Saline Irrigation Effects on *Cynara cardunculus* L. Plants Grown in Mediterranean Soils

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Abstract. *Cynara cardunculus* L., known as cynara for industrial application, is a versatile plant for Mediterranean regions. Irrigation with non-conventional salty water sources is a common practice in these water-scarce regions. However, the research performed on cynara salt-stress response is limited and solely tested under soilless conditions. Thereby, the aims of the current experiment were to ascertain the effect of saline irrigation on cynara growth and mineral nutrition in Mediterranean soils. The influence of soil was considered using two typical agricultural soils, mainly differing in their salinity status. Plants were grown under controlled conditions from November until July in pots filled with soil amended with sewage sludge compost. Three saline irrigation treatments were applied (0.7, 2, and 3 dS·m⁻¹) with increasing concentrations of NaCl (4, 13, and 23 mM). Saline irrigation started in January and ended in June. Plants growth parameters (height, dry biomass, heads number, seed yield) declined with saline irrigation. Above-ground dry biomass of plants irrigated with 3 dS·m⁻¹ was reduced approximately one-third regarding the control value, whereas seed yield was reduced in 57%. Despite growth reduction induced by salinity, no symptoms of nutritional deficiency were observed in leaves. Saline irrigation was the main driving factor regarding cynara mineral concentration, except for potassium (K) and manganese (Mn), which were related to soil type. Chlorine (Cl) and sodium (Na) concentration increased at the whole-plant level, whereas magnesium (Mg) showed the opposing trend. Similar trends were observed in the mineral content of cynara aboveground biomass. Interaction effects between soil type and saline irrigation were marginal. Cynara exhibited high K selectivity, which might be associated with a mechanism of salt tolerance, whereas Mg is suggested as a potential indicator of salt stress in cynara plants grown in calcareous Mediterranean soils. We concluded that cynara growth and mineral nutrition were mainly affected by saline irrigation, probably as a result of the accumulation of Na and Cl.

In the coming decades, non-conventional water sources (e.g., marginal quality waters, saline-sodic drainage waters, wastewaters) will become an important component of agricultural water supplies as a result of global increasing water demand, the impacts of extreme climate events, and climate change (Qadir et al., 2007), especially in water-scarce areas. The Mediterranean region is one of the driest agricultural areas on earth (Jacobsen et al., 2012). Therefore, a shift toward water-saving strategies (e.g., crop irrigation with non-conventional waters) is necessary to meet agricultural water requirements and to

alleviate present and future demand on freshwater sources. Generally, non-conventional water sources in water-scarce regions contain moderate to high salt content, which could increase soil salinity and potentially impair plant growth.

As a general effect, salinity reduces plant growth rate, thus resulting in lower crop yields (Shannon and Grieve, 1999). As discussed by Munns (2002), this reduction occurs over time in two phases: the initial growth reduction phase is quick and is induced by the salt surrounding the plant roots, which impairs water uptake as a result of osmotic effect; the second phase takes more time to develop and results from the excessive ion accumulation in the shoots and the inability to tolerate these accumulated ions. This growth reduction may also arise because of potential nutritional imbalances induced by salinity. For instance, Na is considered to be the primary cause of ion-specific damage for some plant species (Tester and Davenport, 2003) and may impair other ions uptake, especially K, which is essential for plant life (Maathuis and Amtmann, 1999). Thereby, traits like tissue mineral concentration or nutrient uptake of salt-stressed plants can

contribute to identify and clarify potential reductions in biomass production and/or quality (Grattan and Grieve, 1999). To obtain valuable crop yields, it is advisable to study and select crop species that are able to grow under salt stress.

Cardoon (*Cynara cardunculus* L.) is a versatile plant adapted to Mediterranean conditions but limited information about its growth under salt stress is available. Cardoon, known as cynara for industrial applications, has a widespread spectrum of potential applications (liquid biofuel, paper pulp production, green forage, and pharmacological source of active compounds) (Fernández et al., 2006) with growing interest focused in the use of its high epigeal biomass yields (mainly heads and stalk) for energy purposes (Piscioneri et al., 2000; Raccuia and Melilli, 2007). However, studies on the effect of salinity on cynara growth have been limited to the vegetative period (germination stage and leaf development stage) and solely under soilless conditions (Benlloch-González et al., 2005; Colla et al., 2012; Raccuia et al., 2004). Soil plays an important role in plant nutrient availability because the concentration and composition of solutes in the soil solution control the activity of the nutrient ions, especially phosphorus (P), K, and micronutrients (Grattan and Grieve, 1992). Hence, there are still uncertainties regarding cynara growth under salt stress, mainly concerning the impact on cynara reproductive organs (stalk, caulicle leaves, and heads) and the influence of soil as a growing substrate.

The aims of the present study were to characterize the effect of saline irrigation (NaCl-dominated waters) on cynara growth and mineral nutrition in two natural Mediterranean soils. For these purposes, morphological parameters, tissue mineral concentration, and aboveground biomass mineral content of cynara plants irrigated with saline waters were examined. Additionally, the effect of saline irrigation on cynara applications is discussed as a result of its importance for potential growers.

Materials and Methods

An experiment to test cynara growth in Mediterranean soils under saline irrigation was conducted from Nov. 2009 until July 2010 under greenhouse conditions at the University Miguel Hernández (lat. 38°16'2"; N, long. 0°41'51" W; Alicante, southeastern Spain). Maximum air temperature was 39 °C and minimum 9 °C with an average of 21 ± 7 °C, whereas maximum relative humidity was 80% and minimum 65% with an average of 72% ± 6%.

Two Mediterranean calcareous soils were selected from agricultural fields located within the province of Alicante (Spain) according to their salinity status. The reason underlying this selection criterion was to determine if cynara growth was influenced by soil initial salt content. Selected soils differed in their salinity and were identified as S_A (lowest salt content) and S_B (highest

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salt content). Soils were sampled from the top 15 cm, air-dried, sieved (less than 2 mm), and characterized (Table 1). Soil pH and electrical conductivity (EC) determinations were carried out in soil/deionized water suspension of 1/2.5 and 1/5 (w/v), respectively (MAPA, 1986). Organic carbon was determined by the Walkley Black method (Nelson and Sommers, 1996), nitrogen (N) by the Kjeldahl method (Bremner, 1965), available P using the Burriel-Hernando method (Diez, 1982), texture determined by the Bouyoucos method (Gee and Bauder, 1986), and equivalent calcium carbonate by using the Bernard calcimeter (Hulseman, 1966). Micronutrients [copper (Cu), iron (Fe), Mn, and zinc (Zn)] were determined in the DTPA extract (Lindsay and Norvell, 1978), whereas Ca, K, Mg, and Na were determined in the ammonium acetate extract (Knudsen et al., 1982). In these soil extracts, micronutrients, Ca, and Mg were measured by ion absorption spectrometry and Na and K by ion emission spectrometry (Unicam 969 AAS, Unicam, U.K.). Bulk density and saturation percentage were analyzed according to standard methods for soil analysis (MAPA, 1986).

Sewage sludge compost (SSC) was used as cynara seedbed substrate as well as soil organic amendment. The reasons underlying the selection of SSC as a soil amendment were its role as an organic fertilizer and its positive effects on soil physical, chemical, and biological properties, which contribute to ameliorate part of the detrimental effects of salinity on soil properties (Lakhdar et al., 2009). This waste was obtained from the wastewater treatment plant of Aspe (southeast Spain), air-dried, homogenized, sieved (less than 4 mm), and analyzed under the recommended standards methods of R.D. 824/2005 (2005) (Table 2). Regarding plant material, the botanical variety of cynara seeds used was *Cynara cardunculus* L. var. *silvestris* Lam. (wild cardoon). Seeds were collected from plants grown in the agrarian county "Campo del Turia" (altitude: 164 m a.s.l.; precipitation: 450 mm) located in the province of Valencia (eastern Spain).

The preparation of experimental materials started in the first week of Oct. 2009. Cynara seeds were germinated in seedbeds [4% SSC/96% peat (v/v)], whereas soils and SSC were mixed, filling the experimental pots (30 × 30 × 29 cm) with the resulting amended soils. Compost fertilization rate, on a dry weight basis, was 6 kg·m⁻² (0.52 kg of compost/pot). Experimental pots were distributed in a completely randomized design with three replications per irrigation treatment (nine pots per soil type). In the third week of October, two seedlings were transplanted to each pot. The experiment started in the first week of November, when plants were thinned to one per pot.

Plants were irrigated with common irrigation water until 3 Jan. 2010, when saline irrigation started. Three irrigation treatments based on irrigation water EC (EC_w) measured at 20 °C were applied (0.7, 2, and 3 dS·m⁻¹) with increasing concentrations of NaCl (4, 13,

and 23 mmol·L⁻¹). Control treatment (EC_w = 0.7 dS·m⁻¹) consisted of common irrigation water (Table 3), whereas saline treatments (2 and 3 dS·m⁻¹) consisted of the addition of NaCl to common irrigation water until desired EC_w was reached. Saline treatments were selected to emulate the average conductivity of the most common marginal-quality water sources for irrigation in the southeast of Spain. Also, selected EC treatments were within the range of EC that poses slight soil salinity and infiltration risk but potentially high ion toxicity danger for sensitive plants (Ayers and Westcot, 1985; Hillel, 2005).

Every 2 weeks, and before NaCl addition, water used for irrigation was analyzed (Table 3) according to the Standard Methods for the Examination of Waters and Wastewaters (APHA, AWWA, and WEF, 2005). Irrigation frequency was determined on the basis of soil volumetric moisture with a WET sensor (Type WET-2) and a moisture meter (Type HH2) (Delta T Services, U.K.), which were specifically configured for selected soils. Whenever soil volumetric moisture was below 60% of water-holding capacity, pots were irrigated. On average, 31 mm/pot (2.75 L/pot) was supplied within each irrigation. Each pot was irrigated every 3 d from Nov. 2009 until Apr. 2010 and every 2 d from May 2010 until June 2010. In addition to the measurement of soil volumetric moisture, the EC of the soil pore water (EC_p) was recorded to observe soil salinity dynamics. Soil volumetric moisture and EC_p readings were based on the time domain reflectometry technique (Noborio, 2001).

The experiment ended on the first week of July 2010, when, according to the BBCH scale, cynara plants reached growth stage 83 (Archontoulis et al., 2010a). Heads were counted, plant height was recorded, and shoots were divided into heads, stalk, and leaves (basal leaves and caulicle leaves). All the plant fractions were washed with deionized water and dried in a forced-air oven at 60 °C. Then, the dry weight of the different cynara fractions was measured. Dry samples were ground and mineralized according to standards methods for plant analysis (MAPA, 1986) to determine the elemental composition of the different plant organs. In this solution, Ca, Cu, Fe, Mg, Mn, and Zn were measured by ion absorption spectrometry; K and Na by ion emission spectrometry; and P was determined by the vanadomolybdophosphoric acid colorimetric method (APHA, AWWA, and WEF, 2005). In ground non-mineralized plant samples, Kjeldahl N and Cl were determined. Chloride was extracted with hot water (Ghosh and Drew, 1991) and determined by silver-nitrate titration (APHA, AWWA, and WEF, 2005). Seed yield reported in grams per plant (SY) and seed/head weight ratio [seed harvest index (HI_{head})] were calculated according to the models proposed by Archontoulis et al. (2010b). The content of Na, Cl, and macronutrients (Ca, K, Mg, N, P) contained in cynara aboveground biomass was calculated from dry weight and tissue mineral concentration data.

Mean values and sds were calculated for each of the parameters analyzed. Data for each variable were analyzed by two-way analysis of variance ($P \leq 0.05$) to assess saline irrigation, soil type, and interaction (soil type vs. saline irrigation) effects. Differences as a result of saline irrigation treatments within soil type were separated by Duncan's multiple range test ($P \leq 0.05$). Statistical tests were calculated using SPSS software (Version 20; SPSS Institute, Chicago, IL).

Table 1. Physical and chemical properties of selected soils (mean values ± sd).

	Soil	
	S _A	S _B
pH _{1/2.5}	8.64 ± 0.02	8.39 ± 0.02
EC _{1/5} (dS·m ⁻¹)	0.23 ± 0.02	0.64 ± 0.06
OC (g·kg ⁻¹)	8.9 ± 0.3	23 ± 1
N _k (g·kg ⁻¹)	0.8 ± 0.1	2.1 ± 0.2
Phosphorus (mg·kg ⁻¹)	63 ± 1	54 ± 1
SP (%)	48 ± 2	60 ± 2
CO ₃ ⁻² _{eq} (%)	37 ± 1	44 ± 5
Sand (%)	23 ± 3	16 ± 1
Silt (%)	39 ± 2	35 ± 3
Clay (%)	38 ± 5	49 ± 3
BD (g·cm ⁻³)	1.22 ± 0.04	1.06 ± 0.04
Calcium (g·kg ⁻¹)	4.14 ± 0.01	5.13 ± 0.07
Magnesium (g·kg ⁻¹)	0.42 ± 0.02	0.91 ± 0.01
Potassium (g·kg ⁻¹)	0.59 ± 0.01	0.24 ± 0.01
Sodium (g·kg ⁻¹)	0.04 ± 0.02	0.64 ± 0.02
Copper (mg·kg ⁻¹)	3.4 ± 0.2	1.0 ± 0.1
Iron (mg·kg ⁻¹)	1.9 ± 0.1	1.7 ± 0.1
Manganese (mg·kg ⁻¹)	4.5 ± 0.1	4.0 ± 0.2
Zinc (mg·kg ⁻¹)	2.4 ± 0.1	1.1 ± 0.1

OC = organic carbon; N_k = Kjeldahl nitrogen; SP = saturation percentage; CO₃⁻²_{eq} = equivalent calcium carbonate; BD = bulk density; EC = electrical conductivity.

Table 2. Physical and chemical properties of sewage sludge compost (mean values ± sd).

Sewage sludge compost	
pH	6.14 ± 0.04
Electrical conductivity (dS·m ⁻¹)	5.09 ± 0.06
OC (g·kg ⁻¹)	303 ± 7
N _k (g·kg ⁻¹)	27 ± 1
Phosphorus (g·kg ⁻¹)	7.2 ± 0.4
Calcium (g·kg ⁻¹)	12.7 ± 0.3
Magnesium (g·kg ⁻¹)	7.5 ± 0.3
Potassium (g·kg ⁻¹)	5.1 ± 0.4
Sodium (g·kg ⁻¹)	2.7 ± 0.1
Copper (mg·kg ⁻¹)	21 ± 3
Iron (mg·kg ⁻¹)	6470 ± 739
Manganese (mg·kg ⁻¹)	144 ± 3
Zinc (mg·kg ⁻¹)	747 ± 35

OC = organic carbon; N_k = Kjeldahl nitrogen.

Table 3. Irrigation water characteristics (mean values ± sd).

Irrigation water	
pH	8.10 ± 0.09
EC _(20°C) (dS·m ⁻¹)	0.7 ± 0.1
NO ₃ ⁻ (mg·L ⁻¹)	3 ± 1
HCO ₃ ⁻ (mg·L ⁻¹)	117 ± 1
Cl ⁻ (mg·L ⁻¹)	140 ± 22
SO ₄ ⁻ (mg·L ⁻¹)	104 ± 44
Ca ²⁺ (mg·L ⁻¹)	28 ± 12
Mg ²⁺ (mg·L ⁻¹)	18 ± 8
K ⁺ (mg·L ⁻¹)	4 ± 1
Na ⁺ (mg·L ⁻¹)	83 ± 21

EC = electrical conductivity.

Results

Soil salinity dynamics throughout the experiment are shown in Figure 1. From January until the end of March, soil and irrigation water were in the process of reaching equilibrium. Consequently, EC_p values varied differently for each soil type within irrigation treatment. The records of EC_p indicated that equilibrium was reached in April and lasted until the end of June, because no differences were observed between soils irrigated at a certain salinity level. During this period, mean EC_p values for 0.7, 2, and 3 $dS\cdot m^{-1}$ irrigation treatments were 1.5, 2.4, and 4.2 $dS\cdot m^{-1}$ for S_A soil and 1.5, 2.5, and 3.7 $dS\cdot m^{-1}$ for S_B soil, respectively. These data suggest that the level of salinity that cynara plants were submitted to could be assumed to be about the same during the development of reproductive structures in both soils.

The effect of saline irrigation treatments on cynara morphological parameters can be observed in Table 4. Cynara growth was mainly affected by saline irrigation rather than by soil type with no apparent interaction between these factors. The only exception was plant height, which appeared to be affected by soil type ($P \leq 0.05$). Hence, data

of both soils were pooled and irrigation treatment effects were determined. Shoot biomass corresponding to the 3 $dS\cdot m^{-1}$ treatment was significantly reduced ($P \leq 0.001$) in approximately one-third regarding the 0.7 $dS\cdot m^{-1}$ treatment (Table 4). It was observed a significant reduction in stalk dry weight ($P \leq 0.05$), the number of heads ($P \leq 0.05$), head biomass ($P \leq 0.01$), and SY ($P \leq 0.05$). The percentage of seed yield decrease regarding control treatment (0.7 $dS\cdot m^{-1}$) in plants irrigated with 2 and 3 $dS\cdot m^{-1}$ water was 15% and 57%, respectively. Because studied factors did not affect the HI_{head} , data were pooled and the calculated mean value was 0.24 $g\cdot g^{-1}$. Despite growth reduction, no visual symptoms of nutritional deficiencies were observed.

The mineral composition of cynara tissues and the effects of saline irrigation within soil type are shown in Table 5. None of the studied factors significantly affected N concentration. In contrast, P concentration was affected by soil type in stalk tissues ($P \leq 0.01$), showing S_A plants had higher values than S_B plants (Table 5). Also, an interaction effect between saline irrigation and soil type was observed in leaves ($P \leq 0.01$), decreasing with increasing salinity the concentration of P only in S_B plants (Table 5). The primary

effect induced by saline irrigation on the mineral composition of cynara organs was the enhancement of Na and Cl concentration (Table 5). These significant trends were exclusively related to saline irrigation ($P \leq 0.001$). Salinity also affected the concentration of K, which decreased in stalks ($P \leq 0.01$) but remained unaffected in the other cynara organs (Table 5). In addition, the concentration of K was strongly conditioned by soil type in stalks and leaves ($P \leq 0.001$), showing S_A plants had higher K values than S_B plants. In stalk tissues, Ca concentration was significantly affected by saline irrigation ($P \leq 0.01$) and soil type ($P \leq 0.01$), increasing with salinity and evidencing higher values in S_A plants compared with S_B plants (Table 5). Opposite of Ca, the concentration of Mg significantly decreased as a result of saline irrigation in leaves ($P \leq 0.001$) and heads ($P \leq 0.01$) (Table 5). Soil type and the interaction effect between saline irrigation and soil type did not affect Mg concentration. Micronutrients (Cu, Fe, Mn, and Zn) remained unaffected by saline irrigation (Table 5) or the interaction of this factor with soil type. However, Mn was strongly affected by soil type in leaves and heads ($P \leq 0.001$) and to lesser extent in stalks ($P \leq 0.01$). Higher Mn concentration was observed in S_A plants than in S_B plants in all cynara tissues (Table 5).

The content of macronutrients (Ca, Mg, K, N, P), Na, and Cl of cynara aboveground biomass, and the effects of irrigation treatments within soil type are shown in Figure 2. As a general trend, saline irrigation significantly decreased macronutrient content, especially Mg ($P \leq 0.001$), K ($P \leq 0.01$), and P ($P \leq 0.01$), whereas the content of Na ($P \leq 0.001$) and Cl ($P \leq 0.01$) increased (Fig. 1). Soil type clearly influenced the content of K ($P \leq 0.001$), being higher in S_A plants (Fig. 1). The content of Ca ($P \leq 0.05$), N ($P \leq 0.05$), Na ($P \leq 0.05$), and Mg ($P \leq 0.05$) was also affected by soil type, but differences were less evident and only observed between 0.7 $dS\cdot m^{-1}$ plants for Ca and N and between 3 $dS\cdot m^{-1}$ plants for Mg (Fig. 1). No significant interaction effects between salinity and soil type were observed.

Discussion

Soil type only affected cynara plants height, observing taller plants in S_B soil, probably associated with the nutritional status (mainly N) of this soil. Thereby, cynara growth was primarily conditioned by saline irrigation, which had a negative influence in most cynara morphological parameters. The reduction of growth observed is one of the general effects that plants under salt stress exhibit, resulting in decreased biomass production and in shorter plant height (Läuchli and Epstein, 1990; Shannon and Grieve, 1999). It is noticeable that SY was severely affected by salinity, because cynara seeds play a determinant role regarding the cultivation of this crop for energy purposes, either as solid or liquid fuel. Considering higher

Fig. 1. Electrical conductivity (EC) of the soil pore water (EC_p) dynamics throughout the saline irrigation period. Monthly mean values ($n = 6$) for each soil type (S_A = black symbols; S_B = white symbols) within saline irrigation treatment (0.7, 2, and 3 $dS\cdot m^{-1}$) are represented.

Table 4. Cardoon morphological parameters at the end of the experiment (mean values $\pm$ SD, $n = 3$).

Soil	EC_w ($dS\cdot m^{-1}$)	Heads number (no./plant)	Plant ht (m)	Basal leaves		Caulicle leaves		Shoot
				Stalk	Stalk	Heads	Heads	
S_A	0.7	5 $\pm$ 1 b	0.83 $\pm$ 0.18 a	89 $\pm$ 12 a	32 $\pm$ 14 a	28 $\pm$ 11 a	65 $\pm$ 13 b	212 $\pm$ 28 b
	2	4 $\pm$ 0 ab	0.76 $\pm$ 0.18 a	59 $\pm$ 6 a	31 $\pm$ 13 a	21 $\pm$ 10 a	66 $\pm$ 12 b	177 $\pm$ 29 ab
	3	2 $\pm$ 1 a	0.55 $\pm$ 0.28 a	68 $\pm$ 3 a	18 $\pm$ 11 a	22 $\pm$ 9 a	28 $\pm$ 17 a	136 $\pm$ 5 a
S_B	0.7	5 $\pm$ 2 a	1.16 $\pm$ 0.11 b	60 $\pm$ 13 a	52 $\pm$ 8 b	35 $\pm$ 2 b	88 $\pm$ 16 b	235 $\pm$ 28 b
	2	4 $\pm$ 1 a	0.86 $\pm$ 0.18 a	52 $\pm$ 14 a	31 $\pm$ 13 ab	22 $\pm$ 9 a	60 $\pm$ 21 ab	165 $\pm$ 35 a
	3	3 $\pm$ 1 a	0.89 $\pm$ 0.08 a	66 $\pm$ 12 a	27 $\pm$ 3 a	21 $\pm$ 5 a	46 $\pm$ 5 a	159 $\pm$ 10 a

^aDifferent letters within a soil type (S_A , S_B) indicate statistically significant differences at $P \leq 0.05$ as a result of a one-way analysis of variance testing saline irrigation treatments (EC_w). Means were separated by Duncan's multiple range test at $P \leq 0.05$.

Table 5. Elemental composition of cardoon tissues on a dry weight basis (mean values $\pm$ SD, $n = 3$).

Plant tissue	Soil	EC _w (dS·m ⁻¹)	Nitrogen	Phosphorus	Calcium	Magnesium	Potassium	Sodium	Chlorine	Copper	Iron	Manganese	Zinc
			(g·kg ⁻¹)							(mg·kg ⁻¹)			
Stalk	S _A	0.7	2.6 $\pm$ 0.6 a	3.6 $\pm$ 0.5 a	4.7 $\pm$ 0.9 a	3.3 $\pm$ 1.0 a	17 $\pm$ 3 a	16 $\pm$ 2 a	30 $\pm$ 4 a	6 $\pm$ 4 a	55 $\pm$ 23 a	7 $\pm$ 2 a	25 $\pm$ 2 a
		2	3.3 $\pm$ 0.5 a	3.1 $\pm$ 0.4 a	7.1 $\pm$ 2.6 ab	2.5 $\pm$ 1.3 a	12 $\pm$ 1 a	37 $\pm$ 1 b	51 $\pm$ 3 b	2 $\pm$ 1 a	32 $\pm$ 10 a	10 $\pm$ 2 a	26 $\pm$ 1 a
		3	4.2 $\pm$ 1.0 a	4.1 $\pm$ 0.7 a	9.9 $\pm$ 1.4 b	3.2 $\pm$ 0.1 a	6 $\pm$ 1 b	37 $\pm$ 4 b	49 $\pm$ 4 b	8 $\pm$ 8 a	43 $\pm$ 8 a	16 $\pm$ 7 a	25 $\pm$ 1 a
	S _B	0.7	2.9 $\pm$ 0.4 a	2.9 $\pm$ 0.7 a	3.5 $\pm$ 0.2 a	2.3 $\pm$ 0.6 a	7 $\pm$ 3 a	18 $\pm$ 1 a	26 $\pm$ 2 a	4 $\pm$ 2 a	19 $\pm$ 2 a	4 $\pm$ 1 a	25 $\pm$ 1 a
		2	3.9 $\pm$ 1.0 a	2.7 $\pm$ 0.2 a	3.2 $\pm$ 1.0 a	2.3 $\pm$ 0.9 a	6 $\pm$ 2 a	39 $\pm$ 6 b	41 $\pm$ 9 b	4 $\pm$ 2 a	20 $\pm$ 1 a	6 $\pm$ 2 a	25 $\pm$ 2 a
		3	3.9 $\pm$ 0.4 a	2.1 $\pm$ 0.2 a	5.9 $\pm$ 1.9 b	2.2 $\pm$ 0.4 a	4 $\pm$ 3 a	44 $\pm$ 4 b	57 $\pm$ 3 c	2 $\pm$ 1 a	27 $\pm$ 13 a	5 $\pm$ 1 a	25 $\pm$ 1 a
Caulicle leaves	S _A	0.7	7.3 $\pm$ 1.5 a	2.1 $\pm$ 0.2 a	16.1 $\pm$ 4.9 a	3.6 $\pm$ 0.8 a	42 $\pm$ 7 a	17 $\pm$ 5 a	38 $\pm$ 10 a	3 $\pm$ 2 a	56 $\pm$ 21 a	23 $\pm$ 6 a	26 $\pm$ 2 a
		2	8.5 $\pm$ 2.7 a	1.7 $\pm$ 1.0 a	16.1 $\pm$ 9.9 a	2.0 $\pm$ 1.1 ab	44 $\pm$ 16 a	45 $\pm$ 8 b	72 $\pm$ 16 b	3 $\pm$ 1 a	115 $\pm$ 66 a	28 $\pm$ 14 a	24 $\pm$ 1 a
		3	9.9 $\pm$ 3.2 a	2.8 $\pm$ 0.3 a	8.9 $\pm$ 6.3 a	1.5 $\pm$ 0.4 b	32 $\pm$ 6 a	59 $\pm$ 5 b	82 $\pm$ 11 b	3 $\pm$ 1 a	73 $\pm$ 1 a	31 $\pm$ 4 a	24 $\pm$ 1 a
	S _B	0.7	6.5 $\pm$ 2.7 a	3.0 $\pm$ 0.8 a	12.5 $\pm$ 3.6 a	4.7 $\pm$ 0.5 a	22 $\pm$ 3 a	26 $\pm$ 3 a	39 $\pm$ 4 a	3 $\pm$ 1 a	73 $\pm$ 30 a	10 $\pm$ 2 a	24 $\pm$ 1 a
		2	8.8 $\pm$ 2.2 a	2.1 $\pm$ 0.2 ab	10.8 $\pm$ 5.7 a	2.7 $\pm$ 0.6 b	23 $\pm$ 6 a	47 $\pm$ 4 b	64 $\pm$ 4 b	4 $\pm$ 2 a	74 $\pm$ 11 a	11 $\pm$ 4 a	24 $\pm$ 1 a
		3	9.0 $\pm$ 0.9 a	1.1 $\pm$ 0.4 b	11.7 $\pm$ 2.3 a	1.8 $\pm$ 0.6 b	23 $\pm$ 6 a	61 $\pm$ 3 c	85 $\pm$ 3 c	4 $\pm$ 2 a	75 $\pm$ 25 a	16 $\pm$ 3 a	23 $\pm$ 1 a
Heads	S _A	0.7	9.9 $\pm$ 0.8 a	3.1 $\pm$ 0.3 a	2.3 $\pm$ 1.1 a	2.0 $\pm$ 0.1 a	19 $\pm$ 1 a	2 $\pm$ 1 a	15 $\pm$ 2 a	11 $\pm$ 2 a	38 $\pm$ 17 a	12 $\pm$ 2 a	38 $\pm$ 2 a
		2	10.4 $\pm$ 0.1 a	3.1 $\pm$ 0.2 a	2.1 $\pm$ 0.6 a	1.9 $\pm$ 0.1 a	19 $\pm$ 1 a	4 $\pm$ 2 a	14 $\pm$ 1 a	10 $\pm$ 1 a	48 $\pm$ 6 a	13 $\pm$ 1 a	38 $\pm$ 4 a
		3	11.1 $\pm$ 0.7 a	3.3 $\pm$ 0.1 a	1.5 $\pm$ 0.3 a	1.5 $\pm$ 0.0 b	17 $\pm$ 1 a	7 $\pm$ 1 b	16 $\pm$ 1 a	13 $\pm$ 1 a	44 $\pm$ 6 a	15 $\pm$ 2 a	39 $\pm$ 1 a
	S _B	0.7	12.5 $\pm$ 1.4 a	2.8 $\pm$ 0.3 a	1.6 $\pm$ 0.3 a	2.0 $\pm$ 0.3 a	15 $\pm$ 2 a	2 $\pm$ 1 a	11 $\pm$ 1 a	9 $\pm$ 1 a	43 $\pm$ 16 a	10 $\pm$ 1 a	39 $\pm$ 8 a
		2	13.9 $\pm$ 2.6 a	3.3 $\pm$ 0.2 a	1.4 $\pm$ 0.8 a	1.8 $\pm$ 0.2 ab	16 $\pm$ 1 a	5 $\pm$ 2 ab	15 $\pm$ 2 b	13 $\pm$ 1 a	56 $\pm$ 21 a	10 $\pm$ 3 a	42 $\pm$ 6 a
		3	11.1 $\pm$ 1.6 a	3.1 $\pm$ 0.6 a	1.0 $\pm$ 0.2 a	1.5 $\pm$ 0.1 b	17 $\pm$ 6 a	8 $\pm$ 2 b	20 $\pm$ 1 c	12 $\pm$ 3 a	55 $\pm$ 4 a	8 $\pm$ 1 a	34 $\pm$ 9 a

^aDifferent letters within soil type (S_A, S_B) and plant tissue (in columns) indicate statistically significant differences at $P \leq 0.05$ as a result of a one-way analysis of variance testing saline irrigation treatments (EC_w). Means were separated by Duncan's multiple range test at $P \leq 0.05$.

heating values of the different cynara fractions, seeds present the highest value (Fernández et al., 2006). This implies that lower energy yield would be obtained under saline irrigation if the whole aboveground biomass is harvested without separation of seeds. Furthermore, cynara liquid fuel (biodiesel) or oil production would be severely constrained, which can even make these applications unviable.

Regarding cynara mineral nutrition, saline irrigation had a greater influence than soil type. Although N concentration was unaffected by salinity, the content of this element decreased. Generally, the uptake of NO₃⁻ is negatively influenced by the concentration of Cl⁻ present in the soil solution, leading to lower N accumulation in plants (Grattan and Grieve, 1999).

The different P concentration observed in stalks could be related to a comparatively higher content of carbonates and Ca as well as lower P concentration in S_B soil than in S_A soil. The presence of Ca–P minerals, whose solubility is low, is likely to be higher in S_B soil. Hence, with increasing salinity, the availability of P was further restricted for S_B plants than for S_A plants. The interaction effect observed in caulicle leaves may be a result of the additional constraint that increasing salinity implied for S_B plants, which resulted in lower P concentration. Despite these differences in P concentration, the amount of P extracted by shoot biomass was not different between soils, which indicated that P uptake was mainly driven by saline irrigation.

The accumulation of Cl and Na can have detrimental effects on plants. To avoid this damage, salt-tolerant plants sequester Na and Cl in vacuoles (Munns, 2002; Munns and Tester, 2008; Tester and Davenport, 2003). In a soilless culture, Benlloch-González et al. (2005) demonstrated that *Cynara cardunculus* L. osmotic adjustment ability under salt stress conditions was mainly regulated by the inorganic ion content, especially through Na accumulation. Similar results were observed

in our present study because increasing concentrations of Na and Cl produced a concomitant increase in the content of these inorganic ions in cynara tissues.

As a result of the antagonism between Na and K at uptake sites and the effect of Na over K transport into the xylem (Hu and Schmidhalter, 2005), the concentration of K in plant tissues is generally decreased by increasing Na salinity (Grattan and Grieve, 1999). Whereas K shoot content decreased with salinity, cynara plants were able to maintain the concentration of this element in leaves and heads. Regardless of salinity effect, soil type strongly conditioned the concentration and content of K with higher values in S_A plants than in S_B plants. A possible reason that would explain why the concentration and content of K is so closely related to initial soil K fertility is that cynara shows evidence of high K selectivity. If so, cynara plants, up to a certain salinity level, would be able to uptake K according to the soil fertility. This finding is in agreement with Solano et al. (2010), who stated that K content of cynara biomass increases with K fertilization.

Calcium is displaced from its extracellular-binding sites by Na, which implies that Ca availability could be seriously reduced under saline conditions (Grattan and Grieve, 1999). However, neither Ca concentration nor Ca content was adversely affected by increasing NaCl salinity, probably because of the high availability of Ca in these Mediterranean calcareous soils (see Table 1). The increased Ca concentration of stalks might be related to a structural role (e.g., plant cell wall rigidity; Maathuis, 2009) as well as the result of the decline of plant growth resulting from salinity. Thereby, smaller plants (S_A) could present a concentration effect of Ca in stalks.

The decrease observed in plant Mg concentration might be induced by the reduction of Mg in the exchange phase of soils and/or by the inability of the plant to effectively uptake Mg as a result of ion competition

with Na at high concentrations (Grattan and Grieve, 1999). Consequently, shoot Mg content also decreased with increasing salinity. This element might be used as a NaCl salinity indicator for cynara plants grown in calcareous soils because, after Na, it was the element whose concentration exhibited higher sensitivity to salt stress.

Absence of nutritional deficiencies was observed for micronutrients (Cu, Fe, Mn, and Zn), because cynara tissue concentrations were above the critical concentration range for plant deficiency (Marschner, 1995). Besides pH, others factors that influence Mn availability in soils are organic matter, clay, and hydrous oxide content, which can absorb Mn (El-Jaoual and Cox, 1998). The pH was not a differential characteristic between soils, but levels of organic matter and clay in S_B soil were over those observed in S_A soil. Thereby, these factors may have influenced Mn availability by reducing its uptake in cynara S_B plants.

In view of our results, saline irrigation negatively affects cynara cultivation for energy use, because not only biomass quantity, but also biomass quality is reduced. Saline irrigation enhanced the content of Cl and alkali elements such as Na in cynara aboveground biomass. The presence of these elements is related to several problems for power plants such as fouling, slagging, and corrosion, which reduce the plant lifespan (Monti et al., 2008). As an example, the concentration of Cl, even in the control treatment, exceeded the guiding values in solid biofuels for unproblematic combustion indicated by Obernberger et al. (2006). The resultant chloride salts (i.e., NaCl₂ and KCl) might cause corrosion problems in the furnace and be boiler-related. However, saline irrigation might positively contribute to other uses. In a cynara soilless culture study, NaCl salinity irrigation increased the amount of total and individual polyphenols in leaves, compounds with antioxidant properties, thus improving cynara leaf characteristics for

Fig. 2. Aboveground biomass mineral content of cynara plants grown in two Mediterranean soils (S_A , S_B) mainly differing in their initial soil salinity status. The following elements are represented: nitrogen (N; A), phosphorus (P; B), calcium (Ca; C), magnesium (Mg; D), potassium (K; E), sodium (Na; F), and chlorine (Cl; G). Different letters within a soil type indicate statistically significant differences at $P \leq 0.05$ as a result of a one-way analysis of variance testing saline irrigation treatments (EC_w). Means were separated by Duncan's multiple range at $P \leq 0.05$.

phytotherapeutic applications (Colla et al., 2012). Irrespective of lower cynara biomass or energy yields, the growth of cynara under saline irrigation is able to reduce soil erosion and desertification and improve soil physical (i.e., structure) and chemical (i.e., organic matter) properties (Grammelis et al., 2008), which is of special importance in Mediterranean regions.

Conclusions

In the current study, cynara growth and mineral nutrition were primarily affected by saline irrigation. Cynara growth reduction was related to the enhancement of the osmotic effect (increased soil salinity) and the ion toxicity effect (accumulation of Cl and Na in cardoon tissues). With increasing salinity, nutritional disorders may also affect cynara growth. However, no visual symptoms of nutritional deficiency were observed, which suggested that nutritional imbalances were absent or not severe. Cynara exhibits high K selectivity, which seems to be associated with a salt tolerance mechanism. Consequently, the concentration and content of K is highly related with soil fertility, thus with fertilization management. The decrease of the concentration and content of Mg suggest that not only the concentration of Na, but also Mg might be used as an indicator of salt stress in cynara plants grown in calcareous Mediterranean soils. Cynara is a suitable crop species for the Mediterranean region, but the adequacy of its cultivation under saline irrigation is highly dependent on the desired end use.

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