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Elevated CO_2 alleviates negative effects of salinity on broccoli (*Brassica oleracea* L. var Italica) plants by modulating water balance through aquaporins abundance

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ABSTRACT

In the global change scenario, increased CO_2 may favour water use efficiency (WUE) by plants. By contrast, in arid and semiarid areas, salinity may reduce water uptake from soils. However, an elevated WUE does not ensure a reduced water uptake and upon salinity this fact may constitute an advantage for plant tolerance. In this work, we aimed to determine the combined effects of enhanced [CO_2] and salinity on the plant water status, in relation to the regulation of PIP aquaporins, in the root and leaf tissues of broccoli plants (*Brassica oleracea* L. var Italica), under these two environmental factors. Thus, different salinity concentrations (0, 60 and 90 mM NaCl) were applied under ambient (380 ppm) and elevated (800 ppm) [CO_2]. Under non-salinised conditions, stomatal conductance (G_s) and transpiration rate (E) decreased with rising [CO_2] whereas water potential (Ψ_ω) was maintained stable, which caused a reduction in the root hydraulic conductance (L_0). In addition, PIP1 and PIP2 abundance in the roots was decreased compared to ambient [CO_2]. Under salinity, the greater stomatal closure observed at elevated [CO_2] – compared to that at ambient [CO_2] – caused a greater reduction in G_s and E and allowed plants to maintain their water balance. In addition, a lower decrease in L_0 under salt stress was observed at elevated [CO_2], when comparing with the decrease at ambient [CO_2]. Modifications in PIP1 and PIP2 abundance or their functionality in the roots is discussed. In fact, an improved water status of the broccoli plants treated with 90 mM NaCl and elevated [CO_2], evidenced by a higher Ψ_ω, was observed together with higher photosynthetic rate and water use efficiency. These factors conferred on the salinised broccoli plants greater leaf area and biomass at elevated [CO_2], in comparison with ambient [CO_2]. We can conclude that, under elevated [CO_2] and salt stress, the water flow is influenced by the tight control of the aquaporins in the roots and leaves of broccoli plants and that increased PIP1 and PIP2 abundance in these organs provides a mechanism of tolerance that maintains the plant water status.

1. Introduction

Recent estimates indicate that about 7% of the global land area (Szabolcs, 1994) and 20–50% of the global irrigated farmland (Tanji, 2002; Hu and Schmidhalter, 2005) are affected by soil salinisation, one of the major abiotic factors limiting plant growth and the productivity of traditional crop plants (Choukr-Allah and Harrouni, 1996). This problem is expected to be aggravated by global climate change.

Accelerating rates of anthropogenic fossil fuel use and forest clearing have caused mean concentrations of CO_2 in the atmosphere to reach and exceed 380 μmol mol^{-1} (Keeling and Whorf, 2005). It is anticipated that the salinised surface area of the planet will increase and that the atmospheric [CO_2] will double by the end of the century (IPCC, 2007).

Several reports have shown that a CO_2-enriched atmosphere enables plants to counteract better the water stress caused by drought (Robredo et al., 2007, 2011) or saline conditions (Geissler et al., 2009a,b, 2010; Pérez-López et al., 2009a,b, 2010a,b), by increasing photosynthesis and improving their water relations (Robredo et al., 2007; Geissler et al., 2009a,b).

The interaction between salinity and elevated [CO_2] depends on the plant species and results from (i) a compromise between the photosynthetic and respiration rates and (ii) the ability to control stomatal conductance (Pérez-López et al., 2012), which depends on the stomatal density and the opening of the guard cells aperture.

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At elevated [CO_2], plants tend to decrease the leaf conductance to CO_2 and water vapour (Morison, 1985) and to reduce the transpiration rate (Kimball and Idso, 1983). Such regulation would lead directly to partial closure of the stomata (Tyree and Alexander, 1993). However, large differences in the response of stomatal density to elevated [CO_2] seem to exist among species; thus, it was unaffected in wheat (Malone et al., 1993; Estiarte et al., 1994), decreased in tree species (Beerling et al., 1998) and increased in rice (Rowland-Bamford et al., 1990). The combined effects of salinity and elevated [CO_2] on stomatal density and opening await discovery.

Water relations are of special importance since water is considered the primary factor limiting growth and productivity (Schulze et al., 1987). High [CO_2] in the atmosphere allowed plants cultivated in saline conditions to increase their water use efficiency (WUE) and water potential, in order to avoid salinity damage (Geissler et al., 2009a). In addition, elevated CO_2 improves plant tolerance of salinity by increasing the carbon assimilation rate (Rozema et al., 1991) and enhancing the synthesis and activity of enzymes and proteins, which confers on the plants an energy gain that is used mainly for more-efficient detoxification of reactive oxygen species and ion transport/compartmentation (Geissler et al., 2009b, 2010).

In our previous experiments, increased salinity provoked a large reduction in the root hydraulic conductance (L_0) of broccoli plants (López-Pérez et al., 2007; Muries et al., 2011; Zaghdoud et al., 2012) due to high concentrations of Na^+ and Cl^- in the cytoplasm, that reduced water transport through the plasma membrane, and the closely-related changes in the functionality and abundance of aquaporins (López-Pérez et al., 2009; Muries et al., 2013 (in press)). Under non-salinised conditions, elevated [CO_2] caused a reduction in the hydraulic conductance of soybean and alfalfa (Bunce, 1996) and of two rain-forest tree species (Eamus et al., 1995). Recently, Pérez-López et al. (2009a) reported that the combined effects of NaCl and elevated [CO_2] significantly decreased the L_0 of barley plants, the decrease being of the same intensity as that of the salt treatment alone and coinciding with a high decrease in the instantaneous and cumulative transpiration rates.

Aquaporins are intrinsic membrane proteins that mediate water movement across cellular membranes, thus playing an essential role in plant water relations (Chrispeels et al., 2001; Maurel and Chrispeels, 2001; Tyerman et al., 2002). Some aquaporins also facilitate the membrane transport of other small and uncharged molecules (Biela et al., 1999; Baiges et al., 2002; Tyerman et al., 2002; Liu et al., 2003; Jahn et al., 2004; Beitz et al., 2006). Using well-established physiological techniques for the determination of mesophyll CO_2 conductance in leaves, several studies indicated the significance of aquaporins in the regulation of leaf mesophyll conductance under different environmental conditions (Terashima and Ono, 2002; Hanba et al., 2004; Flexas et al., 2006; Uehlein et al., 2008, 2012; Heckwolf et al., 2011). However, less is known about the role of aquaporins in leaf water transport.

Some studies have attributed the enhanced plant growth under the combination of salinity and elevated [CO_2] – with respect to salt stress alone – to an improvement in water relations (Bowman and Strain, 1987; Pérez-López et al., 2009a), in addition to increased net photosynthesis. However, predictions of climate change impacts on plant water relations depend on the cultivar and additional knowledge is needed about the interaction between enhanced [CO_2] and salinity, regarding the mechanisms involved in plant water homeostasis.

Broccoli (*Brassica oleracea* L. var Italica) is a well-recognized health-promoting vegetable, due to its high content of beneficial compounds. Despite the economic importance of broccoli as a plant commodity in the food industry, and the fact that it is moderately salt-tolerant, no data concerning the combined effects of elevated [CO_2] and salt stress on aquaporins abundance and expression and water relations are available. Therefore, this study focused particularly on the interactive effects of elevated [CO_2] and salinity on the regulation of water relations and related parameters (such as root hydraulic conductance, water and osmotic potential and leaf turgor) through the abundance of aquaporins in the roots and leaves of *B. oleracea* in order to determine plant acclimation to both environmental conditions in the frame of climatic change in arid/semiarid regions. Since aquaporins are related to whole plant hydraulics we hypothesised that aquaporins could mediate in the control of hydraulic conductivities in broccoli plants in response to elevated CO_2. The fact that aquaporins may influence the ameliorative effect that an enriched CO_2 atmosphere exerts on water use efficiency under salt stress was also checked. Thus, salt tolerance at an increased [CO_2] is discussed in terms of plant water status and CO_2 assimilation.

2. Materials and methods

2.1. Plant material, culture conditions and experimental design

Seeds of broccoli (*B. oleracea* L. var Italica, cv. Parthenon) (Sakata Seed Ibérica S.L, Valencia, Spain) were pre-hydrated with deionised water and aerated continuously for 12 h, before being placed in an incubator chamber at 28 °C, in darkness. The seeds were placed in trays with vermiculite as substrate. After 2 days, they were transferred to a controlled-environment chamber with a 16-h light and 8-h dark cycle, with temperatures of 25 and 20 °C, respectively, where they were supplied with ambient (380 ppm) or elevated (800 ppm) atmospheric CO_2 concentrations. The CO_2 concentration was supplied and regulated by injection of external compressed CO_2 (bottle [CO_2] ≥ 99.9%), controlled by an infrared gas analyzer (Aerasgard RCO_2, S+S Regeltechnik, Nuremberg, Germany) equipped with an automatic switching solenoid to maintain the CO_2 concentration at the adequate level.

The relative humidity (RH) was 60% (light period) and 80% (dark) and the photosynthetically active radiation (PAR) of 400 $\mu mol\, m^{-2}\, s^{-1}$ was provided by a combination of fluorescent tubes (Philips TLD 36W/83 and Sylvania F36W/GRO) and metal halide lamps (Osram HQI, T 400W). After 3 days, the seedlings were placed in 15-L containers filled with continuously-aerated Hoagland nutrient solution (Hoagland and Arnon, 1950), which was replaced every week. The experimental design was a split plot, using the CO_2 concentration as the main plot. We then allocated randomly the individual experimental units (containers) within each CO_2 concentration to different levels of the other factor (salinity regime). After 2 weeks of growth, different salinity treatments (0, 60 and 90 mM NaCl) were applied for 15 days. The salt treatments were applied to the nutrient solution by the addition of 30 mM NaCl every hour until the final NaCl concentrations of 0, 60 and 90 mM were reached, in order to avoid osmotic shock. After 15 days of NaCl treatment, the plants were harvested for analysis. Five replications of each treatment were used for the determinations.

2.2. Growth parameters and leaf relative water content (RWC)

Plants were harvested 6 h after the beginning of the light phase and immediately separated into shoots and roots. The roots were washed three times with cold, distilled water and blotted with filter paper. The plant fresh weight was recorded directly with a portable balance (Scout Pro 400g, Ohaus Corporation, NJ, USA) and the dry weight (DW) was obtained after drying the fresh organs in an oven at 70 °C until constant weight.

In order to determine the leaf relative water content (RWC), five discs of 1-cm diameter were sampled. The fresh mass (Mf) of the discs was measured immediately; then, they were hydrated for 24 h