Citrus R&T (e-ISSN 2236-3122)
Citrus Research & Technology, v. 37, n. 2, 2016
http://dx.doi.org/10.4322/crt.ICC062

Artigo/Article

Morphological responses of Navelate orange tree grafted on different rootstocks under water deficit

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SUMMARY

Conditions of water deficit can cause morphological changes in plants which consequently affect physiological processes and interfere with plant metabolism. As grafting is a standard process used for citrus trees, these changes depend on the rootstock used and its interaction with the scion; this interaction will determine which plant has the best performance. This study involved assessment of changes in DNA and chlorophyll A and B content in Navelate orange seedlings grafted onto five different rootstocks (Indian and San Diego citrandarin, Swingle citrumelo, Santa Cruz Rangpur lime and Sunki mandarin) under conditions of water deficit. The seedlings from the respective combinations were approximately 12 months-old when they were transferred to 5 L polyethylene bags filled with substrate, comprising standard soil and sand at a 3:1 ratio. Plants were maintained in a greenhouse for three months. After this period, the experiment was set up using a randomized block design with a 5x2x5 factorial scheme based on the following: five rootstocks with and without irrigation, and time-points at 25, 29, 32, 35 and 38 d after stopping irrigation. At each of these time-points, chlorophyll content was assessed by direct reading in cloroLOG CFL1030 equipment and also the DNA content was determined using flow cytometry. The results suggest that severe water deficit can cause morphological changes in DNA content and in chlorophyll concentration, and that the changes are most marked with Sand Diego and Swingle rootstocks.

Index terms: flow cytometry, chlorophyll content.

Respostas morfológicas da laranjeira Navelate enxertada em diferentes porta-enxertos e sob déficit hídrico

RESUMO

As condições de déficit hídrico podem causar alterações morfológicas em plantas que consequentemente afetam os processos fisiológicos e interferem em seu metabolismo. Essas mudanças dependem do porta-enxerto usado e sua relação com a copa uma vez que a enxertia é um processo padrão usado na formação das plantas cítricas. Este estudo teve como objetivo identificar o melhor desempenho da interação de diferentes porta-enxertos com a copa Navelate em condições de estresse hídrico; mediante avaliação do conteúdo de DNA e clorofila A e B. Foram estudados os porta-enxertos citrandarin Indio e San Diego, citrumelo Swingle, limão Cravo Santa Cruz e tangerina Sunki

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utilizando mudas das respectivas combinações com aproximadamente 12 meses de idade. Estas foram transferidas para sacos de polietileno de 5 L preenchidos com substrato, compreendendo solo e areia padrão a uma proporção de 3: 1 e foram mantidas em uma estufa por três meses. Após este período, o experimento foi configurado usando o delineamento em blocos casualizados em esquema fatorial de 5x2x5 sendo cinco porta-enxertos; com e sem irrigação e cinco avaliações no tempo: aos 25, 29, 32, 35 e 38 dias após interrupção da irrigação. Em cada dia de avaliação foi analisado o teor de clorofila pela leitura direta no equipamento cloroLOG CFL1030 e também o conteúdo de DNA por citometria de fluxo. Os resultados sugerem que um déficit de água pode causar alterações morfológicas no conteúdo de DNA e na concentração de clorofila e essas mudanças foram mais evidentes nos porta-enxertos San Diego e Swingle.

Termos para indexação: citometria de fluxo, teor de clorofila.

INTRODUCTION

Water deficit stress can adversely impact on citrus plants and jeopardize many aspects of plant growth and development. However, in order to adapt to this situation some citrus cultivars present anatomical and physiological changes which depend on phenological stage of development and are strongly influenced by rootstock type (Taiz & Zeiger, 2013; Soares et al., 2015). Regarding the interaction between rootstock and canopy, choice of rootstock becomes more significant under conditions of water deficit, since rootstock can influence the degree of drought tolerance of the canopy (Goldschmidt, 2014).

The selection of materials adapted to conditions of water stress is essential for plantations with limited water supply, as well as knowledge of mechanisms related to responses to this condition. Changes in the chlorophyll content of leaves can be a morphological response to water deficit and has been used to identify promising drought-resistant materials (Kitajima & Hogan, 2003; Ciganda et al., 2009). This is because conditions of water deficit cause a reduction in nitrogen absorption, an essential component of chlorophyll. Reduction in chlorophyll pigment content as a consequence of water stress has been reported to be a physiological indicator of stress (Mohawesh & Al-Absi, 2009; Chutia & Borah, 2012).

Drought conditions may also cause changes in DNA by stimulating production of metabolic intermediates which can oxidize membrane lipids, denature proteins and react with DNA; DNA changes may include mutations (Scandalios, 2002; Azevedo Neto et al., 2008).

In view of the influence of rootstocks on citrus plants adaptation to water stress and the need to identify promising materials and improve understanding of the effects of drought on plant behavior, the present study aims to evaluate the morphological responses of different citrus rootstocks under water deficit stress, focusing on alterations in chlorophyll and DNA content.

MATERIALS AND METHODS

Buds from cultivar Navelate (*Citrus sinensis*) were grafted onto the following rootstocks: citrandarin Indio and citrandarin San Diego (*C. sunki* (Hayata) hort.ex Tanaka × *Poncirus trifoliata*), citrumelo Swingle (*C. paradise* × *Poncirus trifoliata*), Santa Cruz rangpur lime (*C. limonia* L. Osbeck) and Sunki mandarin (C. *sunki* (Hayata) hort. Ex Tanaka); cultivars rootstock seeds were provided by EMBRAPA Cassava and Tropical Fruit. After 18 months, standardized seedlings were selected with average height and diameter of 12.53 cm and 0.67 cm, respectively. Seedlings were transplanted into 5L containers with substrate composed of subsoil and sand in a proportion of 3:1. The soil had been previously analyzed and then nutrients were adjusted with basic fertilization performed following citrus recommendations of Mattos Júnior et al. (2005) and Nitrogen (5 g) was applied to each pot. In order to determine the amount of water to be applied in the case of irrigated treatments, soil field capacity was determined by laboratory method (Reichardt, 1988).

Indicators of water stress were assessed, comparing irrigated (control treatment) with unirrigated plants. The irrigated plants were maintained daily at 100% soil field capacity (FC), while plants being evaluated for indicators of water stress were not irrigated. The experiment was conducted using a randomized block design with two plants per plot and five replications, constituting a factorial 2 × 5 × 5 design consisting of 2 irrigated and non-irrigated plants for each of 5 combinations of rootstock grafted with Navalate canopy and 5 evaluation times

(25, 29, 32, 35 and 38 days after stopping irrigation). The research was conducted during the period February to March 2016, with plants grown inside a greenhouse; average temperature and relative humidity data during the evaluation period are presented in Figure 1.

Clorophyll content was determined by direct readings from leaves of plants in each treatment group using a chlorophyllometer clorofiLOG®. The DNA content was evaluated using three samples of approximately 30 mg each from the youngest leaves of plants in each treatment group. These samples were supplemented with same amount of soybean leaf mass (*Glycine max*) as the DNA reference standard (2.50 pg) (Dolezel et al., 1994). The leaves were cut into Petri dishes containing 1 ml of Marie buffer composed of 50 mM glucose, 15 mM NaCl, 15 mM KCl, 5 mM Na_2.EDTA, 50 mM sodium citrate, 0.5% Tween 20, 50 mM HEPES (pH 7.2) and 1% (m/v) polivinilpirrolidona-10 (PVP-10; Marie & Brown, 1993), to obtain a nuclear extract and to maintain nuclear integrity. All procedures were performed over crushed ice. The material was aspirated using a Pasteur pipette, filtered through a 50-µm mesh and 25 µg mL^{-1} fluorochrome propidium iodide was added to the mixture. After 5 min, two readings were taken for each sample, totaling 10,000 nucleus readings to estimate DNA content.

Histograms were obtained using a FacsCalibur® flow cytometer (Becton Dickinson, Biosciense, San Jose, California) with a CellQuest program. Plant nuclear DNA content was estimated from the ratio of fluorescence intensities of the G1-nucleus of the reference standard and the G1-nucleus of the sample, multiplied by the quantity of DNA in the reference standard. All data collected were tabulated and subjected to analysis of variance; mean values for different treatments were compared using the Skott-Knott test, 5% probability by SISVAR - System for Analysis of Variance Version 4.0 (Ferreira, 2011).

RESULTS AND DISCUSSION

With regard to indicators of water stress, there were changes in the chlorophyll content of leaves that were significantly different according to analysis of variance ($P < 0.05$). Interactions between the different treatments (rootstock type and days post-irrigation) were verified for total chlorophyll and chlorophyll B content. For chlorophyll A, there were statistically significant differences for irrigated and non-irrigated treatments and times of evaluation. As shown in Figure 2A, irrigated plants presented highest values for this variable response and these values reduced as the days post-irrigation increased (Figure 2B).

According to Von Elbe (2000), chlorophyll A and B are found in nature at a ratio of 3:1, respectively, which may explain the reduction in these values with conditions of water restriction and their increase over time. It is noted that chlorophyll A is more sensitive to oxidative degradation under conditions of stress compared to chlorophyll B (Streit et al., 2005).

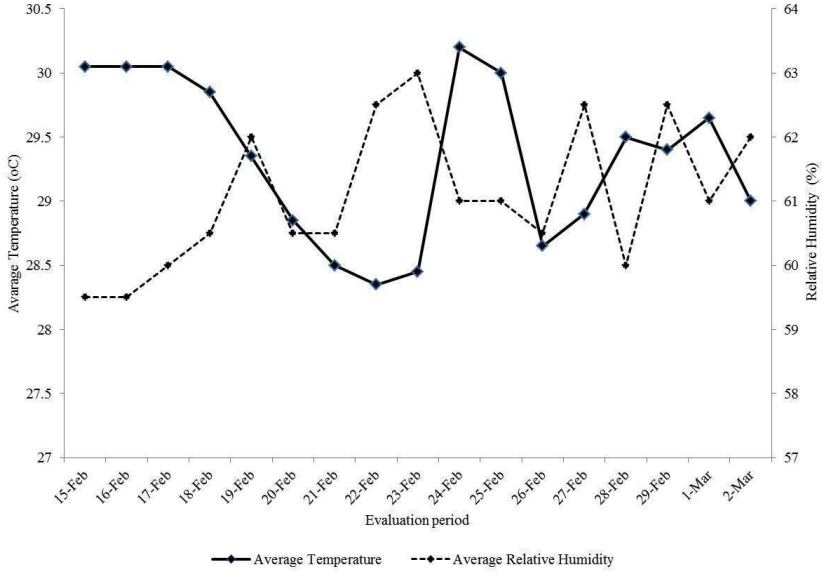

Figure 1. Mean values of temperature (°C) and precipitation (mm) recorded during the evaluation period of Navelate lantern plants grafted onto different rootstocks.

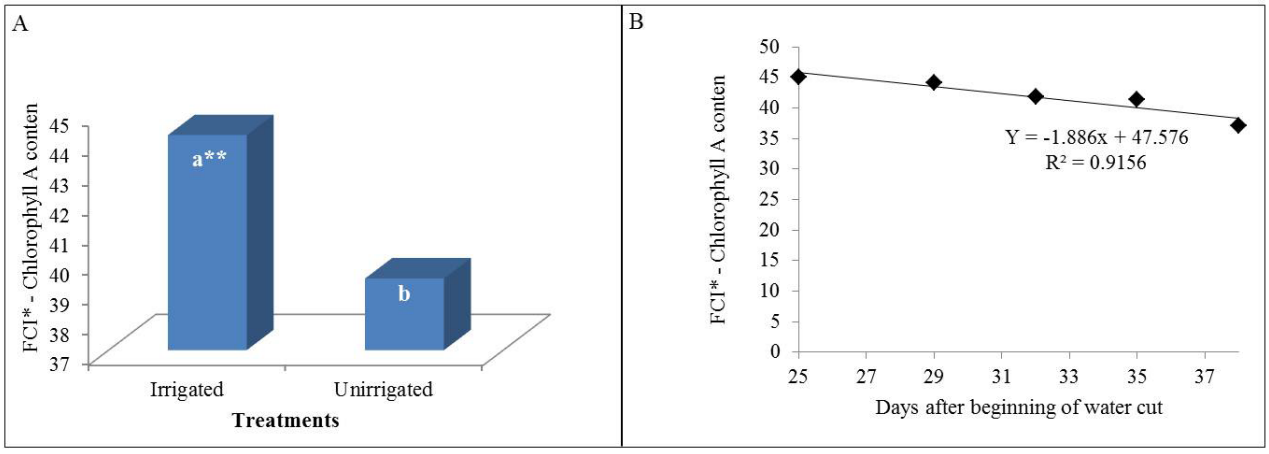

Figure 2. Mean values of chlorophyll A content in plants with irrigated and non-irrigated treatments (A) and its behavior over different evaluated time (B). *Falker chorophyll index (FCI) is a result of three different wave lengths: two in the red range, near the absorption peaks of chlorophyll and one in the near infrared. These were optically measured and transmitted through the leaves using ChlorofiLOG® apparatus. **Significant difference in mean values using Tukeys test (5%).

Unfolding the interaction of studied treatments showed no statistical difference for the chlorophyll B content, reinforcing the presumption that, as it occurs to a lesser extent, chlorophyll B is possibly less influenced by water deficit (Table 1). However, with regard to the total chlorophyll content, San Diego and Swingle rootstocks presented lower values when not irrigated, being more apperant from the third evaluation period -31 days after water cut- (Table 1). After a period, water restriction, Rangpur-lemon Santa Cruz rootstock demonstrated higher values of total chlorophyll content than the others rootstocks which may indicate increased leaf longevity and contribution of the rootstock to improved drought tolerance.

There were no significant differences between rootstocks and evaluation times in results of flow cytometric analysis for irrigated treatments (Table 1). However, for non-irrigated treatments there were variations in DNA content which were proportional to days post-irrigation and were also characterized by an increase in DNA amount. When rootstocks were analyzed at different time-points, an increase in DNA amount was also observed only in non-irrigated plants. Artlip et al. (1995) reported alterations in nuclear DNA content in maize plants under conditions of water stress. Contrasting results were reported by Aldesuquy et al. (2014) in which decreased DNA content was observed in wheat plants when exposed to water deficit.

The variations in the amount of DNA in the plants studied (Table 1), suggests that severe levels of water deficit can cause changes in these parameter. Based on evaluation times, all rootstocks presented variations at 38 days after water cut and San Diego and Swingle seems to be the most sensitive rootstocks to the water deficit, since presented higher values of amount of DNA in 25,29,32,35 and 38 days after water cut. Indio citrandarin and Santa Cruz Rangpur lime appeared to be the most tolerant to water stress, based on genetic stability as evaluated by flow cytometry techniques.

Changes in the amount of DNA are common in plants under water stress due to formation of reactive oxygen species (ROS) which cause oxidative damage to nucleic acids, including base modifications, single- and double-stranded DNA breaks, and changes in cytosine methylation (Imlay, 2003). High concentrations of ROS can cause irreversible damage to plants through oxidation of multiple cellular components, involving lipid peroxidation, protein degradation or DNA fragmentation and, in extreme cases, leading to cell death (Carvalho, 2008; Anjum et al., 2011; Rewald et al., 2013). These changes may explain increases in the DNA content observed in some of the rootstocks studied here, in response to the increased time under conditions of water deficit. In addition, results from the present study also show different levels of genetic instability among the rootstocks studied.

Table 1. Mean values of chlorophyll B, total chlorophyll (FIC – Falker Index chlorophyll) and DNA content (picogram – pg) in different citrus rootstocks, irrigated and non-irrigated, at five evaluation times (ET) – 25, 29, 32, 35 and 38 d post-irrigation

ET (days after water cut)	Chlorophyll B (FIC)									
	Indio citrandarin		San Diego citrandarin		Santa Cruz rangpur lime		Sunki mandarin		Swingle citrumelo	
	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated
25	20.06 A**A**a*	20.08 A**A**a	18.92 A**A**a	19.67 A**A**a	18.87 A**A**a	16.02 A**A**a	18.7 A**A**a	18.02 A**A**a	20.33 A**A**a	16.87 A**A**a
29	19.87 A**A**a	19.82 A**A**a	18.47 A**A**a	17.12 A**A**a	18.82 A**A**a	14.97 A**A**a	18.85 A**A**a	14.12 A**A**a	20.12 A**A**a	13.5 A**A**a
32	19.37 A**A**a	14.15 A**A**a	18.05 A**A**a	14.65 A**A**a	18.77 A**A**a	12.12 A**A**a	18.25 A**A**a	13.92 A**A**a	20.24 A**A**a	11.95 A**A**a
35	19.22 A**A**a	14.8 A**A**a	18 A**A**a	14.19 A**A**a	17.97 A**A**a	12.05 A**A**a	17.95 A**A**a	13.77 A**A**a	20.06 A**A**a	11.01 A**A**a
38	19.35 A**A**a	10.13 A**A**a	17.57 A**A**a	13.97 A**A**a	17.01 A**A**a	11.15 A**A**a	17.93 A**A**a	13 A**A**a	20.15 A**A**a	11.22 A**A**a
ET	Total Chlorophyll (FIC)									
	Indio citrandarin		San Diego citrandarin		Santa Cruz rangpur lime		Sunki mandarin		Swingle citrumelo	
	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated
25	77.22 A**A**a	69.56 A**A**a	73 A**A**a	71.7 A**A**a	66.85 A**A**a	68.17 A**A**a	66.15 A**A**a	64.62 A**A**a	59.02 A**A**a	59.57 A**A**a
29	69.72 A**A**a	63.37 A**A**a	70.52 A**A**a	66.77 A**A**a	60.7 A**A**a	65.67 A**A**a	63.85 A**A**a	61.7 A**A**a	67.2 A**A**a	58.7 A**A**a
32	66 A**A**a	62.52 A**A**a	70.97 A**A**a	59.9 A**B**a	59 A**A**a	61.4 A**A**a	59.35 A**A**a	60.35 A**A**a	72.95 A**A**a	49.05 A**B**a
35	65.87 A**A**a	61.62 A**A**a	65.03 A**A**a	52.33 A**B**a	58.89 A**A**a	60.07 A**A**a	58.65 A**A**a	58.95 A**A**a	71.72 A**A**a	38.72 A**B**a
38	64.42 A**A**a	58.01 A**A**a	63.8 A**A**a	50.2 A**B**a	58.17 A**A**a	60.85 A**A**a	58.05 A**A**a	58.07 A**A**a	61.15 A**A**a	34.52 A**B**a
ET	DNA Content (pg)									
	Citrandarin Indio		San Diego		Rangpur-lemon Santa Cruz		Sunki		Swingle	
	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated	Irrigated	Unirrigated
25	0.87A**A**a	0.85A**A**a	0.86A**A**a	0.89B**A**a	0.87A**A**a	0.85B**A**a	0.86A**A**a	0.84B**A**a	0.84A**A**a	0.84B**A**a
29	0.88A**A**a	0.86A**A**a	0.86A**B**a	0.9B**A**a	0.88A**A**a	0.84B**A**a	0.86A**A**a	0.88A**A**a	0.85A**A**a	0.89B**A**a
32	0.88A**A**a	0.87A**A**a	0.85A**B**a	0.9B**A**a	0.88A**A**a	0.84A**A**a	0.86A**A**a	0.89A**A**a	0.85A**B**a	0.90B**A**a
35	0.88A**A**a	0.88A**A**a	0.88A**B**a	0.91B**A**a	0.88A**A**a	0.89A**A**a	0.87A**A**a	0.89A**A**a	0.86A**B**a	0.93A**A**a
38	0.88A**A**a	0.90B**B**a	0.89A**B**b	0.95A**A**a	0.88A**A**b	0.9A**B**a	0.88A**A**a	0.90A**A**a	0.88A**B**a	0.98A**A**a

*Capital letter compares rootstock values in column at different evaluation times (25.29.32.35 e 38) and treatments (irrigated and non-irrigated); Capital letter in bold and underlined compares rootstocks in row at same evaluation time and with same treatment (irrigated and non-irrigated); Lowercase letter compares rootstocks in each row at same evaluation time with different treatments (irrigated and non-irrigated).

ACKNOWLEDGEMENTS

The authors thank Embrapa-Cassava and Tropical Fruits for providing rootstock seeds used in this study and FAPEMIG to financially supported.

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Received: November 15, 2016
Accepted: May 22, 2017