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Research article

The long-term resistance mechanisms, critical irrigation threshold and relief capacity shown by *Eugenia myrtifolia* plants in response to saline reclaimed water

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ABSTRACT

Salts present in irrigation water are serious problems for commercial horticulture, particularly in semi-arid regions. Reclaimed water (RW) typically contains, among others elements, high levels of salts, boron and heavy metal. Phytotoxic ion accumulation in the substrate has been linked to different electric conductivities of the treatments. Based on these premises, we studied the long-term effect of three reclaimed water treatments with different saline concentrations on *Eugenia myrtifolia* plants. We also looked at the ability of these plants to recover when no drainage was applied. The RW with the highest electric conductivity (RW3, EC = 6.96 dS m^{-1}) provoked a number of responses to salinity in these plants, including: 1) accumulation and extrusion of phytotoxic ions in roots; 2) a decrease in the shoot/root ratio, leaf area, number of leaves; 3) a decrease in root hydraulic conductivity, leaf water potential, the relative water content of leaves, leaf stomatal conductance, the leaf photosynthetic rate, water-use efficiency and accumulated evapotranspiration in order to limit water loss; and 4) changes in the antioxidant defence mechanisms. These different responses induced oxidative stress, which can explain the damage caused in the membranes, leading to the death of RW3 plants during the relief period. The behaviour observed in RW2 plants was slightly better compared with RW3 plants, although at the end of the experiment about 55% of the RW2 plants also died, however RW containing low salinity level (RW1, EC = 2.97 dS m^{-1}) can be effective for plant irrigation.

1. Introduction

The potential for sustainable agricultural activity in many arid and semi-arid regions is limited by the scarce fresh water resources for irrigation (Bezborodov et al., 2010; Cirillo et al., 2016). Reclaimed waters (RWs) used as a non-conventional water resource are of proven agronomic and environmental interest for irrigation of ornamental (Acosta-Motos et al., 2014, 2016; Gómez-Bellot et al., 2015a,b) and other crop plants (Pedrero et al., 2014, 2015; Dorta-Santos et al., 2016; Nicolás et al., 2016), especially in Mediterranean regions where water availability is a limiting factor (Yermiyahu et al., 2008). The use of RW has different benefits, including a reduction in the discharge of pollutants into natural water courses (Zekri and Koo, 1994), which can be particularly important when the treated water is used for landscaping (Dobrowolski et al., 2008). RWs are also characterised by their high

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Abbreviations: APX, ascorbate peroxidase; ASC, ascorbate reduced form; CAT, catalase; DHA, ascorbate oxidized form; DHAR, dehydreascorbate reductase; DW, dry weight; EC, electrical conductivity; ETa, accumulated evapotranspiration; FW, fresh weight; GR, glutathione reductase; GSH, glutathione reduced form; GSSG, glutathione oxidized form; g_s, stomatal conductance; H_2O_2, hydrogen peroxide; J, absorption rate of ions by roots; LP, lipid peroxidation; L_p, root hydraulic conductivity; MDHAR, monodehydroascorbate reductase; NADH, nicotinamide adenine dinucleotide reduced form; NADPH, nicotinamide adenine dinucleotide phosphate reduced form; $O_2^{\bullet -}$, superoxide anion; $^{\bullet}OH$, hydroxyl radicals; PAR, photosynthetically active radiation; POX, peroxidase; P_n, net photosynthetic rate; RH, relative humidity; ROS, reactive oxygen species; RW, reclaimed water; RWC, relative water content; SOD, superoxide dismutase; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive-substances; TW, turgid weight; WFC, weight at field capacity; WUE, water-use efficiency; WUE_i, intrinsic water-use efficiency; Ψ_l, leaf water potential.

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nutrient content, which can preclude the use of fertilizers, thus reducing the risk of environmental contamination (Khajanchi et al., 2015; Dorta-Santos et al., 2016). Despite these advantages, however, RW is of lower quality than fresh water. Furthermore, depending on the origin of the RW, the time of collection and the treatment applied, it may contain certain phytotoxic ions, heavy metals and fecal microorganisms. In such cases, this type of water could be used for landscaping and revegetation projects using ornamental plants where the impact is not as important (Gómez-Bellot et al., 2015b; Acosta-Motos et al., 2016) as it would be in other crops for human consumption (Pedrero et al., 2015; Nicolás et al., 2016).

Salinity is among the other harmful elements present in these waters and can result in a plant damage and reduced plant quality. Salinity in soils and irrigation water is one the main abiotic stresses affecting agriculture worldwide, limiting crop growth and production. In order to mitigate the negative effects of salinity, plants have developed different physiological and biochemical mechanisms including changes in biomass parameters, phytotoxic ion distribution, water relations, photosynthesis and the antioxidative metabolism response (Munns and Tester, 2008). The main negative effects produced by salinity include osmotic stress, related to a decrease in water potential in the roots, and ion toxicity, due to an excessive accumulation of phytotoxic ions in all plant organs, leading to nutritional imbalance resulting from a shortage of calcium, magnesium and potassium ions (Parida and Das, 2005). The response of plants to salinity is different depending on the plant species used. In salinity experiments it is important to select salt-resistant endemic plants adapted to Mediterranean areas, such as *Myrtus communis* (Miralles et al., 2010; Acosta-Motos et al., 2014, 2015a, 2016), or plants adapted to similar climates, such as *Eugenia myrtifolia* (Acosta-Motos et al., 2015b). Moreover, it is also important to set other experimental conditions such as the use of pots for growing of the plants and the drainage conditions applied (Bañón et al., 2012; Acosta-Motos et al., 2014, 2016).

In addition, salinity can limit CO_2 fixation in plants, producing oxidative stress which is mediated by an overproduction and accumulation of reactive oxygen species (ROS) such as superoxide anions ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$) at the subcellular level (Corpas et al., 1993; Hernández et al., 1993, 1995). This response contributes to the appearance of symptoms such as a disruption in cellular metabolisms through membrane lipid peroxidation, enzyme inhibition and damage to nucleic acids (Parida and Das, 2005; Sabra et al., 2012). In order to cope with the ROS, plants have implemented a complex defence system that includes enzymatic and non-enzymatic antioxidant mechanisms (Noctor and Foyer, 1998). In general, salt-tolerant plants have a better response than other plants to oxidative stress, increasing the activity and/or the expression of antioxidant enzymes, as has been observed in different crops (Hernández et al., 2000, 2001; Demiral and Türkan, 2006; Moradi and Ismail, 2007; Duarte et al., 2013; Gil et al., 2014; Acosta-Motos et al., 2015a,b). This increase may also occur, however, in salt-sensitive species (Arbona et al., 2003; Lee et al., 2013). Other authors have correlated salt tolerance with higher constitutive levels of certain antioxidants (Hernández et al., 2003; López-Gómez et al., 2007). Overall, there is scarce and inconsistent information on the effect of RW with high salt levels on the antioxidative metabolism of ornamental crops.

In a previous work, under controlled environmental conditions, we studied the short-term effect of NaCl on *Eugenia myrtifolia* plants (an interesting plant useful for xeriscaping and landscaping projects in public areas) and their ability to recover (Acosta-Motos et al., 2015b). In the present work, the aim was to determine the long-term effect of the same responses when RWs containing different salt concentrations are used as an unconventional alternative water resource. This work thus evaluates the effect of salt accumulation due to the different RW treatments applied during a long period of time (23 weeks) and the plants' ability to recover following a salinity relief period (9 weeks) (with no drainage applied). To this effect, plant growth, ornamental quality parameters, water relations, gas exchange, mineral nutrition and antioxidative metabolism were evaluated. Furthermore, we established a set of guidelines to be considered by nurseries. These "lines of action" indicate how long irrigation should be applied and, on the one hand, which salt threshold levels are critical for optimum growth and even for improving the visual and ornamental qualities of the plants (positive approach), and, on the other hand, the levels that can cause irreversible damage and plant death (negative approach).

2. Materials and methods

2.1. Plant and experimental conditions

Single rooted cuttings (120) of native *Eugenia myrtifolia* plants were transplanted into 14 × 12 cm pots (1.2 l) filled with a mixture of coconut fibre, sphagnum peat and perlite (8:7:1) and amended with Osmocote plus (2 g l^{-1} substrate) (14:13:13 N, P, K + microelements) supplied by Agrosolmen S.L., Lorca (Murcia), Spain. The experiment was conducted in a controlled environment growth chamber set to simulate natural conditions. The temperature in the chamber was 23° C during the light phase (16 h photoperiod) and 18° C during darkness. Relative humidity (RH) values ranged between 55 and 70%. A mean photosynthetic active radiation (PAR) of 350 $\mu mol\ m^{-2}\ s^{-1}$ at canopy height was supplied during the light phase (08:00 h-00:00 h) by cold white fluorescent lamps.

2.2. Water irrigation treatments, substrate analyses and experimental design

At the beginning of the experimental period three water samples from each irrigation water source were collected in glass bottles, transported in an ice chest to the laboratory and stored at 5 °C in order to determine the irrigation water quality. A chemical analysis of the waters used for each irrigation treatment was performed, and the results obtained are shown in Table 1.

The electrical conductivity (EC) was measured with a multi-range Cryson-HI8734 electrical conductivity meter (Cryson Instruments, S.A., Barcelona, Spain). The pH was calculated with a Cryson-507 pH-meter (Cryson Instruments, S.A., Barcelona,

Table 1
Chemical analyses of the water used in different treatments. Data are values collected at the beginning of the experimental period.

Parameters	Irrigation water			
	Control	RW1	RW2	RW3
EC (dS m^{-1})	0.88	2.97	4.38	6.96
pH (-log[H^+])	7.72	8.07	8.25	7.85
SDT (mg L^{-1})	–	754.02	1679.17	5340.00
OD (mg L^{-1})	–	5.10	9.05	6.20
SS (mg L^{-1})	–	2.56	8.65	5.46
Turbidity	–	7.00	3.22	1.65
Na^+ (mmol L^{-1})	2.26	11.31	15.78	64.90
Cl^- (mmol L^{-1})	1.96	20.68	24.28	43.86
Ca^{2+} (mmol L^{-1})	2.35	1.72	4.14	5.05
B^{3+} (mmol L^{-1})	0.01	0.02	0.05	0.12
K^+ (mmol L^{-1})	0.09	0.85	0.96	3.00
Mg^{2+} (mmol L^{-1})	1.72	1.67	4.08	8.50
S (mmol L^{-1})	2.67	1.17	6.38	8.79