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Tolerance of young (*Ceratonía siliqua* L.) carob rootstock to NaCl

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ABSTRACT

One-year-old carob (*Ceratonía siliqua* L.) rootstock was grown in fertilised substrate to evaluate the effects of NaCl salinity stress. The experiment consisted of seven treatments with different concentrations of NaCl in the irrigation water: 0 (control), 15, 30, 40, 80, 120 and 240 (mmol L⁻¹), equivalent to electrical conductivities of 0.0, 1.5, 2.9, 3.9, 7.5, 10.9 and 20.6 dS m⁻¹, respectively. Several growth parameters were measured throughout the experimental period. At the end of the experiment, pH, extractable P and K, and the electrical conductivity of the substrate were assessed in each salinity level. On the same date, the mineral composition of the leaves was compared. The carob rootstock tolerated 13.4 dS m⁻¹ for a period of 30 days but after 60 days the limit of tolerance was only 6.8 dS m⁻¹. Salt tolerance indexes were 12.8 and 4.5 for 30 and 60 days, respectively. This tolerance to salinity resulted from the ability to function with concentrations of Cl⁻ and Na⁺ in leaves up to 24.0 and 8.5 g kg⁻¹, respectively. Biomass allocation to shoots and roots was similar in all treatments, but after 40 days the number of leaves was reduced, particularly at the larger concentrations (120 and 240 mmol NaCl L⁻¹). Leaves of plants irrigated with 240 mmol NaCl L⁻¹ became chlorotic after 30 days exposure. However, concentrations of N, P, Mg and Zn in leaves were not affected significantly ($P > 0.05$) by salinity. Apparently, K⁺ and Ca²⁺ were the key nutrients affected in the response of carob rootstocks to salinity. Plants grown with 80 and 120 mmol L⁻¹ of NaCl contained the greatest K⁺ concentration. Na⁺/K⁺ increased with salinity, due to an elevated Na⁺ content but K⁺ uptake was also enhanced, which alleviated some Na⁺ stress. Ca²⁺ concentration in leaves was not reduced under salinity. Salinization of irrigation water and subsequent impacts on agricultural soils are now common problems in the Mediterranean region. Under such conditions, carob seems to be a salt as well as a drought tolerant species.

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1. Introduction

Soil salinization is one of the most severe causes of yield reduction in modern agriculture (Paranychianakis and Chartzoulakis, 2005). It is estimated that from 4 to 7% of arable land surface is adversely affected by excessive salt concentration (Ebert, 2000). Around 380 million ha are potentially usable for agriculture, but production is severely restricted by salinity (Lambers, 2003). Salt-affected areas are a prevalent problem, particularly in arid and semi-arid regions. In such locations expansion of agriculture into arid zones with high evapotranspiration rates, leads to rising saline water tables, a concept known as “dryland salinity” (Lambers, 2003). Over the next two decades soil salinity may become a serious problem in southern Portugal, since saline groundwater

continues to rise in catchments, declining the quality of water resources and consequently affecting the traditional agro-ecosystems in the Mediterranean area (Ben Asher et al., 2002).

The adverse effects of salinity result from the inhibition of water uptake, because of the low soil water potentials and the toxicity effects of specific ions at cellular level (Ebert, 2000). Higher plants have distinctive behaviours when faced with salinity so that they can be segregated into two groups: includer (mostly halophytes) and excluder-plants (mainly non-halophytes including fruit trees). Includer-plants rely on the accumulation of significant amounts of ions to ensure a sufficient water uptake. In contrast, the classification of salt tolerance of excluder-plants depends on the ability of these plants to avoid ion translocation or to compartmentalize ions within the cell (Marschner, 1995). According to Kozłowski (1997) salt tolerance of horticultural crops is difficult to quantify because it depends on environmental (e.g., soil fertility, soil physical conditions, irrigation methods, and climate) and plant factors (stage of growth, variety, and rooting

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characteristics). However, tolerance of fruit trees to salinity can be classified according to their different growth responses to electrical conductivity (EC). For example, in Mediterranean habitats it is possible to find moderately salt tolerant species, such as fig (*Ficus carica* L.) and pistachio (*Pistacia vera* L.) that can grow in soil with an electrical conductivity (EC_{soil}) of 6 dS m^{-1} as well as very sensitive species such as almond (*Prunus dulcis* Mill.) that cannot grow in soil with $EC_{soil} > 1 \text{ dS m}^{-1}$ (Ebert, 2000). In olive (*Olea europaea* L.), a moderately salt tolerant tree crop (Maas and Hoffman, 1977), relative growth of salt-treated plants ranged from 16 to 70% of control plants showing a high intraspecific variability for salt tolerance (Marín et al., 1995).

In saline habitats, the vegetative and reproductive growths of fruit trees are affected (Noble and West, 1989; Kozłowski, 1997). Relative to growth in other habitats their shoot development and leaf initiation are suppressed as well as leaf expansion and internode growth. The presence of NaCl alters the nutritional balance of plants (Ruiz et al., 1997, 1999; Savvas and Lenz, 2000) and elevated concentrations of Na^+ and Cl^- in the soil solution may decrease the activities of various ions leading to nutritional imbalances of Ca^{2+} , K^+ , Mg^{2+} and NO_3^- (Grattan and Grieve, 1999). A particular result of salinity stress induced by Na^+ salts is the reduction in availability and transport of Ca^{2+} to the growing parts of the plant (Grattan and Grieve, 1999).

Since an important part of salinity management strategy (Lambers, 2003) could be “living with salt” it is important to know what species within the biodiversity of Mediterranean agroecosystems are particularly tolerant to soil salinity. Due to sea water intrusion in deep wells, particularly during dry years, it is likely that available irrigation water will carry excessive salts. Carob (*Ceratonia siliqua* L.) is a sclerophyllous leguminous evergreen tree species cultivated in Mediterranean ecosystems, notably in marginal and calcareous soils (Martins-Loução, 1990). Water-use strategies and nutrient (N, P and K) dynamics are already documented (Correia and Martins-Loução, 2005) but some ecological traits of this species are not yet known. Carob appears to grow successfully in saline soils (tolerance to a soil salt content of up to 3% of NaCl; Batlle and Tous, 1997). Cruz et al. (1996) studied several nutrients interactions used to overcome the adverse effect of NaCl, but there is no information about the mechanism of salt tolerance including impacts on nutrient balance and biomass allocation. In this crop, seeds produced by domestic female cultivars carry *cultivated* × *wild hybrid* combinations since female flowers are fertilised by pollen from different unknown genetic sources. Seedlings are transplanted to the field and after two to four years, rootstocks are grafted with female buds of chosen productive trees (Batlle and Tous, 1997). Carob is a crop that is productive even when its water use is less than 50% of the daily class pan evaporation. However, like other more productive tree crops, rootstocks need irrigation during the first two to three years to achieve good vegetative growth and reach suitable size for grafting. The effect of water quality (specifically salinity level) on the earlier stages of growth has never been studied. In this work, a pot experiment was established in which young carob rootstock was irrigated with saline water to assess tolerance to salinity. It was hypothesized that this crop would adapt its growth patterns and adjust its ionic balance to overcome the adverse effect of salinity.

2. Materials and methods

The experiment was conducted at the University of Algarve, in Faro ($37^\circ 02' \text{N}$; $7^\circ 58' \text{W}$). Carob (*C. siliqua* L.) rootstock plants, one-year-old, were acquired from a commercial nursery. As carob is dioecious, the seeds from any female plant are genetically variable as are the rootstocks formed from them. Each plant was transferred

to 16 cm-diameter plastic pots that contained a mixture of calcareous soil, vermiculite and peat in a 2:1:1 proportion. During substrate preparation two different NPK fertilisers were supplied and mixed with the substrate: 12:12:17 (+2% MgO) and a slow release fertiliser (11:22:9) to ensure non-limiting amounts of these elements throughout the experimental period.

The plants were placed outdoors from January until the experiment began in June 2005. The experiment ended in August 2005. The site had a typical Mediterranean climate, with hot summers and mild winters. During the experimental period (June–August) precipitation was null and the mean air temperature varied between 19.2°C (minimum) and 28.1°C (maximum).

2.1. Experimental design

The experiment consisted of seven treatments using: distilled water (control) and solutions with $15 \text{ mmol NaCl L}^{-1}$ (1.5 dS m^{-1}), $30 \text{ mmol NaCl L}^{-1}$ (2.9 dS m^{-1}), $40 \text{ mmol NaCl L}^{-1}$ (3.9 dS m^{-1}), $80 \text{ mmol NaCl L}^{-1}$ (7.5 dS m^{-1}), $120 \text{ mmol NaCl L}^{-1}$ (10.9 dS m^{-1}) and $240 \text{ mmol NaCl L}^{-1}$ (20.6 dS m^{-1}). Each treatment comprised 10 plants, giving to a total of 70 plants in the experiment. NaCl was used as the sole salinizing agent. As Grattan and Grieve (1999) pointed out, such a procedure might limit the interpretation of data, due to an unrealistic composition of irrigating water. As this study was the first attempt to assess the salt tolerance of a carob rootstock, it seemed reasonable to use irrigation water composition that had electrical conductivity comparable to that found in groundwater wells where carob has been found to grow. The NaCl solutions were prepared in the laboratory and used to irrigate the plants every two days. Each plant received a total of 9 L of distilled water or NaCl solution throughout the experimental period. Leaf wetting due to irrigation was avoided. The electrical conductivity of each solution was measured using a conductivity meter (Hanna Instruments) and the values were: 0.0, 1.5, 2.9, 3.9, 7.5, 10.9 and 20.6 dS m^{-1} , respectively in 0, 15, 30, 40, 80, 120 and $240 \text{ mmol NaCl L}^{-1}$ treatments (Table 1). Carob is grown in an area where the conductivity of the water from deep wells can reach 2.4 dS m^{-1} but is still used to irrigate crops.

2.2. Substrate parameters

Three random substrate samples were taken before salinization treatments, oven-dried for 48 h at 40°C then passed through a 2-mm sieve. Substrate potassium (K) and phosphorus (P) were extracted using a solution of ammonium acetate (Riehm, 1958) and sodium bicarbonate (Olsen and Sommers, 1982), respectively. The P content in the extracts was quantified colorimetrically, and K by atomic absorption spectrometry. Substrate pH was determined in substrate–water suspensions (1:2.5) and organic carbon by oxidation using dichromate (Walkley and Black, 1934).

Table 1

Effect of NaCl concentration (mmol L^{-1}) on pH (H_2O), electrical conductivity (EC_s), K and P concentrations in the substrate at the end of the experiment (60 days exposure to salinity). The electrical conductivity of the irrigation water (EC_i) of each salinity treatment is also presented. Treatments with the same letters are not significantly different at $P < 0.05$ (Duncan multiple range test). ns: not significant.

Salinity treatments					
NaCl (mmol L^{-1})	EC_i (dS m^{-1})	EC_s (dS m^{-1})	pH (H_2O)	P (mg kg^{-1})	K (mg kg^{-1})
0	0	0.5 f	7.66 abc	32 ns	280 a
15	1.5	1.8 e	7.65 abc	24 ns	225 ab
30	2.9	3.6 d	7.59 bc	32 ns	246 a
40	3.9	5.1 c	7.51 c	25 ns	237 a
80	7.5	5.6 c	7.73 ab	21 ns	238 a
120	10.9	7.9 b	7.78 a	48 ns	247 a
240	20.6	10.8 a	7.70 ab	33 ns	175 b

Substrate conductivity was measured with a conductivity meter (Hanna Instruments) in 1:5 soil to demineralised water mixtures. Total carbonates were measured by the quantification of the CO₂ produced from the reaction of the substrate with HCl (Allison and Moodie, 1965). The substrate had 5.9% of organic matter, 19% of total calcium carbonate, an electrical conductivity of 0.86 dS m⁻¹, and a pH in water (1:25) of 7.2. Just after the application of the fertilisers, the substrate had 47.8 mg P kg⁻¹ and 457.4 mg K kg⁻¹.

2.3. Plant parameters

The number of leaves was counted every 10 days. Plants were harvested 30 and 60 days after exposure to the salinity treatments. Roots were gently washed from the growth medium and surface water removed with tissue. Fresh and dry weights of the leaves, shoots and roots were determined for five plants randomly selected from each treatment. However, some plants grown in the 80 and 240 mmol L⁻¹ NaCl treatments did not survive for 60 days, so fewer plants from these treatments were available for analysis. Dry weight of leaves, shoots and roots were used to calculate several biomass parameters as indicated by Ryser and Lambers (1995): leaf weight ratio (LWR; leaf dry weight per unit plant dry weight), shoot weight ratio (SWR; shoot dry weight per unit plant dry weight) relative root weight (RWR; root dry weight per unit plant dry weight) and root to shoot ratio (R/S; root dry weight per unit shoot dry weight). Visual symptoms of salt effects on leaves were assessed. Total dry weight (TDW) was calculated as the sum of leaves, shoots and roots dry weight.

2.4. Leaf nutrients

To determine leaf nutrients concentration, three plants were randomly selected in each treatment at the final harvest date (August). Complete leaves (leaflets plus petiole) were removed from shoots and washed with tap water, followed by distilled water containing a non-ionic detergent, and then with 0.01 M HCl. Finally, three rinses were carried out with distilled water.

After drying to constant weight at 60 °C the leaves were then ground and ashed at 450 °C, and digested in 10 cm³ HCl 1 M. Standardized procedures (AOAC, 1990) were used to determine mineral ion concentration. Nitrogen was analysed by the Kjeldahl method and the concentrations of K, Ca, Mg, Na, Zn, Cu, Mn and Fe were determined by atomic absorption spectrometry. P was determined colorimetrically by the molybdo-vanadate method using a 375 nm wavelength light source (Dickman and Bray, 1940). Chloride was extracted from 0.1 g of ground material with 50 mL of distilled water and measured with an ion selective electrode. Subsequently, nutrient ratios (Na⁺/Ca²⁺ and Na⁺/K⁺) were calculated (Chartzoulakis, 2005; Tabatabaei, 2006).

2.5. Salinity response models and salt tolerance index

To model carob response to salinity, yield response functions were studied (Maas and Hoffman, 1977; Steppuhn et al., 2005a). Because yield can be obtained from different plant components, it should be expressed on a relative basis. Since we investigated one-year-old carob rootstocks material (no fruit production), total plant dry weight (roots and shoots with leaves) was used as yield (*Y*) in all tested models. Yield functions were assessed in two different sampling dates: 30 and 60 days from salinization. To compare these dates, the absolute yield (*Y*) was converted into relative yield (*Y_r*) by employing a scaling divisor (*Y_m*) based on the maximum value of total biomass where salinity has no influence on yield (Maas and Hoffman, 1977). Since the maximum yield (*Y_m*) was obtained with 15 mmol NaCl L⁻¹, the plants irrigated without NaCl

(0 mmol NaCl L⁻¹) were not considered. The *Y_r* value for each salinity treatment was determined separately for 30 and 60 days growth according to:

$$Y_r = \frac{Y}{Y_m} \quad (1)$$

After data transformation using Eq. (1), to test the yield response to salinity, an exponential model was selected on the basis of the best fit according to the square of the correlation coefficient (*r*²):

$$Y_r = ae^{bEC_i} \quad (2)$$

where *EC_i* is the electrical conductivity of irrigation water, *a* is a constant that reflects the shape of the curve, and *b* is always negative and defines the slope of the model. For each coefficient (*a* and *b*) the standard errors were also calculated. Similar approaches have been used for other tree crops (Steppuhn et al., 2005a). Steppuhn et al. (2005b) proposed a salt tolerance index (ST-Index) as an indicator of the inherent salinity tolerance of agricultural crops to root-zone. For *Y_r* = 0.5, the yield that was 50% of the maximum yield, Eq. (2) allowed the corresponding electrical conductivity of the irrigation water (*EC_{i50}*) to be calculated. The ST-Index was then computed as proposed by Steppuhn et al. (2005a):

$$ST\text{-Index} = EC_{i50}(1 + b) \quad (3)$$

where *b* was the same as in Eq. (2).

2.6. Statistical analysis

The experiment had a completely randomised design, and the values obtained for each plant and each variable were considered as independent replicates. In some treatments, the number of plants sampled on the last date decreased from 5 to 2 due to the death of plants induced by salinity.

The means were compared by one-way analysis of variance and by using the Duncan multiple range test at *P* < 0.05, using the SPSS Software (v. 12.0).

3. Results

3.1. Substrate measurements

At the end of the experiment, the pH values of the substrate of all treatments (Table 1) increased slightly in relation to the initial value (7.2). As expected, electrical conductivity of the soil, *EC_s* (Table 1), increased due to salinity, but treatments with 40 and 80 mmol L⁻¹ showed similar values, 5.1 and 5.6 dS m⁻¹, respectively. Extractable P ranged from 21 to 48 mg kg⁻¹ and K ranged from 175 to 280 mg kg⁻¹ (Table 1). K decreased only for the treatment with the highest level of salinity. Apparently the P concentration was not affected by salinization.

3.2. Biomass and visual symptoms

Salt stress led to marginal chlorosis of mature leaves and then to inter-vein chlorosis and spots of necrotic tissue. These symptoms were firstly observed in plants irrigated with 240 mmol L⁻¹ after 30 days of exposure and were followed by dieback of the shoot tips.

Fifty days after exposure, a NaCl concentration of 240 mmol L⁻¹ triggered severe injury leading to the death of all plants. At the end of the experiment only two plants survived the 80 mmol NaCl L⁻¹ treatment.

There were no significant effects of salinity treatment on the number of leaves during the first 30 days in all treatments (Fig. 1). Thereafter, the number of leaves on plants irrigated with 0, 15 and 30 mmol NaCl L⁻¹ increased, while leaf numbers in the

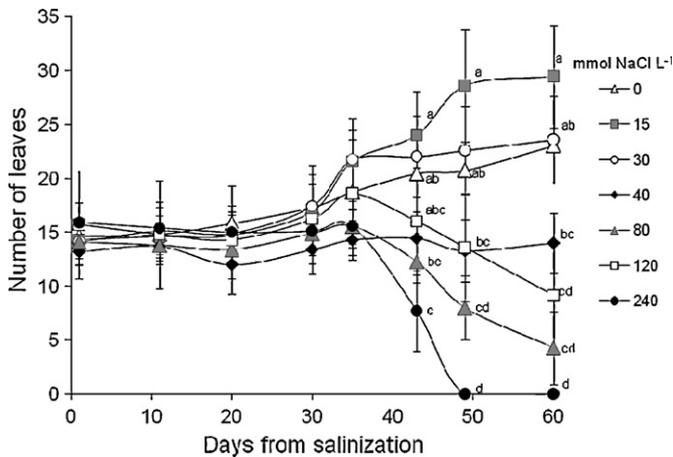


Fig. 1. Salinity effect (0, 15, 30, 40, 80, 120, 240 mmol NaCl L⁻¹) on the number of leaves of carob over a period of 60 days. Means with the same letter indicate no significant difference at $P < 0.05$ (Duncan multiple range test). Vertical bars indicate the standard error.

40 mmol NaCl L⁻¹ treatment remained constant but after 40 days leaf shedding occurred in plants irrigated with 80, 120 and 240 mmol NaCl L⁻¹. At the end of the experiment, it was clear that salinity had a great effect on leaf number (coefficient of correlation $r = -0.88$; $P < 0.001$).

Changes in biomass parameters were noticeable 30 days from salinization (Table 2) since total weight (TDW) was affected. Biomass allocation to shoots (SWR) and roots (RWR) were similar in all saline treatments, but 60 days from salinization the biomass allocated to leaves (LWR) was severely affected, particularly in the highest concentration (240 mmol NaCl L⁻¹). Root to shoot ratios were also similar for all treatments.

Table 2
Effect of NaCl concentration on total plant dry weight (TDW), leaf weight ratio (LWR), shoot weight ratio (SWR), relative root weight (RWR) and root to shoot ratio (R/S). n : number of plants per treatment. Treatments with the same letters are not significantly different at $P < 0.05$ (Duncan multiple range test). ns: not significant.

Salinity treatments (mmol NaCl L ⁻¹)	n	TDW	LWR	SWR	RWR	R/S
30 days from salinization						
0	5	16.0 abc	0.36 ns	0.32 ns	0.32 ns	0.52 ns
15	5	21.8 a	0.33 ns	0.24 ns	0.43 ns	0.82 ns
30	5	13.7 bc	0.40 ns	0.21 ns	0.41 ns	0.67 ns
40	5	19.0 ab	0.34 ns	0.24 ns	0.42 ns	0.74 ns
80	5	15.0 abc	0.37 ns	0.24 ns	0.39 ns	0.67 ns
120	5	12.4 bc	0.35 ns	0.24 ns	0.42 ns	0.71 ns
240	5	8.6 c	0.25 ns	0.33 ns	0.42 ns	0.75 ns
60 days from salinization						
0	5	25.3 a	0.37 ab	0.20 ns	0.43 ns	0.78 ns
15	5	25.9 a	0.33 b	0.26 ns	0.41 ns	0.71 ns
30	5	22.9 a	0.35 ab	0.22 ns	0.42 ns	0.74 ns
40	5	23.9 a	0.32 b	0.22 ns	0.46 ns	0.89 ns
80	2	14.2 b	0.45 a	0.21 ns	0.34 ns	0.52 ns
120	5	22.2 a	0.28 bc	0.25 ns	0.47 ns	0.93 ns
240	3	17.1 ab	0.21 c	0.29 ns	0.51 ns	1.11 ns

Table 3
Mineral composition of macronutrients and chloride (expressed in g kg⁻¹) in carob leaves treated with different concentrations of NaCl (mmol L⁻¹) at the end of the experiment (60 days exposure to salinity). Treatments with the same letters are not significantly different at $P < 0.05$ (Duncan multiple range test). ns: not significant.

Salinity treatments (mmol NaCl L ⁻¹)	N	P	K	Mg	Ca	Na	Cl
0	14.6 ns	1.3 ns	5.0 d	1.7 b	7.4 b	0.7 d	7.7 c
15	16.8 ns	1.2 ns	9.6 bc	2.1 a	9.7 b	3.3 cd	20.1 b
30	17.8 ns	1.4 ns	9.7 bc	2.0 ab	9.4 b	8.5 abc	29.5 ab
40	15.4 ns	1.2 ns	8.9 c	2.0 ab	9.4 b	5.5 bcd	24.0 b
80	16.8 ns	1.2 ns	11.8 a	2.0 ab	8.5 b	11.4 ab	29.0 ab
120	15.8 ns	1.2 ns	14.1 a	2.2 ab	9.6 b	13.0 a	36.0 a
240	12.2 ns	1.4 ns	9.1 bc	2.6 a	13.9 a	12.4 ab	33.7 a

3.3. Mineral composition of leaves

Nitrogen and P concentrations in leaves were not affected by salinity (Table 3). However, Mg concentrations in leaves of plants from 15 and 240 mmol NaCl L⁻¹ treatments were greater than those from the control treatment (0 mmol NaCl L⁻¹). The largest value of the Ca²⁺ concentration was observed in plants irrigated with 240 mmol NaCl L⁻¹. In control plants, the K⁺ concentrations were also smaller than in other treatments. Plants with 80 and 120 mmol NaCl L⁻¹ had the greatest K⁺ concentration. Leaf Na⁺ concentrations increased significantly in response to the salt treatments (more than 15 times). Plants treated with greater salt concentrations (80, 120 and 240 mmol NaCl L⁻¹) showed the largest Na⁺ concentrations. Leaf chloride increased with the sodium concentration ($r = 0.95$; $P < 0.001$), but the proportion was less than unity. Zinc was not affected by salinity and although Mn concentrations tended to increase with salinity there was no clear response (Table 4). The concentration of Fe was greater in plants treated with 80 and 120 mmol L⁻¹.

Plotting Na⁺/K⁺ and Na⁺/Ca²⁺ ratios versus salinity of irrigation water led to two quadratic models (Fig. 2). Ratios of Na⁺/Ca²⁺ greater than 1 were obtained in treatments over 80 mmol NaCl L⁻¹, but for Na⁺/K⁺, an extreme imbalance was only observed for the 240 mmol NaCl L⁻¹ treatment (Fig. 2).

3.4. Salinity response models and salt tolerance index

Total dry weight (TDW, Table 2) was related to electrical conductivity of irrigation water (EC_i) in both 30 and 60 days exposure to salinity. The best models (those with the smallest root mean square error and the largest r^2 value) obtained for each sample date were, respectively:

$$Y_{T(30 \text{ days from salinization})} = (0.92 \pm 0.095)e^{(-0.041 \pm 0.01)EC_i} \quad (4)$$

$$Y_{T(60 \text{ days from salinization})} = (3.49 \pm 3.03)e^{(-0.344 \pm 0.09)EC_i} \quad (5)$$

Table 4
Mineral composition of micronutrients (expressed in mg kg^{-1}) in carob leaves treated with different concentrations of NaCl (mmol L^{-1}) at the end of the experiment (60 days exposure to salinity). Treatments with the same letters are not significantly different at $P < 0.05$ (Duncan multiple range test). ns: not significant.

Salinity treatments (mmol NaCl L^{-1})	Mn	Zn	Fe
0	13.6 b	10.2 ns	82.7 b
15	17.6 ab	10.2 ns	47.5 d
30	17.7 ab	11.5 ns	58.9 cd
40	13.3 b	9.5 ns	80.8 b
80	12.9 b	9.5 ns	104.5 a
120	16.1 ab	13.2 ns	119.3 a
240	20.2 a	15.8 ns	70.5 bc

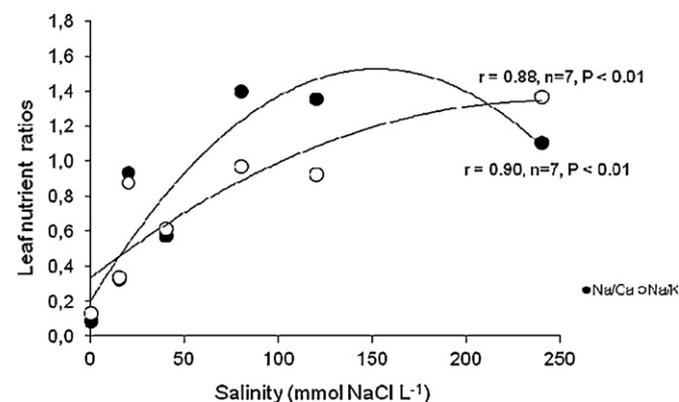


Fig. 2. Relationship between salinity (mmol NaCl L^{-1}) and leaf Na/Ca and Na/K ratios at the end of the experiment.

where Y_r is relative yield and EC_i is the electrical conductivity (dS m^{-1}) of irrigation water (30 days from salinization: $r^2 = 0.81$; $F_{(1,4)} = 17.0$; $P = 0.015$; 60 days from salinization: $r^2 = 0.80$; $F_{(1,4)} = 16.2$; $P = 0.016$). (Regression coefficients are given \pm standard error).

The relative yields decreased exponentially as salinity increased (Fig. 3) but the impact on relative yield was much greater after 60 days exposure to salinity than after 30 days (Fig. 3).

Based on these models, it was possible to estimate the EC_{i50} (the salinity level associated with a 50% decrease of the maximum

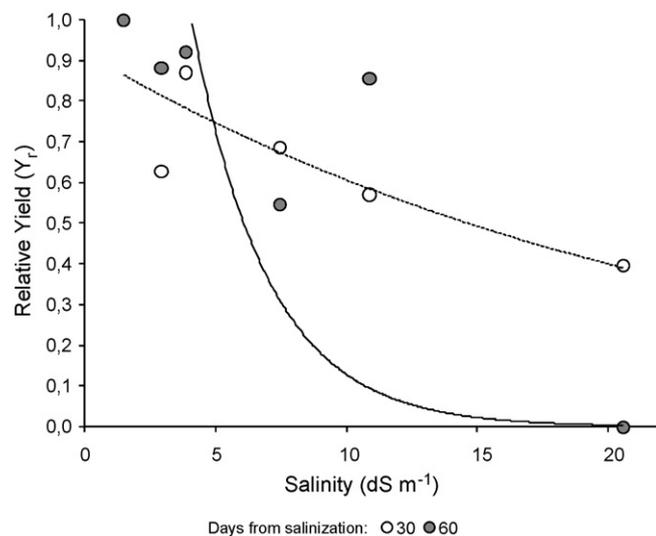


Fig. 3. Relationship between the electrical conductivity of irrigation water and relative yield (expressed as total dry weight) after 30 days and 60 days exposure to salinity.

Table 5
Mid-yield salinity (EC_{i50}) and salinity tolerance index (ST-Index) for 30 and 60 days exposure to salinity.

Exposure to salinity (days)	EC_{i50} (dS m^{-1})	ST-Index (dS m^{-1})
30	13.4 ± 0.2	12.8
60	6.5 ± 1.4	4.3

yield) for each date (Table 5). Carob rootstock may tolerate 13.4 dS m^{-1} for a period of 30 days of exposure but for 60 days the limit of tolerance was only 6.5 dS m^{-1} . The corresponding stress tolerance indices were 12.8 and 4.3, respectively for 30 days and 60 days exposure to salinity.

4. Discussion

Based on total plant dry weight, this experiment showed that carob rootstock after two months of exposure to salinity was able to tolerate up to 6.5 dS m^{-1} (EC_i) or more than $40 \text{ mmol NaCl L}^{-1}$. Thus, this crop is one of the most tolerant of those found in typical Mediterranean orchards (Ebert, 2000; Tabatabaei, 2006). This tolerance was obtained through strategies that differed from those already described for this crop to cope with nitrogen and drought stress (Martins-Loução and Cruz, 1999). The decrease in LWR after 60 days exposure to salinity suggests that leaves were more sensitive to this stress than were shoots or roots. After 40 days exposure, leaf shed occurred in plants receiving 80, 120 and $240 \text{ mmol NaCl L}^{-1}$, while there were no new leaves produced in $40 \text{ mmol NaCl L}^{-1}$ treatment. Biomass balance between root and shoot was not significantly affected. Salinity had no effect on root/shoot ratio in maritime pine (Loustau et al., 1995) but it increased in grain legumes (Tejera et al., 2006). Correia et al. (2003) found increased root/shoot ratios in carob grown in nutrient deficient hydroponic cultures.

In citrus, biomass allocation to leaves (LWR) varied between rootstocks in response to salinity (Ruiz et al., 1997; Fernandez-Ballester et al., 2003). For the almond \times peach hybrid, GF-667, and the almond cultivar ‘Ferragnes’ leaf number was affected by salinity (maximum of $60 \text{ mmol NaCl L}^{-1}$), but not in the almond cultivar ‘Trudito’ (Noitsakis et al., 1997). Decreased leaf number, including shedding of leaves, is a normal strategy of carob plants to cope with environmental stress such as drought (Correia and Martins-Loução, 2005), nutritional deficiencies (Correia et al., 2003) and other abiotic conditions (Cruz et al., 1993).

Plant ontogeny and growth stage can affect the tolerance of horticultural crops to salinity (Kozłowski, 1997), and it is generally accepted that the longer the crop is exposed to root-zone salinity the greater the reduction in vegetative growth (Steppuhn et al., 2005a). In this work, carob showed a large ST-Index (12.8) for a 30 days period of exposure to salinity. This value was only 4.3 when the growth period was extended to 60 days. Nevertheless a yield reduction of 50% occurred when the EC_{i50} was 6.5 dS m^{-1} . In *Citrus*, a fast growing tree crop, a 60-day exposure to $10 \text{ mmol NaCl L}^{-1}$ had a significant impact on growth (Ruiz et al., 1997).

In saline conditions, Na^+ and Cl^- concentrations in the plant often exceed those of most nutrients. Sodium concentration in leaves ($0.66 \text{ g kg}^{-1} \text{ dw}$) in control plants ($0 \text{ mmol NaCl L}^{-1}$) was similar to those found in other non-salinized tree crops, like *Citrus* ($0.90 \text{ g kg}^{-1} \text{ dw}$, Syvertsen et al., 1993) and almond ($0.50 \text{ g kg}^{-1} \text{ dw}$, Noitsakis et al., 1997). In olive, Na^+ concentration in leaves was less in the salt stress tolerant cv. Mission compared with a less tolerant cv. Manzanillo (Tabatabaei, 2006). Tabatabaei (2006) suggested that tolerance was associated with the ability to reduce the transport of saline ions. In carob irrigated with, at least, $80 \text{ mmol NaCl L}^{-1}$ (7.5 dS m^{-1}), Na^+ concentration in leaves was significantly greater than in control plants, suggesting that above

this concentration, plants are no longer able to compartmentalize saline ions. Uptake and accumulation of Cl^- also increased with increasing concentrations of NaCl in the irrigation water. These were within the range observed for leaves of hybrid *almond x peach*, GF-677, grown under saline conditions but greater than found in almond leaves (Noitsakis et al., 1997). Salt-stressed plants have nutritional imbalances, due to a complex net of interactions. Normally, elevated Na^+ and Cl^- levels in soil solutions depress nutrient-ion activities and produce extreme ratios of $\text{Na}^+/\text{Ca}^{2+}$ and Na^+/K^+ (Grattan and Grieve, 1999). The present results showed that N, P and Zn concentrations in carob leaves were not clearly affected by salinity. Several studies showed reduced P in plant tissue as salinity increased (Grattan and Grieve, 1999) but others did not report any effect (Ruiz et al., 1997). Phosphorus and Zn were present in non-limiting concentrations for vegetative growth, and P was present in concentrations greater than typically found in field-grown trees (Correia et al., 2002). Chemical analysis of the substrate at the end of the experiment indicated that the P concentration was small (Table 1). However, leaf analysis did not indicate P deficiency (Table 3), which suggests that endogenous P pools were not limiting (Correia et al., 2002). Moreover, field-grown carob can efficiently take up P from solutions containing low levels of this ion (Correia and Martins-Loução, 2005).

There were only very small differences between treatments in the Mg^{2+} concentration in leaves. It is known that increases in salinity are not always associated with decreases in leaf Mg^{2+} (Grattan and Grieve, 1999). NaCl can cause Ca deficiency (Ebert, 2000) and Na^+ may inhibit Ca^{2+} and K^+ absorption resulting in Na^+/K^+ antagonism (Tejera et al., 2006). In this study, salinity increased K^+ leaf content, particularly in treatments with 80 and 120 mmol NaCl L^{-1} . Normally, a decrease in K^+ uptake is found in salt-stressed plants, but in *Citrus macrophylla* leaves Ruiz et al. (1997) reported that K^+ increased with salinity. They attributed this effect to an exchange between Na^+ and K^+ in the base of the stem close to the root, whereby K^+ is released from the root to the xylem, and then to the leaves. It is possible that such a response occurred in these carob rootstocks. In fact, with the onset of salt stress, fruit trees perform an osmotic adjustment by enhancing inorganic ions uptake (Ebert, 2000). As a strategy for salt stress resistance most crop species may show a selectivity of K^+ over Na^+ and a preferential loading of K^+ rather than Na^+ into the xylem (Hu and Schmidhalter, 2005). This agreed with the decrease of soil K^+ concentration at the end of experiment with salt increase. The maintenance of a high K^+ concentration in leaves has an important role in cation regulation (Tejera et al., 2006). As been reported for other crops (Tabatabaei, 2006), Na^+/K^+ in carob increased with salinity, due to the high level of Na^+ but in some way K^+ uptake was enhanced and somewhat alleviated the Na^+ stress. The ionic balance between Na^+ and Ca^{2+} was severely affected (Fig. 2) due to Na^+ accumulation, implying retention of Ca^{2+} in the leaf tissue. Normally, Na^+ displaces Ca^{2+} from its extracellular binding sites and Ca availability can be reduced under salinity. The ability of plants to retain Ca under salt stress conditions may be associated with their salt resistance as suggested by Hu and Schmidhalter (2005). It appears that the salt tolerance mechanism of carob was related to the capability to withstand larger leaf Cl^- and Na^+ concentrations of 24.5 and 8.5 g kg^{-1} , respectively. Moreover this species retained Ca^{2+} and increased leaf K^+ even in the largest salt treatments.

Salt effects on the micronutrient nutrition of plants are variable (Grattan and Grieve, 1999), and in this study it was also not possible to found a clear pattern. In *Citrus*, NaCl decreased the concentration of all micronutrients (Ruiz et al., 1997), but in this experiment with carob at the higher levels of salinity (80 and 120 mmol NaCl L^{-1}), leaves had greater Fe concentrations than at lower levels of salinity.

5. Conclusions

According to these results, carob can maintain the major physiological processes at 40 mmol NaCl L^{-1} ($\text{EC}_i = 4 \text{ dS m}^{-1}$) and a yield reduction of 50% was observed only for $\text{EC}_i = 6.8 \text{ dS m}^{-1}$. Given the theoretical relationship between the electrical conductivity of irrigation water and of the substrate, it is possible to conclude that carob rootstock is more tolerant than most of the fruit tree crops, such as *Citrus*, mango, avocado and peach (Zuazo et al., 2004). Moreover, for the 30-day exposure, salinity tolerance was even greater ($\text{EC}_i = 13.4 \text{ dS m}^{-1}$), which may provide an important tool for irrigation management with saline water. As a result it is expected that carob could withstand summer irrigation using saline water since it was able to cope with the toxic effects of Na^+ and Cl^- , maintaining unaltered some important physiological (leaf nutrients pools) and morphological (root and shoot biomass) parameters. Therefore, under field conditions, it is expectable that mature trees will have the ability to recover following winter rainfall since no permanent damage will have occurred. The recovery rate in field-grown trees needs further investigation, as do the responses of rootstock/scion combinations to salinity.

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