Journal of Plant Physiology 185 (2015) 65–74

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Physiology

Comparison of individual and combined effects of salinity and deficit irrigation on physiological, nutritional and ornamental aspects of tolerance in *Callistemon laevis* plants

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ARTICLE INFO

Article history:
Received 16 April 2015
Received in revised form 5 June 2015
Accepted 14 July 2015
Available online 5 August 2015

Keywords:
Gas exchange
Ion uptake
Ornamental potted plant
Water relations
Water use efficiency
Elastic modulus

ABSTRACT

The effect of water deficit, salinity and both applied simultaneously on several physiological and morphological parameters in the ornamental plant *Callistemon laevis* was studied to identify the tolerance mechanisms developed by this species to these sources of stress and to evaluate their adaptability to such conditions. *C. laevis* plants were grown in pots outdoors and subjected to four irrigation treatments lasting ten months: control (0.8 dS m^{-1}, 100% water holding capacity), water deficit (0.8 dS m^{-1}, 50% of the amount of water supplied in control), saline (4.0 dS m^{-1}, same amount of water supplied as control) and saline water deficit (4.0 dS m^{-1}, 50% of the water supplied in the control). Water and saline stress, when applied individually, led to a reduction of 12% and 39% of total biomass, respectively, while overall plant quality (leaf color and flowering) was unaffected. However, saline water deficit affected leaf color and flowering and induced an excessive decrease of growth (68%) due to leaf tissue dehydration and a high leaf Cl and Na concentration. Biomass partitioning depended not only on the amount of water applied, but also on the electrical conductivity of the water. Water stress induced active osmotic adjustment and decreased leaf tissue elasticity. Although both Na and Cl concentrations in the plant tissues increased with salinity, Cl entry through the roots was more restricted. In plants submitted to salinity individually, Na tended to remain in the roots and stems, and little reached the leaves. However, plants simultaneously submitted to water and saline stress were not able to retain this ion in the woody parts. The decrease in stomatal conductance and photosynthesis was more marked in the plants submitted to both stresses, the effect of which decreased photosynthesis, and this together with membrane damage delayed plant recovery. The results show that the combination of deficit irrigation and salinity in *C. laevis* is not recommended since it magnifies the adverse effects of either when applied individually.

1. Introduction

Salinity and drought are the major constraints affecting physiological processes, and their effects may have severe consequences for plant growth and survival in semiarid regions (Vilagrosa et al., 2003; Álvarez et al., 2012). Therefore, in these regions it is important to consider the use of salt- and drought-tolerant species for gardening or landscaping (Slama et al., 2007; Razzaghi et al., 2012). Drought and salt tolerance in plants may be explained by functional and structural adaptations, such as growth regulations, osmotic adjustment, regulation of stomatal conductance, changes in cell wall elasticity, mineral nutritional and hormone balance, all of which may help alleviate the harmful effects of both stresses (Zheng et al., 2010; Suárez, 2011). However, although salinity and drought stress are physiologically related and some of the tolerance mechanisms overlap, other aspects of plant physiology and metabolism may differ if the plant experiences saline and water individually or both stresses simultaneously (Sucre and Suárez, 2011). In relation to the comparative physiological processes in saline versus drought, both stresses reduce the ability to take up water, but

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Abbreviations: C, control; C*, chroma; DW, dry weight; EC, electrical conductivity; g_s, stomatal conductance; h°, hue angle; J, absorption rate of ions by the root system; L*, lightness; P, significance; P_n, net photosynthesis rate; P–V, pressure-volume; RWC_{tpl}, relative water content at turgor loss point; S, saline treatment; W, deficit irrigation treatment; WUE, water use efficiency of production; W + S, simultaneous saline and water stress treatment; Ψ_l, leaf water potential; Ψ_s, stem water potential; Ψ_{100s}, leaf osmotic potential at full turgor; Ψ_{tpl}, leaf water potential at turgor loss point; ϵ, bulk modulus of elasticity.

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http://dx.doi.org/10.1016/j.jplph.2015.07.009
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when high Na^+ and Cl^- concentrations are present in the irrigation water, ion toxicities (associated with an excessive Na and Cl uptake and/or transport to aerial parts of the plant) and nutritional deficiencies may arise because of competition between cations or anions, depending upon the composition of the saline solution (Acosta Motos et al., 2014).

Water stress and salinity often occur simultaneously in arid regions because as soils dry, the salts become concentrated in the remaining soil solution (Munns, 2002; Chaves et al., 2009). The ability to overcome multiple and simultaneously stresses is of great importance for plant growth and survival in a stressful environment (Slama et al., 2008; Glenn et al., 2012). Numerous studies have investigated drought and salt stress separately, but fewer have examined their interactions. Several studies have demonstrated that the molecular and metabolic responses of plants to the combination of drought and salinity are unique and cannot be directly extrapolated from the corresponding response of plants to either when applied individually (Suzuki et al., 2014; Mittler, 2006). The response by plants is more complicated than a simple additive effect of these two stress factors (Brown et al., 2006; Glenn et al., 2012). Drought may magnify the adverse effects of salinity and, when combined with salinity may interfere with nutrient accumulation, contributing to further growth inhibition or even reduce plant survival (Brown et al., 2006; Slama et al., 2008). Moreover, some studies have shown that the interaction of saline and water stress strongly reduces the capacity of plants to recover the water and carbon balance even after stress alleviation compared with plants subjected to a single source of stress (Omami and Hammes, 2006; Pérez-Pérez et al., 2007). By contrast, other studies have found that the addition of salt to plants subjected to a water deficit stress ameliorates the negative effects of deficit irrigation, improves the plants' ability to cope with water stress and enhances drought tolerance. For examples, Martinez et al., (2003, 2005), Glenn et al. (2012) and Alla et al. (2011), demonstrated that salinity actually has a protective effect on biomass production in a variety of species deficit-irrigated with saline water, and Sucre and Suárez (2011) reported that the water and carbon balance were enhanced when both stresses were applied simultaneously. Similarly, other studies have shown that the physiology of plants affected by a combination of salinity and drought is less altered than in the case of plants affected by drought only and that plant survival is enhanced (Glenn and Brown, 1998; Pérez-Pérez et al., 2007). While most studies have been conducted in halophytes plants, it is also important to investigate the physiology of salt and drought tolerance in non-halophytes species to understand the limits and trade-offs between drought and salt tolerance, and the traits that are associated with tolerance to both factors. Indeed, plant salt tolerance differs significantly between species. Halophytes are able to complete their life cycle in 200 mM NaCl or more, while some non-halophytes can be injured by one tenth these salt concentrations (Cassaniti et al., 2009).

Among Australian ornamental plants, one of the most widely used genera is *Callistemon*, which includes several species with interesting ornamental features (Mitchem, 1993). In Europe, the most widely used *Callistemon* species are *C. citrinus* Skeels and *C. laevis* Anon, both with a great potential for urban landscaping and gardening due to their good tolerance to environmental stresses (Mugnai et al., 2009). In *Callistemon citrinus*,the effect of drought and salinity on physiological and morphological parameters and the mechanism this species uses to confront both sources of stress have been well established by Álvarez and Sánchez-Blanco (2013, 2014), *C. citrinus* being seen particularly salt and drought tolerant. In these studies it has been reported that both salinity and soil drying led to reductions in dry matter accumulation (a similar slight reduction of 16% of total biomass), stomatal conductance and transpiration and improvements in water use efficiency and root system, while overall quality are unaffected. In addition, salinity induced a slight osmotic adjustment and root storage of Na and Cl. It was accompanied by reductions in photosynthesis and intrinsic water use efficiency due to the cumulative effect of irrigating with saline water (Álvarez and Sánchez-Blanco, 2014). However, it has been documented that the degree of response to salt stress may vary considerably within a genus (Sánchez-Blanco et al., 2002; Lippi et al., 2003). Indeed, Vernieri et al. (2006) found that *Callistemon laevis* appeared to be moderately tolerant to water stress, but less resistant to salt stress, at least using irrigation water of 23 dS m^{-1} (200 mM NaCl). It is well known that plant responses to salt, in addition to being species-dependent, also depend on the length of exposure and the severity of the salt treatment (electrical conductivity (EC) of the saline water used). Both factors must be considered when saline water is used for irrigation water, as the interaction of both parameters will determine the physiological and molecular changes that take place. Since the growing season also seems to affect the response of shrubs to salt (Valdez-Aguilar et al., 2011), the research described in this paper was carried out over a period of 10 months in *C. laevis* using a salt level (4 dS m^{-1}) similar to that of the irrigation water commonly applied in the Mediterranean horticultural sector (nurseries, growers, gardeners; Pedrero et al., 2010; Álvarez and Sánchez-Blanco, 2014). Moreover, no studies have evaluated the effects of both stresses applied simultaneously to *C. laevis* plants and the mechanism involved during a combination of salinity and drought require more study (Martinez et al., 2003; Pérez-Pérez et al., 2007; Slama et al., 2008; Sucre and Suárez, 2011).

Based on the discussion above, it was hypothesized that combined salinity and deficit irrigation may interfere with nutrient uptake and may therefore modify the tolerance mechanisms developed by this species to confront both sources of stress, enhancing or minimizing their drought and salt tolerance compared with plants subjected to a single source of stress. Consequently, the primary aim of our investigation was to quantify the long-term effects on growth, ion uptake, water relations and the parameters obtained by pressure-volume analysis in plants of *C. laevis* exposed to both saline and water stress and to throw light on the mechanisms the plants use to confront the same. Knowledge of the salt and drought response of ornamental plants may help the horticultural sector (growers and gardeners) to select species that are tolerant to salt and/or water stress, while maintaining an acceptable appearance. The results may also be of great interest for planning irrigation strategies in the Mediterranean area, where low quality waters are very often used in deficit irrigation strategies.

2. Materials and methods

2.1. Plant material and experimental conditions

Rooted cuttings of 2-year-old *Callistemon laevis* Anon grown in 14 × 12 cm pots by a specialized nursery were transplanted into 5 L plastic pots (20 × 16 cm) filled with an 8:7:1 (v/v/v) mixture of coconut fibre:black + sphagnum perlite: perlite, amended with 2 g L^{-1} of Osmotocote Plus (14:13:13 N, P, K plus microelements). Plants were placed outdoors in a plot at the CEBAS-CSIC experimental station in Santomera, Murcia, Spain (38°06íN, 1°02íW, 110 m a.s.l.). All the plants were watered daily for 4 weeks to field capacity prior to starting the treatments. The micro-climatic conditions, registered with an automatic weather station located about 50 m from the experimental site, were 13.6 °C (mean minimum), 24.9 °C (mean maximum), and 18.7 °C (average) temperature; and 1.02 Kpa (average) vapor pressure deficit. Additional information about evolution of the daily mean values of air temperature and vapor pressure deficit recorded during the experimental period is detailed in Figure S1.