Environmental and Experimental Botany 143 (2017) 1–9

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Moderate salinity improves stomatal functioning in rose plants grown at high relative air humidity

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ARTICLE INFO

Keywords:
Abscisic acid
Salt stress
Rosa × *hybrida*
Stomatal physiology
Vapor pressure deficit
Water loss

ABSTRACT

Plants grown at high relative air humidity (RH ≥ 85%) show hampered stomatal closure in response to closing stimuli. We hypothesized that a moderate salinity during growth could trigger a stress response and stimulate stomatal functioning due to an increased leaf abscisic acid concentration ([ABA]). Cut rose 'Prophyta' was grown at moderate (63%) or high (89%) RH combined with three electrical conductivities (EC) in the nutrient solution (2, 4 and 6 dS m^{-1}; adding NaCl). High RH resulted in higher pore area per leaf area in intact leaves, and higher stomatal conductance (g_s) both in leaves subjected to desiccation and to light/dark transition, as compared to moderate RH. Increasing EC in high RH-grown plants lead to higher stomatal density but it enhanced stomatal closure in response to leaflet desiccation. This enhanced stomatal functioning was associated with increased [ABA] and [ABA + metabolites]. Nonetheless, plants grown at EC6 showed a significantly lower chlorophyll content, total plant dry weight and total leaf area. This negative effect on plant growth is related to ionic stress as the sodium and chloride concentrations increased in plants grown at EC6 compared to EC2 (up to 111- and 14-fold, respectively). This is the first study on the interactive effects of RH and salinity on stomatal functioning and anatomy during leaf development. It is shown that, when these two environmental factors that influence stomatal responsiveness in an opposite way are combined, moderate EC is able to improve stomatal responsiveness to leaflet desiccation in high RH-grown plants due to increased leaf [ABA].

1. Introduction

High relative air humidity (RH ≥ 85%) during leaf development is well described to disturb the stomata's capacity to close in response to water stress, darkness and abscisic acid (ABA) (Torre et al., 2003; Carvalho et al., 2016; Fanourakis et al., 2016). This leads to uncontrolled water loss when plants are further subjected to conditions of high evaporative demand, namely during postharvest, reducing plants longevity (Mortensen and Gislerød 1999; Fanourakis et al., 2012). Stomatal physiology has been pointed out as the major cause for this negative water balance (Arve et al., 2013; Fanourakis et al., 2013; Aliniaeifard et al., 2014; Carvalho et al., 2015) and the phytohormone ABA is closely involved in this process. In fact, lower ABA concentrations ([ABA]), associated with poor stomatal functioning, were found in leaves of *Trasdescantia virginiana* (Rezaei Nejad and van Meeteren 2007), *Vicia faba* (Aliniaeifard et al., 2014) and *Rosa* × *hybrida* (Arve et al., 2013; Giday et al., 2013; Carvalho et al., 2015) developed at high RH compared to moderate RH.

The tissue [ABA] is determined by its biosynthesis and catabolism (Nambara and Marion-Poll 2005). ABA can be permanently inactivated via oxidation to form 8′-hydroxy ABA which rearranges to phaseic acid (PA) and is further reduced to dihydrophaseic acid (DPA) (Cutler and Krochko 1999; Nambara and Marion-Poll 2005). In addition, ABA can also be temporarily inactivated by covalent conjugation with

Abbreviations: ABA, abscisic acid; ABA-GE, ABA-glucosyl ester; DPA, dihydrophaseic acid; EC, electrical conductivity; g_s, stomatal conductance; PA, phaseic acid; RH, relative air humidity; RWC, relative water content; VPD, vapor pressure deficit

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http://dx.doi.org/10.1016/j.envexpbot.2017.07.018
Received 29 May 2017; Received in revised form 26 July 2017; Accepted 26 July 2017
Available online 27 July 2017
0098-8472/

monosaccharides (e.g. ABA-β-D-glucosyl ester; ABA-GE), which accumulates in vacuoles and it is, hence, hypothesized to be a storage form of ABA (Dietz et al., 2000; Lee et al., 2006; Arve et al., 2013). High RH has been shown to increase the inactivation of ABA to PA in *Arabidopsis thaliana* leaves (Okamoto et al., 2009), whereas in roses ABA-GE remained high during the night, indicating that the conversion to ABA following a light-dark transition did not occur (Arve et al., 2013). Besides RH, there are other environmental factors, such as salinity, that influence ABA metabolism (Maggio et al., 2007; Lovelli et al., 2012). An increased [ABA] associated with a lower stomatal conductance has been reported in tomato plants in response to high levels of salinity (Maggio et al., 2007; Lovelli et al., 2012).

Soils are considered saline when the EC is above 4 dS m^{-1} (George et al., 2012). According to FAO (Food and Agriculture Organization of the United Nations), a conductivity of the saturation extract between 4 and 8 (dS m^{-1}) corresponds to a moderately saline soil class, where yields of several crops are restricted (Website FAO, 2017). The three major constrains for plant growth under salinity are: (i) osmotic stress, i.e., water deficit arising from the low water potential in the rhizosphere; (ii) ionic stress, i.e., ion toxicity resulting from the excessive uptake mainly of Na^+ and Cl^-; and (iii) nutrient imbalance by reduction in uptake and/or shoot transport and impaired distribution of nutrients (Greenway and Munns, 1980). During the initial phase of root exposure to excessive [NaCl], water uptake may be inhibited, causing a physiological drought stress (Shalhevet and Bernstein, 1968) and leading to stomatal closure and a reduction in plant growth (Munns and Tester, 2008). Moreover, salinity can affect stomatal and leaf anatomy. In cotton, a decrease in stomatal density was compensated by an increase in stomatal size and mesophyll surface area (Jafri and Ahmad, 1995), and in strawberry salinity reduced transpiration flux due to low stomatal density (Orsini et al., 2012). Ionic stress results in leaf chlorosis and/or necrosis due to the accumulation of Na^+ which disrupts protein synthesis and interferes with enzymatic activity (Munns and Termaat, 1986; Hasegawa and Bressan, 2000; Munns, 2002). Furthermore, salinity may also affect the homeostasis of certain elements, including K and Ca (George et al., 2012) which are essential ions for the regulation of stomatal functioning. It is also known that the ratio K^+:Na^+ translates the metabolic competence of the cells, and to maintain it favorable under increased salinity plants might either restrict the Na^+ accumulation in the tissues, or prevent K^+ lowering (Shabala and Cuin, 2008). Calcium contributes to the exclusion of Na^+ and the decrease in K^+ efflux, despite a low uptake of Ca^{2+} is likely to occur in response to salinity (Francois et al., 1991). In tomato, it has been shown that high shoot K^+:Na^+ and Ca^{2+}:Na^+ ratios may enhance salt tolerance or resistance (Dasgan et al., 2002). Plant responses to salinity during cultivation depend on the species, salt concentration, duration of exposure, plant developmental stage and other environmental conditions (Munns, 2002). The modern rose cultivars are regarded as moderately tolerant to salinity in soilless culture (Cabrera and Perdomo, 2003).

The exclusion mechanism for Na^+ and Cl^- seem to be rather efficient in roses due to a reduced ion uptake by the roots and further loading into the xylem avoiding toxic concentrations within the leaves (Sonneveld et al., 1999; Davenport et al., 2005). Moreover, to the best of our knowledge, the effect of high RH combined with moderate salinity on ABA metabolism and stomatal functioning has not yet been studied. The objective of this work is to investigate the interactive effects of these two environmental factors during leaf development on stomatal physiology and anatomy of cut roses, since they influence stomatal responsiveness in an opposite way (i.e., high RH impairing stomatal responsiveness to closing stimuli and moderate salinity enhancing stomatal closure). Here, it is hypothesized that when grown at high RH, a moderate salinity during growth triggers a mild stress response and stimulates stomatal functioning in response to closing stimuli due to an increase in the leaf [ABA].

2. Materials and methods

2.1. Plant material and growth conditions

Rooted cuttings of the cut rose *Rosa* × *hybrida* 'Pink Prophyta' (hereafter named 'Prophyta') were planted in 3.5 l pots containing a mixture (2/1, v/v) of cocopeat (Pelemix, Murcia, Spain) and perlite (Otavi, Neuss, Germany). Sixty plants were randomly distributed over two walk-in climate controlled growth chambers (length × width × height = 2.0 × 1.6 × 2.0 m; 5000 EH, Aralab, Albarraque, Portugal) at a density of 19 plants m^{-2} (one plant per pot; single stem). During cultivation, the RH in one of the growth chambers was 63 ± 2% (moderate RH) while in the other one it was 89 ± 3% (high RH). Constant day and night temperature was 22.2 ± 1.5 °C in both growth chambers, resulting in vapor pressure deficits (VPDs) of 0.99 ± 0.02 kPa (moderate RH) and 0.29 ± 0.06 kPa (high RH). The CO_2 concentration was 350 ± 20 μmol mol^{-1} (IAQ 910, TSI Incorporated, Shoreview, MN, USA). Fluorescent lamps (Osram L58W/840, Lumilux, Cool White, Munich, Germany) provided a 20 h photoperiod of 130 ± 5 μmol m^{-2} s^{-1} photosynthetic active radiation (Li-1000 datalogger, Li-Cor, Lincoln, Nebraska, USA) measured 20 cm above the root-shoot interface. Plants were watered daily with a nutrient solution containing both (i) macronutrients (mM) [NH_4 1.0, K 4.0, Ca 3.5, Mg 1.38, NO_3 10.5, SO_4 1.5, H_2PO_4 1.25] and (ii) micronutrients (μM) [Fe 25, Mn 5, Zn 3.5, B 20, Cu 0.75, Mo 0.5]. The nutrient solution had an electrical conductivity (EC) of 2 dS m^{-1} (EC2, control) (Cond 6+, Eutech Instruments, Eutech Instruments Europe bv, Nijkerk, The Netherlands). In each growth chamber ten plants were directly watered with the standard nutrient solution (EC2, control), while the other 20 plants were watered with the standard nutrient solution adjusted to an EC of 4 dS m^{-1} (EC4) or 6 dS m^{-1} (EC6) by adding 18.3 mM and 31.6 mM of NaCl, respectively. To prevent EC build-up in the substrate throughout plant development, the drained solution was monitored twice per week. When the values from the soil saturation extract were above the ones from the irrigation water, plants were watered with their corresponding nutrient solution as much as necessary to lower the EC from the soil saturation extract to the levels of the irrigation water (EC2, EC4 or EC6), meaning that, in this experiment, the EC from the soil saturation extract was similar to the EC from the irrigation water. The salinity treatment lasted 10 weeks, i.e., during the whole cultivation period, starting with the planting of the rooted cutting till flowering. A pH of 5.3 (pH 5+, Eutech Instruments, Eutech Instruments Europe bv, Nijkerk, The Netherlands) was kept in all treatments.

2.2. Stomatal conductance and responsiveness to desiccation

Stomatal conductance (g_s) was measured in intact plants on the terminal leaflets of fully grown tri-foliated leaves (i.e., tri-foliate immediately above the first penta-foliated counting from the apex), with a porometer (AP4, Delta-T Devices, Cambridge, United Kingdom), 2 h after the beginning of the light and of the dark periods. Dark-induced stomatal closure was calculated according to Eq. (1).

$$\text{Dark} - \text{induced stomatal closure}(\%) = \frac{g_s(\text{light}) - g_s(\text{dark})}{g_s(\text{light})} \times 100 \quad (1)$$

Stomatal responsiveness to desiccation was assessed in fully grown terminal leaflets (i.e., first penta-foliated counting from the apex) detached from fully grown plants (i.e., flower bud with cylindrical shape and pointed tip). Leaflet petioles were recut under water (to prevent cavitation induced-embolism) and placed in flasks filled with degassed water. Leaflets were further incubated in a saturated RH (≈ 100%) environment at 20.4 ± 1.0 °C (i.e., VPD close to 0) for 1 h to establish their saturated fresh weight (Fanourakis et al., 2011). The rehydration took place under fluorescent light (40 ± 2 μmol m^{-2} s^{-1}) to induce