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Research article

Foliar potassium nitrate application improves the tolerance of *Citrus macrophylla* L. seedlings to drought conditions

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ABSTRACT

Scarcity of water is a severe limitation in citrus tree productivity. There are few studies that consider how to manage nitrogen (N) nutrition in crops suffering water deficit. A pot experiment under controlled-environment chambers was conducted to explore if additional N supply via foliar application could improve the drought tolerance of *Citrus macrophylla* L. seedlings under dry conditions. Two-month-old seedlings were subjected to a completely random design with two water treatments (drought stress and 100% water/field capacity). Plants under drought stress (DS) received three different N supplies via foliar application (DS: 0, DS + NH_4NO_3: 2% NH_4NO_3, DS + KNO_3: 2% KNO_3). KNO_3-spraying increased leaf and stem DW as compared with DS + NH_4NO_3 and DS treatments. Leaf water potential (Ψ_w) was decreased by drought stress in all the treatments. However, in plants from DS + NH_4NO and DS + KNO_3, this was due to a decrease in the leaf osmotic potential, whereas the decrease in those from the DS treatment was due to a decrease in the leaf turgor potential. These responses were correlated with the leaf proline and K concentrations. DS + KNO_3-treated plants had a higher leaf proline and K concentration than DS-treated plants. In terms of leaf gas exchange parameters, it was observed that net assimilation of CO_2 (A_{CO_2}) was decreased by drought stress, but this reduction was much lower in DS + KNO_3-treated plants. Thus, when all results are taken into account, it can be concluded that a 2% foliar-KNO_3 application can enhance the tolerance of citrus plants to water stress by increasing the osmotic adjustment process.

1. Introduction

Citrus is one of the most important tree crops in many countries (Borroto et al., 1996b; Albrigo et al., 2005; Lagoda, 2007), including Spain (García-Sánchez et al., 2007). Irrigation is essential in some periods of the citrus fruit production cycle (Borroto et al., 1996a). However, high-quality irrigation water is not always available, and, therefore, many citrus orchards suffer severe drought periods, mainly in the summer and spring seasons (García-Sánchez et al., 2007). Under these conditions, fruit quality and yield are reduced, but such reductions depend, of course, on the duration and severity of the stress (Hockema and Etxeberria, 2001). Moderate drought can retard growth and increase fruit abscission, and fruit that reaches maturity has low juice content and inferior quality (Carr, 2012). Leaf abscission usually occurs in field-grown trees when pre-dawn leaf water potentials reach −2.75 MPa (Ribeiro and Machado, 2007). The physiological effects of drought stress in citrus trees include a reduction of stomatal conductance (g_s), leaf transpiration rate (E_{leaf}), and net assimilation of CO_2 (A_{CO_2}) (Arbona et al., 2005). The biochemical inhibition of A_{CO_2} in citrus can be linked to changes in leaf anatomy, interactions with leaf nutrients (Romero-Aranda et al., 1998; Arbona et al., 2005), and/or reductions in electron transport (López-Climent et al., 2008).

Plants have developed various mechanisms to withstand drought, such as higher shoot/root ratios, fewer and smaller leaves, concentrated solutes, or increased activity of oxidative stress enzymes in leaf cells (Lei et al., 2006). The accumulation of solutes to decrease leaf osmotic potential (Ψ_π) allows plants to maintain a favourable water potential gradient as the soil becomes drier. Thus, lower leaf osmotic potentials can maintain the positive leaf turgor

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required to keep stomata open and sustain gas exchange and growth (White et al., 2000; Hoekstra et al., 2001; García-Sánchez and Syvertsen, 2006). All of these responses have been described in citrus but do not always occur simultaneously, as some are rootstock dependent (Perez–Perez et al., 2008a, 2008b; Garcia-Tejero et al., 2010). In citrus leaves, the regulation of proline has not been studied in full detail but is likely to be atypical and different from other plant species, which accumulate high proline levels in response to salinity and drought stress (Verslues et al., 2006). In previous experiments in citrus, very heterogeneous responses were observed, indicating that the accumulation of these compounds can depend on genotype, intensity, and stress duration (Perez–Perez et al., 2007; García-Sánchez et al., 2007).

Citrus is an intensively managed crop that requires fertiliser applications to reach a good fruit crop and a healthy tree status. Most fertilisers are inorganic salts applied in either solid form or with irrigation water. In Mediterranean areas, most of the N fertiliser applied is in form of ammonium nitrate (NH_4NO_3) and potassium nitrate (KNO_3) (Quiñones et al., 2009). In addition, previous studies have demonstrated that an adequate N status in plants can alleviate the negative effects of drought stress by increasing organic solutes such as proline and enhance the activities of some antioxidant enzymes (Khammari et al., 2012). However, the application of these fertilisers in irrigation water during water-stress conditions could have negative effects on the citrus growth mainly due to the increased osmotic effect by increasing salt concentrations in the root medium due to their high electrical conductivity values. Until now, studies in citrus to evaluate the effects of N fertilisation to mitigate the negative effects of drought stress have not been conducted. In this experiment, we hypothesised that N applications via foliar spray could contribute to osmotic adjustment of citrus rootstock and alleviate the effects of drought stress. To test this hypothesis, we evaluated the physiological responses of *Citrus macrophylla* seedlings submitted to drought stress and two foliar-applied N-forms fertilisation treatments: 2% KNO_3 and 2% NH_4NO_3. We used *C. macrophylla* plants because they are the most popular rootstock used in scion x rootstock lemon trees in Spain. Currently, citrus nurseries produce a large amount of these plants in protected areas, but in summer and spring periods, these plants usually suffer drought stress due to the scarce water available to the nursery growers. Under these conditions, appropriate fertilisation can help obtain healthy and vigorous plants.

2. Material and methods

2.1. Plant material and experimental conditions

Uniform, two-month-old *C. macrophylla* L. seedlings were transplanted into 1-L plastic pots containing a universal substrate consisting of Canadian blond peat moss blended with coconut fibre and perlite (Compost Reciclable S.L. Spain). The plants were then grown in a controlled-environment chamber with a 16/8-h light/dark cycle and an air temperature of 28 °C/21 °C (day/night). The relative humidity was 55% (day) and 85% (night), and the photosynthetic photon flux density at plant height was 550 μmol m^{-2} s^{-1}. The seedlings were watered daily with Hoagland's nutrient solution, which contained 6 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2 mM KH_2PO_4, 2 mM $MgSO_4$, 20 μM Fe^{3+} masquolate, 25 μM H_3BO_3, 2 μM $MnSO_4 \cdot H_2O$, 2 μM $ZnSO_4$, 0.5 μM $CuSO_4$, and 0.4 μM $((NH_4)_6Mo_{27}O_{24} \cdot H_2O)$.

Water-stress treatments were imposed in the plants for four weeks beginning two weeks after transplanting. The amount of water applied to the plants was decreased 25% every week, starting at 75% of the water lost due to evapotranspiration and ending with complete water withdrawal, whereas the well-watered controls were maintained at field capacity. The water lost from evapotranspiration for every plant was calculated weekly as the difference between the mass of the pot just watered and the mass of the pot 24 h later. This method of applying drought treatments allows for knowing the effects of N fertilisation in plants exposed to drought with the same water deficit regardless of size of the plants (Morgan, 1984; García et al., 2007). The N foliar treatments consisted of applying foliar-N solution (2%) weekly either as NH_4NO_3 or KNO_3 to the drought-stressed plants. The experimental design was a complete block that included four treatments: well-watered plants, corresponding to the control treatment (WW); drought-stressed plants without additional N applications (DS); drought-stressed plants supplied with N applications as NH_4NO_3 (DS + NH_4NO_3); and drought-stressed plants supplied with N applications such as KNO_3 (DS + KNO_3) with six replicate plants in each treatment. The plants were shuffled within the growth chamber every week to avoid any potential positional effects. The experiment was performed two times.

2.2. Plant–water relationships

All measurements of water relations were performed on the leaves using a single mature leaf from the mid-stem region of each of the six replicate plants. Pre-dawn leaf water potential (Ψ_w) was measured at 1, 7, 14, 21, and 28 days after starting the drought treatment using a Scholander-type pressure chamber (PMS instrument, Corvallis, OR (Scholander et al., 1965)). After Ψ_w was measured, the leaves were immediately wrapped tightly in aluminium foil, frozen by immersion in liquid nitrogen, and subsequently stored in airtight plastic bags at −18 °C. After thawing, the Ψ_π of the extracted sap was measured at 25 ± 1 °C with an osmometer (Digital Osmometer, Wescor, Logan, UT). Turgor potential (Ψ_P) was calculated as the difference between Ψ_w and Ψ_π. Leaf osmotic potential at full turgor (Ψ_{II}^{100}) at the end of the experiment was also measured for one leaf per plant after full hydration overnight with the same procedures described for Ψ_π.

2.3. Leaf gas-exchange parameters

The net assimilation of CO_2 (A_{CO_2}), stomatal conductance (g_s), instantaneous leaf water-use efficiency (WUE = A_{CO_2}/leaf transpiration), and ratio of intercellular to ambient CO_2 (C_i/C_a) were measured at 1, 7, 14, 21, and 28 days after starting the drought treatments using a portable photosynthesis system (model LCA-4, ADC Bioscientific Ltd., Hoddesdon, U.K.) with a PLC-4N leaf chamber (11.35 cm^2) configured to an open system. All measurements were performed in the morning from 09:00 to 11:00 h using a single mature leaf in the mid-stem region of each of the six replicate plants. During all measurements, the leaf temperature was 29 ± 2 °C, and the leaf-to-air vapour pressure difference was 2.4 ± 0.4 kPa within the cuvette.

2.4. Growth and nutrient concentration

At the end of the experiment, the plants were harvested and separated into leaves, stems, and roots. The tissues were briefly rinsed with deionised water, oven-dried at 60 °C for at least 48 h, weighed, and ground into a fine powder. The dry weight (DW) of leaves, stems, and roots was used to calculate the shoot (leaf + stem)/root DW ratio. Samples of the leaves, stem, and root tissues were thus used to determine the nutrient concentrations. Samples were submitted to acid digestion with HNO_3:H_2O_2 (5:3 by volume) in a microwave (CEM Mars Xpress, North Carolina, USA) at 190 °C for 2 h. Next, the tissue K^+, Mg^{2+}, Ca^{+2}, and P concentrations were determined by inductively coupled plasma emission optical