± 44.2 c	1,072.7 ± 107.3 e	1×10^{-4}
Taltal	312.4 ± 20.8 e	1,722.6 ± 97.8 c	6×10^{-6}
Azapa	396.4 ± 41.6 d	1,637.1 ± 97.8 cd	4×10^{-9}
SP II	435.3 ± 31.4 d	2,635.5 ± 141.3 a	4×10^{-6}
Frantoio	677.2 ± 38.2 a	2,152.8 ± 256.3 b	2×10^{-4}
Lluta	212.3 ± 23.3 f	2,791.2 ± 282.7 a	3×10^{-6}

Table 3. Cl^- contents of the roots of eight olive accessions due to the effect of saline-boron stress. Values represent the means of measurements on 5 plants of each accession and their standard deviations. Different letters represent significant differences between the means of different accessions (Fisher test, $P \leq 0.05$), and the *p-value* represents the difference between the means of the same accession (Student test, $P \leq 0.05$).

	Cl^- in roots (mg L^{-1})		
Accession	Control	Saline-boron	P - value
Suca	7,141.3 ± 52.2 b	8,854.5 ± 113.5 g	1×10^{-9}
Chiza	6,551.2 ± 146.9 c	22,161.3 ± 844.1 d	2×10^{-6}
SP I	8,435.4 ± 179.6 a	9,767.9 ± 618.1 g	5×10^{-3}
Taltal	5,733.5 ± 210.4 d	27,682.3 ± 1688.2 a	8×10^{-6}
Azapa	7,016.5 ± 120.8 b	23,544.6 ± 514.3 c	2×10^{-7}
SP II	9,838.9 ± 151.9 a	26,473.3 ± 813.7 b	1×10^{-6}
Frantoio	9,655.8 ± 109.0 a	20,857.2 ± 798.5 e	6×10^{-6}
Lluta	7,169.5 ± 99.3 b	15,979.9 ± 419.3 f	1×10^{-6}

and Chiza, at 6.3, 7.8 and 7.8 mg L^{-1}, respectively. Frantoio and Taltal exhibited intermediate values. Boron accumulated to a greater extent in the roots than in the leaves. The largest increment of boron in the roots was found in SPII, SPI and Frantoio at 73.2, 57.2 and 55.7 mg L^{-1}, respectively, whereas the least accumulation occurred in Azapa, Suca and Chiza at 11.1, 12.6 and 16.8 mg L^{-1}, respectively.

Water potential

Ψ_w was significantly influenced by both saline-boron stress and the response of each accession (Table 5). Saline-boron stress decreased Ψ_w in all accessions. The accessions exhibiting the greatest change in Ψ_w were Suca, SPI, Frantoio and Azapa with -1.80, -2.28, -1.63 and -1.70 MPa, respectively, compared to their controls, whereas SPII and Lluta exhibited smaller changes in Ψ_w due to saline-boron stress. The accession Taltal alone did not present a significant difference between the control and the saline-boron stress treatment; these findings indicate that these last three accessions named exhibit a high capacity to regulate water potential. SPI exhibited the greatest increase in water potential, with and without stress.

Table 4. B contents of the roots and leaves of eight olive accessions due to the effect of saline-boron stress. Values represent the means of measurements on 5 plants of each accession and their standard deviations. Different letters represent significant differences between the means of different accessions (Fisher test, $P \leq 0.05$), and the *p-value* represents the difference between the means of the same accession (Student test, $P \leq 0.05$).

	B in roots (mg L^{-1})			B in leaves (mg L^{-1})		
Accession	Control	Saline-boron	P - value	Control	Saline-boron	P -value
Suca	7.4 ± 0.3 d	12.6 ± 0.4 de	9×10^{-9}	4.4 ± 0.5 d	6.3 ± 0.7 e	8×10^{-4}
Chiza	8.0 ± 0.8 d	16.8 ± 0.9 d	2×10^{-7}	3.5 ± 0.3 e	7.8 ± 0.7 de	1×10^{-6}
SP I	10.0 ± 0.4 b	57.2 ± 4.4 b	1×10^{-5}	5.9 ± 0.5 b	33.6 ± 2.1 a	9×10^{-6}
Taltal	3.2 ± 0.1 e	39.9 ± 3.4 c	1×10^{-5}	2.9 ± 0.3 f	19.1 ± 2.6 c	1×10^{-4}
Azapa	9.8 ± 0.5 b	11.1 ± 0.4 e	1×10^{-3}	4.9 ± 0.6 c	7.8 ± 0.7 de	1×10^{-4}
SP II	10.0 ± 0.4 b	73.2 ± 6.5 a	2×10^{-5}	3.2 ± 0.3 ef	33.0 ± 2.0 a	4×10^{-6}
Frantoio	11.4 ± 0.4 a	55.7 ± 3.9 b	1×10^{-5}	6.8 ± 0.5 a	21.1 ± 1.7 b	9×10^{-6}
Lluta	9.0 ± 0.7 c	41.8 ± 2.6 c	1×10^{-6}	5.1 ± 0.5 c	9.4 ± 1.1 d	4×10^{-5}

Relative water content (RWC)

All of the olive accessions submitted to saline-boron stress exhibited a significant decrease in RWC compared to the controls (Table 5); a significant difference in RWC was also observed among the accessions with and without stress. The greatest variations in RWC produced by stress were found in the accessions Lluta and Azapa, with values of 50.9% and 52.9% (20.5% and 17.3% less than the relevant controls, respectively). Chiza with 57.3% exhibited the smallest decrease compared to its control (6.1% less than the relevant control).

Discussion

Vegetative growth

The significant decrease in growth caused by saline-boron stress in all accessions may be due to several causes; first, as indicated by Chinnusamy *et al.* (2005), the presence of salts in the soil may decrease osmotic potential (Ψs), thereby decreasing water flow to the root cells and, consequently, the absorption of essential ions, whose entrance into cells is limited by excessive Cl^- and Na^+ in the soil. Second, the decrease in growth might result from toxicity caused by the absorption of toxic ions such as Na^+ and Cl^- from the substrate (Kumar and Bandhu, 2005). Kchaou *et al.* (2010) showed that shoot growth in five olive cultivars (Chemlali, Chetoui, Koroneiki, Arbequina I18 and Arbosana I43) was significantly reduced when treated with 200 mM NaCl. This reduction ranged from 64% for Chemlali to 85% for Arbequina I18. Shaheen *et al.* (2011) also obtained results indicating that reduced growth in olive trees is directly proportional to increases in salinity and obtained minimal growth at 200 mM NaCl. Chartzoulakis *et al.* (2002) observed significant reductions in shoot length at 50 mM NaCl for olive cultivars such as Koroneiki, Megaritiki and Kalamata; in other cultivars, such as Mastoidis, Amphissis and Kothreiki, a significant decrease was observed at 100 mM NaCl. At 200 mM NaCl, decreases from 42% (Megaritiki) to 78% (Mastoidis) were observed compared to the control. In our research, shoot growth was reduced by saline-boron stress in the range from 83.9% (Suca) to 99.3% (Taltal and SPII). Moreover, excessive levels of boron may reduce the growth of olives (Benlloch *et al.*, 1991); this decrease in growth principally affects the number of leaves and the number of lateral shoots, and shoot length and plant height are also decreased (Chatzissavvidis and Therios, 2010). In addition, cell wall expansion and root and leaf (area) growth are decreased (Reid *et al.*, 2004). At high concentrations, salinity and boron can be toxic by themselves; however, some authors suggest that the combined action of B and salinity are less toxic to the growth of plants. This relationship can be considered antagonistic; an additive action can be discarded, and the results suggest (1) that the absorption of B is reduced in the presence of Cl^- and (2) that the absorption of Cl^- is reduced in the presence of B (Yermiyahu *et al.*, 2008; Camacho *et al.*, 2008).

Dry leaf matter

Greenway and Munns (1980) indicate that saline-boron stress affects the accumulation of dry leaf matter to a small extent; nevertheless, our results show that the effects of both saline-boron stress and accession on dry material were significant. Accessions such as Lluta and Azapa exhibited low production under saline-boron stress compared to SPI, Chiza and Suca, which were the most efficient accessions in terms of producing dry matter. Kchaou *et al.* (2010) found that 200 mM NaCl severely reduced dry matter production in olives; this value was decreased by 25% in Arbosana I43 and 74% in Koroneiki relative to the control. Chartzoulakis *et al.* (2002) indicate that 50 mM NaCl may cause a significant reduction in dry weight for the olive cultivars Mastoidis, Kalamata, Kothreiki, and Megaritiki and that 100 mM NaCl may affect the cultivars Koroneiki and Amphissis. Among these, Kalamata exhibited the lowest dry weight reduction at 200 mM

NaCl (48.3%). In this work, the leaf dry weight decreased from 25.3% (Chiza) to 43.8% (Lluta) compared to the appropriate controls.

Chlorine in the leaves and roots

Saline stress is mainly caused by Na^+ and Cl^- and may decrease the water potential and cause ionic imbalance and toxicity. Saline stress may also alter the water balance of a plant, decrease its growth and limit productivity (Kumar and Bandhu, 2005). Olive trees are considered moderately resistant to salinity (Mass and Hoffman, 1997; Loupassaki *et al*., 2002). The toxic concentration levels of Cl^- depend on the cultivar; it has been estimated that this level may be approximately 2,000 mg L^{-1} in dry leaves (Aragüéz *et al*., 2005), and a level of 350 mg L^{-1} in irrigation water did not result in toxicity (Chartzoulakis, 2005). The olive cultivar Chemlali, when irrigated with water containing 660 mg L^{-1} Na^+ and 710 mg L^{-1} Cl^-, exhibited low leaf contents of both elements, indicating resistance to salinity (Poli, 1986). The accessions Suca and SPI were the most resistant to Cl^- because they had lower leaf and root Cl^- contents than other accessions after exposure to saline-boron stress. Leaf Cl^- content increases when plants are subjected to salinity stress, and this increase depends on the cultivar and plant organ tested (Kchaou *et al*., 2010). In this study, Suca and SPI exhibited leaf Cl^- contents of 1,446.3 and 1,072.7 mg L^{-1}, respectively, under saline-boron stress (Table 2). The Cl^- levels in the roots were greater than those in the leaves (Table 3). The greater Cl^- content of roots may indicate a physiological exclusion mechanism that regulates the passage of this anion to the foliage, thereby avoiding toxic concentrations (Chartzoulakis, 2005). Kchaou *et al.* (2010) reported that cultivars Chemlali, Koroneiki, Arbequina I18, and Arbosana I43 exhibited Cl^- contents in their leaves of 3,500, 4,400, 4,100 and 3,800 mg L^{-1} and Cl^- contents in their roots of 7,600, 6,500, 8,200 and 6,500 mg L^{-1}, respectively, when treated with 200 mM NaCl. The Cl^- contents in leaves and roots in the study of Kchaou *et al*. (2010) were greater than those found in the present study. Although genotypic differences between olive cultivars have been documented (Chartzoulakis *et al*., 2002; Perica *et al*., 2008), the main reason for the difference may be the type of stress applied; Kchaou *et al*. (2010) only varied the concentration of NaCl, whereas the stress applied in this study combined 200 mM NaCl with 0.49 mM boron.

Boron in leaves and roots

The absorption of B was different among the accessions, and although the leaf B content increased in some, all accessions contained levels much lower than the critical level for most plants. In fact, the observed levels of B were very low in the studied accessions (Table 4); under conditions of saline-boron stress, B levels varied from 6.3 to 33.5 mg L^{-1}. The level of leaf B can be as high as 70.0 mg L^{-1} without toxicity, although this may be related to the duration for which plants are exposed to this element (Brown and Hu, 1997; Szwerdszarf, 2003). B levels increased in the roots and leaves in response to higher levels of B in the irrigation water, possibly because the transport of B by the xylem is influenced by the rate of transpiration (Raven, 1980). The levels of B were higher in the roots than in the leaves, possibly indicating the existence of an exclusion mechanism in that roots, thus restricting the passage of B to the leaves (Gucci *et al*., 1999). The accessions Azapa, Suca and Chiza exhibited the greatest tolerance to the absorption of B ions at the levels of the roots and leaves. However, although the Azapa, Suca and Chiza accessions absorbed only low levels of B in their leaves and roots, the Lluta accession contained less B in the leaves and more in the roots, indicating that this accession appears to have an efficient system for B exclusion or an important mechanism to limit B transport from the root zone to the aerial parts.

Water potential

Significant variability in water potential among the studied accessions was observed during saline-boron stress. Most accessions exhibited a significant decrease in water potential, although the accession Taltal exhibited no change in this parameter. Lluta and SPII were the least altered accessions, whereas Suca, Chiza, SPI, Azapa and Frantoio exhibited significant increases in water potential. The effects of water restriction depend upon climatic conditions, phenological state, type of soil, salt concentration and plant genotype (Montaldi, 1995). The values of this parameter observed under saline-boron stress affected the water tension in the xylem in the majority of the olive accessions studied; however, the most negative value observed (-2.28 MPa in SPI) may still be considered within the range that does not cause osmotic stress or cell dehydration (Bastías *et al*., 2002). In irrigated field-grown olive trees (cultivar Memecik), leaf water potential values were approximately -2.5 MPa; for the stress treatment, these values decreased to below -4 MPa (Akkuzu *et al*., 2010). Levels below -0.9 MPa may reduce activities such as transpiration, photosynthesis, stomatal conductance and internal CO_2 content, causing a suspension of these activities at extreme values such as -7.0 MPa (Xiloyannis *et al*., 1999; Ennajehe *et al*., 2008). As a tree, the olive can absorb water from the soil at values as low as -2.5 MPa by regulating the water potential differential between the leaves and roots; this physiological condition classifies the olive as tolerant to water deficit (Ennajehe *et al*., 2008). The accessions Lluta and SPII exhibited little modification of water potential under saline-boron stress, whereas the other accessions exhibited greater alterations (Table 5). The most important accession is Taltal, which exhibited no significant change in leaf water potential under saline-boron stress. Despite these differences, the accessions with the lowest water potential did not present visual symptoms of external structural damage. This might indicate that the reduction of water potential is not really a deficiency but rather an important mechanism adopted by these accessions that allows them to adjust their water balance to overcome saline-boron stress.

Relative water content (RWC)

The values of RWC observed in the controls ranged from 61.0% to 67.2%, but under saline-boron stress, the values ranged between 50.9% and 57.3%. Although the controls exhibited RWC values considered normal for olives (Larsen *et al*., 1989), plants submitted to saline-boron stress exhibited moderate water stress (González, 2004). Lluta and Azapa exhibited more sensitivity of RWC to saline-boron stress, with reductions of 20.5% and

Table 5. Water potential and RWC of eight olive accessions due to the effect of saline-boron stress. Values represent the means of measurements on 5 plants of each accession and their standard deviations. Different letters represent significant differences between the means of different accessions (Fisher test, P≤0.05), and the *p-value* represents the difference between the means of the same accession (Student test, P≤0.05).

	Water potential (MPa)			RWC (%)		
Accession	Control	Saline-boron	P - value	Control	Saline-boron	P - value
Suca	-0.39 ± 0.04 d	-1.80 ± 0.2 bc	1×10^{-4}	66.8 ± 3.0 ab	56.8 ± 2.0 ab	2×10^{-4}
Chiza	-0.85 ± 0.07 b	-1.81 ± 0.1 b	2×10^{-7}	61.0 ± 1.5 d	57.3 ± 1.4 a	7×10^{-4}
SP I	-1.19 ± 0.04 a	-2.28 ± 0.1 a	1×10^{-8}	65.0 ± 1.9 abc	54.8 ± 2.0 bc	3×10^{-5}
Taltal	-0.80 ± 0.10 b	-0.85 ± 0.1 d	3×10^{-1}	63,7 ± 2.3 c	53.9 ± 2.2 c	1×10^{-4}
Azapa	-0.56 ± 0.06 c	-1.70 ± 0.2 bc	3×10^{-7}	64.0 ± 2.6 bc	52.9 ± 2.0 cd	6×10^{-5}
SP II	-0,58 ± 0.10 b	-0.79 ± 0.1 e	1×10^{-2}	65.7 ± 1.8 abc	54.8 ± 1.7 bc	9×10^{-6}
Frantoio	-0.46 ± 0.07 d	-1.63 ± 0.2 c	3×10^{-7}	67.2 ± 1.5 a	53.7 ± 2.6 c	8×10^{-6}
Lluta	-0.43 ± 0.05 d	-0.81 ± 0.1 d	4×10^{-5}	64.1 ± 2.0 bc	50.9 ± 2.0 d	5×10^{-6}

17.3%, respectively, with respect to the controls. Frantoio is globally considered tolerant to water stress (Chartzoulakis, 2005); in this study, the RWC observed in Frantoio was comparable to that in Taltal and slightly superior to that in Azapa. It is worth noting that Frantoio was the only accession that was not obtained from northern Chile.

The key findings of the study are as follows. Saline-boron stress of 200 mM NaCl and 0.49 mM $B(OH)_3$ may have negative effects on several of the measured parameters. In general, this stress reduced biomass and increased the B and Cl^- contents in all of the studied accessions; however, the damage caused by this type of stress was expressed with different intensities depending on the accession. The different capacities of the accessions with respect to growth, water and ionic conditions indicate that they contain specific systems that are used to avoid the excessive absorption of B and Cl^- and to distribute these chemicals adequately within the plants. All accessions had greater B and Cl^- contents in the roots, thereby avoiding greater concentrations in the sensitive leaf tissues. The accessions Suca, Chiza, Azapa and Lluta exhibited the lowest levels of B in the leaves; Suca and SPI had the lowest concentrations of Cl^- in the leaves. From another perspective, some accessions contained low concentrations of Cl^- and B in the roots, indicating that the plant has the capacity to avoid or minimize the absorption of these chemicals. Suca and SPI contained the lowest Cl^- concentrations in their roots, whereas Suca and Azapa had the lowest B concentrations in their roots. The accessions Taltal, SPII and Lluta were the most efficient at regulating the water potential, and although the majority of the accessions decreased their water potential due to saline-boron stress, normal levels (for olives) were maintained.

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Resumen

H. Escobar, N. Lara, Y. Zapata, C. Urbina, M. Rodriguez y L. Figueroa. 2013. Estrés salino-boro en accesiones de olivos del norte de Chile: relaciones hídricas, contenido de B y Cl^- e impacto en el crecimiento de las plantas. Cien. Inv. Agr. 40(3): 597-607. Este trabajo tiene por objetivo analizar el efecto del estrés salino-boro en el crecimiento vegetativo, peso seco de hojas, potencial hídrico (Ψ_w), contenido relativo de agua (RWC), contenido foliar y radical de B y Cl^- en 8 accesiones (acc.) de olivo. Plantas enraizadas de un año de edad fueron cultivadas por un periodo de 132 días en condiciones de 50% de sombra, en macetas de 5 L con sustrato de arena y regadas con una solución nutritiva de Jensen. Después de 8 días de fertirrigación uniforme, las plantas fueron expuestas a un estrés salino-boro, el cual se suministró en tres etapas sucesivas para acondicionar las plantas al estrés final correspondiente a 0.49 mM de $B(OH)_3$ y 200 mM de NaCl. Las acc. se identifican según su lugar de procedencia como Suca, Chiza, San Pedro I (SPI), Taltal, Azapa, San Pedro II (SPII), Frantoio y Lluta. Los resultados indican que el estrés salino-boro causa una disminución del crecimiento vegetativo y de la materia seca en todas las acc. El nivel de Cl^- en hojas y raíz aumenta significativamente, siendo menor en las acc. Suca y SPI. El B aumenta en hoja y raíz sin alcanzar niveles tóxicos. El potencial hídrico disminuye, excepto en la acc. Taltal y SPII. El CRA (RWC) disminuye en todas las accesiones. El cv Frantoio, conocido internacionalmente por su alta tolerancia a la salinidad, es utilizado como una referencia de las respuestas observadas en las demás accesiones.

Palabras clave: Boro, cloruro, olivo, relaciones hídricas, salinidad.

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