Scientia Horticulturae 197 (2015) 483–489

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Effects of salinity on physiological parameters of grafted and ungrafted citrus trees

Catherine R. Simpson[a,*], Shad D. Nelson[a,b], Juan C. Melgar[c], John Jifon[d], Greta Schuster[b], Astrid Volder[e]

[a] Texas A&M University-Kingsville Citrus Center, Weslaco, TX 78599, USA
[b] Texas A&M University-Kingsville, Kingsville, TX 78363, USA
[c] Department of Agricultural and Environmental Sciences, Clemson University, Clemson, SC 29634, USA
[d] Texas A&M AgriLife, Weslaco, TX 78599, USA
[e] University of California-Davis,Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 3 June 2015
Received in revised form 1 October 2015
Accepted 5 October 2015
Available online 21 October 2015

Keywords:
Citrus aurantium L.
Citrus sunki Hort. Ex Tan. × *Poncirus trifoliata* L. Raf.'Swingle'
Citrus sinensis 'Olinda'
Grafting
Salinity

ABSTRACT

Subtropical and tropical climates are becoming more vulnerable to drought and salinity problems. Of the crops produced in these climates, citrus is especially sensitive to increasing salinity. In times of water scarcity and periodic drought alternative water sources that may be lower in quality may be required for irrigation, leading to reduced yield unless a mechanism of increasing salinity tolerance is found. The salinity tolerance of citrus rootstocks differs depending on species and cultivar in their ability to exclude or sequester toxic ions. Identifying rootstocks that are more salt tolerant is important to citrus production in susceptible areas. 'Olinda' Valencia trees budded to three rootstocks (sour orange [SO], C22, and C146) and the ungrafted rootstocks were subjected to 6 months of saline irrigation. Simulated brackish water at 1, 3, 5, and 10 dS m^{-1} along with a 0 dS m^{-1} control were used to irrigate trees over the course of the study. Throughout this time period chlorophyll fluorescence, stomatal conductance, electrolyte leakage, and SPAD were measured to identify how trees responded to different levels of salinity stress. Chlorophyll fluorescence, stomatal control, and SPAD decreased with increased salinity, while electrolyte leakage increased. These responses were more pronounced in grafted trees than ungrafted trees. C22 and C146 rootstocks showed more tolerance to increases in salinity than the SO rootstocks. The 'Olinda' Valencia scion reduces rootstock tolerance to salinity, however, trees grafted to the C22 and C146 rootstocks showed more salinity tolerance than those grafted to SO rootstocks. This study emphasizes that both rootstock and scion should be considered when selecting trees for saline conditions.

Published by Elsevier B.V.

1. Introduction

As salinity of arable lands becomes a major concern due to climate change and drought, crops sensitive to salt are more susceptible than ever. Worldwide citrus is cultivated in sub-tropical to tropical climates, which are vulnerable to drought and subsequently salinity problems. Identifying cultivars tolerant to these challenging conditions is vital for crop production in the future. Citrus is classified as sensitive to saline irrigation water with a sensitivity threshold of 1.7 dS m^{-1} (Maas, 1993; Spiegel-Roy and Goldschmidt, 1996). The ability of citrus rootstocks to restrict uptake and accumulation of toxic ions in the leaves is one of the determining factors of their salinity tolerance and is dependent upon mechanisms that regulate sodium (Na) and chloride (Cl) absorption and transport throughout the plant. Toxic accumulation of Na and Cl lead to reduced growth and can impair physiological and biochemical processes, depending on the variety sensitivity (García-Sánchez et al., 2006; Pérez-Pérez et al., 2007). The development of new rootstocks has been suggested as a strategy to improve salt tolerance in citrus (Saleh et al., 2008). Salinity is known to reduce CO_2 assimilation, stomatal conductivity, and PSII efficiency as well as interfere with nutrient ion uptake and assimilation (Behboudian et al., 1986 Garcia-Legaz et al., 1993). Salinity has also been known to increase cell membrane permeability leading to higher electrolyte leakage from affected cells (Ashraf and Harris, 2004; Lutts et al., 1996). These physiological factors indicate plant response to salinity stress and overall ability of a rootstock to tolerate saline conditions. Sour orange (SO) is con-

* Corresponding author.
E-mail address: catherine.simpson@tamuk.edu (C.R. Simpson).

http://dx.doi.org/10.1016/j.scienta.2015.10.010
0304-4238/Published by Elsevier B.V.

sidered a good Na and Cl excluder (Gimeno et al., 2009) and is commonly used in many citrus-producing regions in the world, especially in areas with high pH and calcareous soils; nevertheless, SO is highly susceptible to citrus tristeza virus (CTV; Moreno et al., 2008) and other trifoliate rootstocks such as Bitters (C22) and C146 have been proven tolerant to CTV and maintain good yields, although there are no research studies on the effect of salinity on physiological parameters of citrus grafted on these rootstocks. Various trifoliate rootstocks have been shown to have high vigor and resistance to biotic and abiotic stresses (Albrecht et al., 2012; Niles et al., 1995; Walker, 1986). Additionally, certain trifoliate orange cultivars (*Poncirus trifoliata* (L.) Raf.) were found to have a greater ability to restrict Na accumulation in the leaves as well as the ability to exchange K for Na in the xylem, effectively sequestering Na in the woody tissues of the root and basal stem compared to Cleopatra mandarin (Walker, 1986). Due to the ability of trifoliate orange to sequester and limit toxic ion translocation throughout the plant we hypothesize that the trifoliate hybrids, C22 and C146 (*Citrus sunki* Hort. Ex Tan. × *Poncirus trifoliata* L. Raf 'Swingle'), would be more tolerant to Na and Cl ions than SO as measured by certain physiological parameters. As a second part of the manuscript "growth response of grafted and ungrafted citrus trees to saline irrigation" the objective of this manuscript is to further explain the effects of salinity on the physiological parameters of grafted and ungrafted citrus.

2. Materials and methods

2.1. Plant materials and growing conditions

Citrus trees and rootstocks were originally propagated from seed at the Texas A&M University-Kingsville Citrus Center in Weslaco, TX as described in the previous manuscript by Simpson et al. (2014). From January to July 2011 citrus trees were grown in an experimental greenhouse located at Texas A&M University-Kingsville. Citrus rootstocks Sour Orange (SO) (*Citrus aurantium* L.), Bitters (C22) and C146 (both are crosses of *C. sunki* Hort. Ex Tan. × *P. trifoliata* L. Raf 'Swingle') were used. Olinda Valencia (*Citrus sinensis* 'Olinda') scions were budded onto half of the rootstocks whereas the other half remained ungrafted. Trees were grown in 10 cm × 10 cm × 36 cm tall pots filled with potting soil and treated with an Osmocote® fertilizer (Indoor/Outdoor Smart-Release® Plant food, The Scotts Company, LLC, Columbus, OH) every 3 months throughout the experiment. Plants were irrigated with salinity treatments of 0, 1, 3, 5, or 10 dS m^{-1} twice weekly throughout the study period. Irrigation volume was designed to replenish transpirational water losses with less than 10% of total applied water allowed to drain from pots. Saline solutions were prepared using Instant Ocean© sea salt (United Pet Group, Blacksburg, VA) and deionized water. Trees were set up in a random complete block design with five replications for each rootstock and treatment. Greenhouse temperatures were regulated to remain between 10 °C (January) and 36.5 °C (July) to maintain sufficient survival and growing temperatures throughout the study although temperatures sufficient for active growth usually occur by early February in Kingsville, TX.

2.2. Data collection

The following physiological parameters were collected at designated times throughout the experiment. Leaf measurements were taken on recent fully expanded mature leaves for each tree. Leaf chlorophyll fluorescence was measured using a chlorophyll fluorometer model OS1p (Opti-Sciences, Inc., Hudson, NH) using methodology described by Melgar et al. (2008). Stomatal conductance data was collected using a steady state diffusion leaf porometer (Model SC-1, Decagon Devices, Pullman, WA). Electrolyte leakage was determined using the method described by Lutts et al. (1996). SPAD (Soil Plant Analysis/Analytical Development) is a unit-less, objective measurement of leaf greenness, (Grosser et al., 2012), and an indirect measurement of chlorophyll content indicated by leaf color. SPAD measurements were conducted on two leaves per tree per treatment using a chlorophyll meter (SPAD-502, Spectrum Technologies, Plainfield, IL).

2.3. Statistical analysis

Treatments were conducted using five replications of each rootstock per treatment and were set up in a complete randomized block design. Repeated measures ANOVA analysis was used to analyze time series data. Treatment interactions were analyzed using full factorial and bivariate fit models and statistical significance ($P < 0.05$) was determined using ANOVA with JMP® Pro 10.0.0 software (SAS Institute, Cary, NC).

3. Results

3.1. Chlorophyll fluorescence

Chlorophyll fluorescence ($F_vF_m^{-1}$) declined over time at higher salinities (5 and 10 dS m^{-1}) (P duration of stress × salinity = 0.001; Fig. 1). Grafted trees showed a more pronounced decrease in fluorescence when irrigated with higher salinity solutions by the end of the study (P grafting × salinity = 0.04) compared to ungrafted trees (Fig. 1). Additionally, rootstocks showed significantly different reactions to salinity (P rootstock = 0.001, P duration of stress × rootstock × salinity × 0.02); with SO showing the most susceptibility to salinity treatments. Trees grafted to the SO rootstock had lower chlorophyll fluorescence when treated with saline water at 3 dS m^{-1} than trees grafted to C22 and C146 rootstocks, as well as its ungrafted counterpart. Marked differences in chlorophyll fluorescence were not obvious at the lower salinity levels, with most trees remaining between 0.7 and 0.8 $F_vF_m^{-1}$, which generally indicates no negative effects on the functioning of PSII. Overall, non-grafted C146 and C22 rootstocks fared better than SO rootstocks when exposed to salinity, with no significant decrease in chlorophyll fluorescence after irrigating with 5 dS m^{-1} after 6 months.

3.2. Stomatal conductance

Overall, the stomatal conductance of trees exposed to salinity treatments was variable and mainly dependent upon the salinity and duration of exposure to salinity stress (P duration of stress × salinity < 0.001). However, after 6 months of salinity treatments the stomatal conductance differed based on rootstock cultivar, grafting treatment, and salinity level (P rootstock × grafting × salinity = 0.015). By the time of the last measurement, ungrafted trees showed a steady decline of stomatal conductance with increased salinity with the exception of the 10 dS m^{-1} irrigated C22 rootstock, although these were all above measurements seen in the controls (Fig. 2). The C22 and C146 ungrafted rootstocks showed higher stomatal conductance than that of the 0 dS m^{-1} controls (denoted by the solid horizontal line, Fig. 2) and the ungrafted SO rootstocks. The grafted trees varied depending on salinity treatment and rootstock cultivar, however, the trees grafted to C22 and C146 generally showed increased stomatal conductance as the salinity treatments increased. Ultimately, SO ungrafted rootstocks and grafted trees showed lower stomatal conductance values when compared to the 0 dS m^{-1} control except when exposed to the 1 dS m^{-1} salinity treatment.