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Similarities and differences between the responses to osmotic and ionic stress in quinoa from a water use perspective

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ABSTRACT

Faced with the consequences of climate change, such as increased drought and salinization of soils, the species *Chenopodium quinoa* may be a good alternative crop because of its high tolerance to these conditions and its high nutritional value. The objective of this work was to analyze the response of the quinoa plant in drought and salinity conditions. Under conditions of drought and severe saline stress (500 mM NaCl), highly similar reductions in growth and relative water content were observed. However, the strategies implemented by the plants in either stress condition and their relative importance were different. Under salt conditions, responses related to osmotic adjustment were more prominent than under drought conditions, where more dehydration was detected. In addition, despite a similar reduction in stomatal conductance in drought and saline conditions, a greater non-stomatal effect was observed in drought conditions, which was demonstrated by the fact that the intercellular CO_2 concentration was increased. Moreover, the antioxidant metabolism also responded differently to the two stresses. Photoassimilate allocation was also different between treatments: the root/shoot ratio remained constant independent of salt concentration, whereas under drought conditions, this ratio increased. A similar trend between treatments was detected for water use efficiency, which was maintained under salt stress and increased under drought conditions, indicating that under reduced water conditions, quinoa can use lower amounts of water per unit of biomass production. These results suggest that *C. quinoa* could be irrigated with brackish or even higher salinity water without severely affecting the growth during its early growth stage, thereby making *C. quinoa* a promising alternative crop for arid and semi-arid regions.

1. Introduction

Climate change is projected to cause a change in the amount of rainfall, increasing in some areas and significantly decreasing in others. Additionally, it is expected to increase extreme events of drought and flooding due to the changes in the distribution of rainfall patterns (IPCC, 2014), and these extreme events are more harmful to ecosystems than changes in the annual mean precipitation (Smith et al., 2005). Furthermore, a rise in temperature is estimated; therefore, higher rates of water evaporation will occur, expanding even more the drought areas. These phenomena, together with agricultural practices, are also causing an increase in the salinization of crop soils (IPCC, 2014). Worldwide, more than 11% of irrigated land are estimated to be salt-affected, a figure that has been rising each year (FAO, 2011a). Water availability is the most important factor in the development of plants (Boyer, 1982; Chaves et al., 2003), therefore under drought- and salt-affected soils, plants suffer water scarcity, and in recent years decreases in crop productivity have been observed.

In addition to the negative impact of climate change causing decreases in productivity, the world population is expected to increase by 52% by the end of the century (UN, 2015), which will require an increase in agricultural production to cope with growing food demand (Tilman et al., 2011). Since 1991, the total agricultural area has remained constant (O'Mara, 2012), so the productivity of crops must increase to meet the growing demand. The increase of drought- and salt affected-soil area, together with the increase in world population, force the research community to find varieties or species that are more tolerant to water limitation or salinity to maintain or increase agricultural production. Recently, efforts have been made in order to improve water use efficiency (*WUE*) of crops to reduce the water needed for agricultural practices (Araus, 2004; Morison et al., 2008; Medrano et al., 2015).

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Quinoa, *Chenopodium quinoa* Willd, is a halophyte that has been historically cultivated in various regions of South America due to its high adaptability to extreme environments (Bazile et al., 2016). Various authors have observed that quinoa is a highly salt-tolerant crop (Jacobsen et al., 2003; Adolf et al., 2013). Razzaghi et al. (2015) determined that the EC50 for quinoa is 25 dS m^{-1}, and thus confirmed the classification of quinoa as a highly salt-tolerant crop. Additionally, quinoa possesses extraordinary nutritional characteristics, such as high content of protein and essential amino acids (Miranda et al., 2012). All these characteristics led FAO to consider quinoa to be one of the crops that might contribute to global food security during the 21st century (FAO, 2011b).

However, despite the importance of this crop, the studies done till know have analyzed the impact of drought and/or salt stress on the physiological processes separately; for example in water relations (Jensen et al., 2000; Cocozza et al., 2013), in photosynthesis and related parameters (González et al., 2011; Geissler et al., 2015; Razzaghi et al., 2015; Talebnejad and Sepaskhah, 2016), in antioxidant metabolism (Panuccio et al., 2014; Amjad et al., 2015) and in growth (González et al., 2009; Ruiz-Carrasco et al., 2011; Adolf et al., 2012; Ruiz et al., 2016) but never before have been measured all the aforementioned parameters together in just one study. Besides, the studies done comparing the response of quinoa plants to drought and to salt stress are scarce (Bosque-Sanchez et al., 2003; Pulvento et al., 2012; Cocozza et al., 2013; Riccardi et al., 2014; Amjad et al., 2015; Ince Kaya and Yazar, 2016). Thus, the objectives of this study were to determine the different physiological mechanisms that are activated in response to drought and salt stress provoked growth reductions, as well as to compare the response of quinoa to drought and salt stress, highlighting the similarities and differences in the mechanisms that quinoa plants use to cope with the stresses through analysis of various physiological processes as a whole.

2. Materials and methods

2.1. Plant material and experimental design

A Bolivian cultivar of *Chenopodium quinoa* Willd (cv. Real Blanca) was used in this study. Its origin is near the Salar de Uyuni and belongs to *Altiplano* of the Andes ecotype characterized by growing in areas between 3500 and 3900 m above sea level with an annual rainfall of 400–800 mm. Seeds of this cultivar were sown in a 3:1 mixture of perlite/vermiculite in 3 L pots with one seed per pot. Plants were grown from sowing until the end of the experiment in a Conviron PGR15 controlled-environment growth chamber (Conviron, Manitoba, Canada) under a daily 14 h light regimen, an average day/night temperature of 24/20 °C, and a relative day/night humidity of 70/80%. During the light period, the photosynthetic photon flux density (PPFD) was 400 μmol m^{-2} s^{-1}. Light was provided by a combination of incandescent bulbs and warm-white fluorescent lamps. To minimize the effects of intrachamber environmental gradients, plants were randomly repositioned within the chamber each week.

Plants were watered with Hoagland's solution (Arnon and Hoagland, 1940) three times per week maintaining at field capacity until the beginning of the stress treatment, when the plants were 28 days old. Stress treatment was imposed for 14 days. Plants were divided into six groups: five groups were watered every 2 days with 250 mL of Hoagland's solution supplemented with a range of NaCl concentrations (0 mM = control, 60 mM, 120 mM, 240 mM, and 500 mM), as salinity treatments and the sixth group was maintained without watering for 14 days, as drought treatment.

2.2. Measurements of plant water parameters

The leaf relative water content (*RWC*) was measured by gravimetric methods (Pérez-López et al., 2009a). The leaf osmotic potential (Ψ_o) was measured through the freezing point of the cellular sap by an osmometer (Osmomat 030, Gonotec, Germany). The leaf osmotic potential at full turgor (Ψ_o^{100}) was measured following the same procedure as for Ψ_o. To obtain full turgor, leaves were cut from the plants, incubated for 24 h in deionized water, and stored in the dark at 4 °C to avoid the loss of dry mass by respiration or the synthesis of new dry mass by photosynthesis. Dehydration was calculated as the difference between Ψ_o^{100} and the Ψ_o for each treatment. The osmotic adjustment was calculated as the difference between Ψ_o^{100} of the control plants and Ψ_o^{100} of the stressed plants.

The proline concentration was measured following the procedure of Bates et al. (1973). Aliquots of leaf tissue (20 mg lyophilized) were homogenized with 2 mL sulfosalicylic acid at 3%. The homogenates were centrifuged at 16,100*g* for 5 min, and the supernatant was kept on ice. To 0.75 mL of the above supernatant, 0.75 mL of ninhydrin acid was added, consisting of 1.25 g of ninhydrin dissolved in 20 mL of 6 M phosphoric acid and 30 mL of glacial acetic acid. Subsequently, to the mixture of the supernatant and ninhydrin acid, 0.75 mL of glacial acetic acid was added. Samples were incubated for 1 h at 100 °C. Once the tubes had cooled, 1.5 mL of toluene was added, and the mixture was shaken vigorously for 20 s. The fluid separated into two phases, and the upper phase was recovered. Then, the absorbance was determined at 517 nm.

The cumulative plant water transpiration was calculated by gravimetric method. Each pot was weighed every 2 days at the same time, before and after watering (De Luis et al., 1999).

2.3. Gas exchange parameters and chlorophyll a fluorescence

Leaf gas exchange parameters were determined using a Li-6400 open gas exchange system (Li-Cor Inc., Lincoln, NE, USA). Leaf gas exchange rates were measured as described by Pérez-López et al. (2013). Measurements were done 3 h after dawn with a cuvette temperature held at 24 °C and at a relative humidity of 60%. The photosynthetic photon flux density was 400 μmol m^{-2} s^{-1}, provided by red/blue LED light source (model LI 6400-02B, Li-Cor Inc.). The CO_2 concentration of the cuvette (*Ca*) was the same as in growth conditions. Li-Cor software was used to calculate stomatal conductance (*gs*) and the intercellular CO_2 concentration (*Ci*) from net photosynthetic rate (*A*) and instantaneous transpiration rate (*E*) according to the method of von Caemmerer and Farquhar (1981). The intrinsic water use efficiency (*A*/*gs*) was calculated by dividing *A* by *gs*.

The actual photochemical efficiency of photosystem II ($\Phi PSII = (Fm' - Fs)/Fm'$) was determined by measuring steady-state fluorescence (*Fs*) and maximum fluorescence during a light-saturating pulse of ~8000 μmol m^{-2} s^{-1} (*Fm'*) following the procedures of Genty et al. (1989). The electron transport rate (*ETR*) indicates the proportion of light used in photochemical processes of the total of energy that reaches the leaf per unit of time and surface (PPFD), and it was calculated according to the following formula: $ETR = \Phi PSII x PPFD x 0.85 \times 0.5$. The absorption coefficient of the leaves was taken as 0.85 and the fraction of the excitation energy distributed to PSII was taken as 0.5 (Genty et al., 1989).

Chlorophylls and carotenoids were extracted in dimethylsulfoxide (DMSO) as described by Barnes et al. (1992). The absorbance was measured at 665, 649, and 480 nm to quantify the pigment concentration using the formulas proposed by Wellburn (1994).

2.4. Antioxidant enzyme activities

All operations were carried out at 0–4 °C. Aliquots of leaf tissue (0.15 g fresh weight) were ground in a cold mortar using extraction buffer (3 mL). For catalase (CAT) activity, the extraction buffer consisted of 50 mM Tris–HCl (pH 7.8), 0.1 mM ethylenediaminetetraacetate (EDTA), 0.2% Triton X-100 (v/v), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 2 mM dithiotreitol (DTT). The