Citrus R&T (e-ISSN 2236-3122)
Citrus Research & Technology, v. 37, n. 1, 2016
http://dx.doi.org/10.4322/crt.ICC110

Artigo/Article

Short hypoxia period affects photosynthesis of citrus scion leaves under different rootstocks

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SUMMARY

We evaluated the effects of soil hypoxia for eight days on two-yr-old citrus scions and rootstock combination in a greenhouse. The citrus scion cultivars were Salustiana orange [*Citrus sinensis* (L) Osb] and Fukumoto navel orange [*C. sinensis* (L) Osb] grafted either onto *Poncirus trifiolata* (L) Raf or Citrange Carrizo [*C. sinensis* (L) Osb x *P. trifiolata* (L) Raf]. Scion/rootstock flooding tolerance was determined by measuring plant water relations, leaf chlorophyll and fluorescence parameters, net assimilation of CO_2 (ACO_2), stomatal conductance (gs), leaf mesophyll to air CO_2 ratio (Ci/Ca) and water use efficiency. Soil oxygen depletion reduced the leaf relative water content (RWC) in all scion/rootstock combination. The RWC decreased in Fukumoto leaves three days after the commencement of the flooding treatment irrespective of the rootstock. Leaf chlorophyll content was reduced by hypoxia after five days of flooding regardless the rootstock used. Leaf ACO_2 and gs sharply decreased 24 h after the beginning of the experiment for all scion/rootstock combination in flooded plants. Higher values of Ci/Ca found after 6 days of flooding were associated with a reduction of maximum quantum efficiency (Fv/Fm), indicating that non-stomatal factors were more limiting to ACO_2 than stomatal conductance after six days of flooding. Based on timing set reduction of leaf net gas exchange, chlorophyll fluorescence and water relations, the harmful response to hypoxia by the grafted rootstock speed up at 25 °C, irrespective of the scion cultivar and the Citrange Carrizo and Trifoliate rootstock.

Index terms: rootstock-scion combination, hypoxia, flooding, leaf gas exchange.

Curto período de hipoxia afeta a fotossíntese de variedades de copa de citros sobre diferentes porta-enxertos

RESUMO

Em casa de vegetação, foram avaliados os efeitos da hipoxia do solo durante oito dias em plantas cítricas, com dois anos de idade, em diferentes combinações entre variedade copa e porta-enxertos. As cultivares de copa de citros foram as laranjeiras Salustiana [*Citrus sinensis* (L) Osb] e Fukumoto [*C. sinensis* (L) Osb] enxertados em *Poncirus trifiolata* (L) Raf ou citrange Carrizo [*C. sinensis* (L) Osb X *P. trifiolata* (L) Raf]. A tolerância à inundação pelas variedades copas e porta-enxertos foram determinadas pela medição das relações de água da planta, teor de clorofila e parâmetros de fluorescência, assimilação de CO_2 (ACO_2), condutância estomática (gs), relação entre

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condutância do mesófilo foliar e taxa de CO_2 no ar (Ci/Ca) e eficiência de uso da água. A redução de oxigênio no solo reduziu o conteúdo relativo de água da folha (RWC) em todas as combinações de copa/porta-enxerto. O RWC diminuiu em Fukumoto após três dias do início do tratamento de inundação, para ambos os porta-enxertos. O teor de clorofila foi reduzido por hipoxia após cinco dias de inundação, independentemente do porta-enxerto utilizado. Nas folhas, a ACO_2 e gs diminuíram drasticamente 24 h após o início do experimento para todas as combinações de variedades copa/porta-enxerto nas plantas inundadas. Os valores mais elevados de Ci/Ca, encontrados após seis dias de inundação, foram associados a uma redução da eficiência quântica máxima (Fv/Fm), indicando que fatores não estomáticos eram mais limitantes para ACO_2 do que a condutância estomática após 6 dias de inundação. Com base na redução do intervalo de tempo das trocas gasosas das folhas, fluorescência da clorofila e relações de água, a resposta prejudicial da hipoxia nas plantas enxertadas aumenta a 25 °C, independentemente da variedade de copa ou dos porta-enxertos citrange Carrizo e trifoliata.

Termos de indexação: combinação copa e porta-enxerto, hipóxia, inundação, trocas gasosas da folha.

INTRODUCTION

Trifoliate rootstock is the most commonly rootstock used in Uruguay (81%; Goñi, 2008). In this region, significant amounts of rainfall can cause temporary and locally soil flooding conditions, which interacting with soil temperature follows in different intensity of citrus damage (Otero et al., 2015).

Citrus rootstocks vary in their tolerance to flooded soil conditions (Camacho-Bustos, 1972; Syvertsen et al., 1983; Garcia-Sanchez et al., 2007; Arbona et al., 2009). Flooding tolerance in seedlings of citrus rootstocks have been linked to their ability to maintain higher root conductivity (Camacho-Bustos, 1972) and greater water relations (Syvertsen et al., 1983) along with higher maintenance of photosynthetic machinery and chlorophyll fluorescence in leaves than flooding susceptible rootstocks (Arbona et al., 2009). Leaf chlorophyll content and chlorophyll fluorescence have been used as screening tools to determine flood tolerance (Smethurst & Shabala, 2003).

Citrus rootstock seedlings evidence differences in the physiological response and metabolic mechanism to avoid waterlogging stress. The ranking for waterlogging tolerance from the seedling of citrus rootstock studies and for young grafted plant, do not seem to have the same results.

The objective of this study was to evaluate the relationship between short term soil hypoxia on different citrus cultivars, grafted onto *P. trifoliata* and Citrange Carrizo rootstock.

MATERIAL AND METHODS

We evaluated the effects of the soil hypoxia for 8 days on two-yr-old citrus scions and rootstock combination in a greenhouse at the Instituto Nacional de Investigación Agropecuaria, Salto Grande Experiment Station located in North West Uruguay (31°16’20’S, 57°53’25’W). The citrus scion cultivars used were Salustiana orange [*Citrus sinensis* (L) Osb] and Fukumoto navel orange [*Citrus sinensis* (L) Osb] grafted both onto *P. trifiolata* (L.) Raf and Citrange Carrizo [*Citrus sinensis* (L.) Osb. x *Poncirus trifoliata* (L.) Raf]. The grafted plants grew in 5 L plastic pots in the greenhouse filled with fine sand/peat substrate (60/40 by vol.; Novarbo, Biolan, Fi) in a well-ventilated greenhouse under maximum photosynthetically active radiation (PAR) of 1100 μmol m^{-2} s^{-1} and natural 11-14 h photoperiods from the spring to fall. All pots had good drainage and they were well-fertigated three times per week with 150 ml of half-strength Hoaglands solution. Each plastic pot was placed in a reservoir of 1.5 m × 2.5 m × 0.30 m surrounding by sand, where the soil temperature was automatically maintained by cooling or heating the sand to reach a constant 25 °C inside the pots.

The eight treatments designed represent the scion-rootstock combination (two scions × two rootstocks) under flooding and non-flooding (control), with nine replicates. Half of the citrus scions and rootstock combination were flooded maintained soil saturation, with a constant water level over 1-2 cm above the soil surface. The other half of the plants remained well-drained without flooding as a control group and continued to receive fertigation.

Scion/rootstock flooding tolerance was determined by measuring plant water relations, leaf chlorophyll and fluorescence parameters, leaf CO_2 net assimilation (ACO_2), stomatal conductance (gs), and leaf mesophyll to air CO_2 ratio (Ci/Ca).

Gs, ACO_2 and the ratio Ci/Ca, were periodically measured from 4 to 6 times using a portable photosynthesis system (CIRAS-1, PP Systems, USA) with an additional external light supplied PAR 900-1000 μmol m^{-2} s^{-2}.

All leaf measurements were made in the morning from 08:30 to 10:30 h using single mature leaf in the mid-stem region of each plant to avoid high temperatures and low humidity in the afternoon.

Leaf chlorophyll a fluorescence was periodically measured from 4 to 6 times with a pulse-modulated fluorometer (Model OS5-Fl, Opti-Sciences, Tyngsboro, MA). Maximum quantum efficiency (Fv/Fm) of PSII was calculated as Fv/Fm =(Fm - Fo)/Fm, where Fm and Fo were maximum and minimum fluorescence in dark-adapted leaves after being covered for at least 20 min with light-tight leaf clips (FLDC, Opti-Sciences; Jifon & Syvertsen 2003, Maxwell & Johnson 2000).

Relative water content (RWC) was estimated at midday using three leaves by tree. Leaf samples were covered in plastic bag inside a cooler with ice, during the next half hour the leaves were weighted and deep in water for 24 h following the González & González-Vilar (2001) procedure and calculation. Soil redox potential and soil temperature were recorded (Patrick, et al. 1996).

A completely randomized design was used as experimental design, each of the eight treatments represent a scion-rootstock combination under flooding and non-flooding (control). Data were analyzed using the Proc GLM procedure (SAS Inst. Inc., Cary, N.C.) to determine the main effects of rootstock, scion and flooding on response variables. Means were separated by Tukey´s Test at $p < 0.05$.

RESULTS

Flooded soil at 25 °C became completely anaerobic (redox = 0 mV) by about 8 days after flooding (DAF) but soil oxygen depletion started immediately after the pots were flooded (Figure 1). Soil oxygen depletion reduced leaf relative water content (RWC) in all scion/rootstock combination. No differences were found between flooded and non flooded plants until day 6 of flooding (Table 1). After the 6th day the oxygen shortage in the soil solution reduced the leaf RWC, but there was no difference between the scions and the rootstocks.

Leaf scion chlorophyll content was reduced by hypoxia after 5 days of flooding regardless of the rootstock used (Figure 2). Flooded plants of Fukumoto grafted onto Carrizo, decreased leaf chlorophyll content faster than the other combinations, between the 3th and the 6th day (Figure 2).

Leaf maximum quantum efficiency (Fv/Fm) of PSII values were similar for scion and rootstock in the treatments throughout the experiment. However, as leaf chlorophyll content decreased, the Fv/Fm ratio also decreased from the 6th day (Table 2), when the effect of flooding on leaf became evident. There was no evidence of significant interaction between the main effect of scion, rootstock or flooding pot condition.

ACO_2 and gs sharply decreased 24 h after the beginning of the experiment for all scion/rootstock combination in flooded plants; but no interaction was significant between flooding condition, scion and rootstock (Figure 3). Leaf gas exchange variables decreased after 2 days regardless of the scion/rootstock combination (Figure 3). Higher values of Ci/Ca were recorded 6 days after flooding, from this time the plants in the pots became wilt and died. No leaf gas exchange measurement was possible after the day 8th in any plant in the flooded pots (Figure 3).

Higher values of Ci/Ca after 6 days of flooding were associated with a reduction of maximum quantum efficiency (Fv/Fm), indicating that non-stomatal factors were more limiting to ACO_2 than gs after 6 days of flooding.

DISCUSSION

ACO_2, gs and plant water status are very sensitive indicators of hypoxia and they were proposed as a tool for flooding tolerance screening in rootstock breeding programs (Otero et al., 2015). The most frequent studies in citrus flooding were done on seedling (Smethurst & Shabala, 2003; Garcia-Sanchez et al., 2007; Arbona et al., 2009; Martínez-Alcántara et al., 2015) or

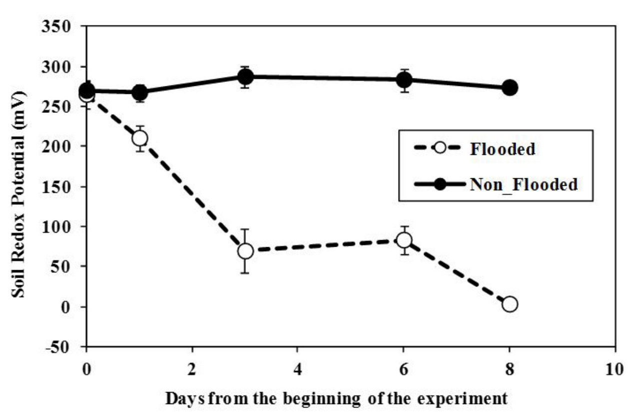

Figure 1. Soil redox potential (mV) evolution on flooded pots. Bars represent the standard error of the mean.

Table 1. Leaf relative water content (%) from the beginning of the experiment

Scion	Rootstock	Flooding	Day after experiment beginning							
			1		3		6		8	
Fukumoto			80.0	ns [1]	74.9	ns	69.1	ns	64.0	ns
Salustiana			78.9		73.8		72.9		65.5	
	Carrizo		79.8	ns	75.3	ns	71.1	ns	64.2	ns
	Trifoliate		79.1		73.4		70.9		65.3	
		Flooded	79.2	ns	74.4	ns	57.9	b	48.3	b
		Non Flooded	79.7		74.3		84.2	a	81.2	a
Scion [2]			0.2630		0.371		0.075		0.349	
Rootstock			0.5180		0.102		0.919		0.484	
Flooding			0.6920		0.949		<.0001		<.0001	
Scion*Rootstock			0.0900		0.275		0.472		0.025	
Scion*Flooding			0.5520		0.068		0.006		0.112	
Rootstock*Flooding			0.7510		0.829		0.874		0.057	
Scion*Rootstock*Flooding			0.1610		0.519		0.393		0.059	

[1] Column means followed with different letter are significant different by Tukey test ($p<0.05$); [2] Main effects and interaction significance (p< value); ns= non significant.

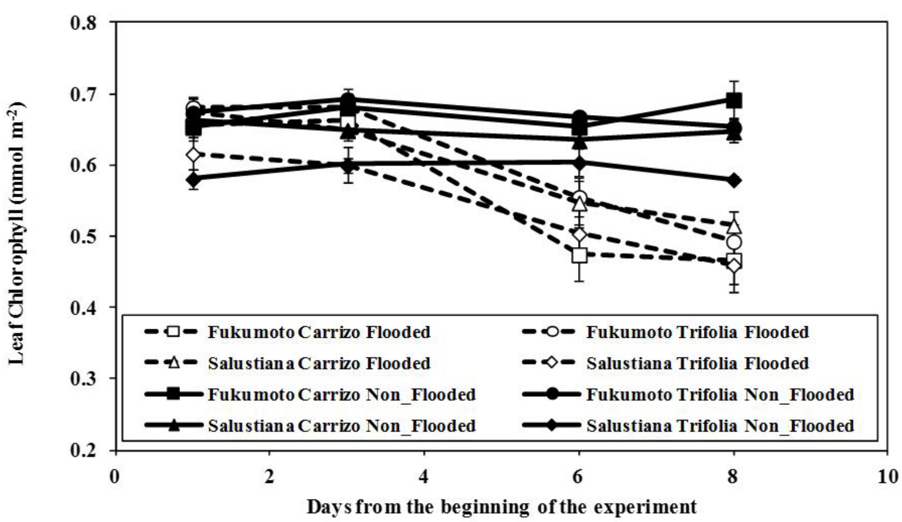

Figure 2. Leaf chlorophyll content (mmol m^{-2}) from the beginning of the experiment. Bars represent the standard error of the mean.

in bearing trees (Ortuño et al., 2007). Nonetheless, there is a lack of information about comparing young citrus plants grafted onto different rootstocks varieties/cultivars.

Flooding tolerance in seedling of citrus rootstock is been linked to the ability to longer maintain higher root conductivity (Camacho-Bustos, 1972), water relations (Syvertsen et al., 1983) together with photosynthetic machinery and chlorophyll fluorescence in seedling leaves (Arbona et al., 2009). In seedlings, leaf photosynthetic machinery decay is evident after 6 to 10 days of hypoxia (Syvertsen et al., 1983; Arbona et al., 2009; Martínez-Alcántara et al., 2015) depending of soil temperature

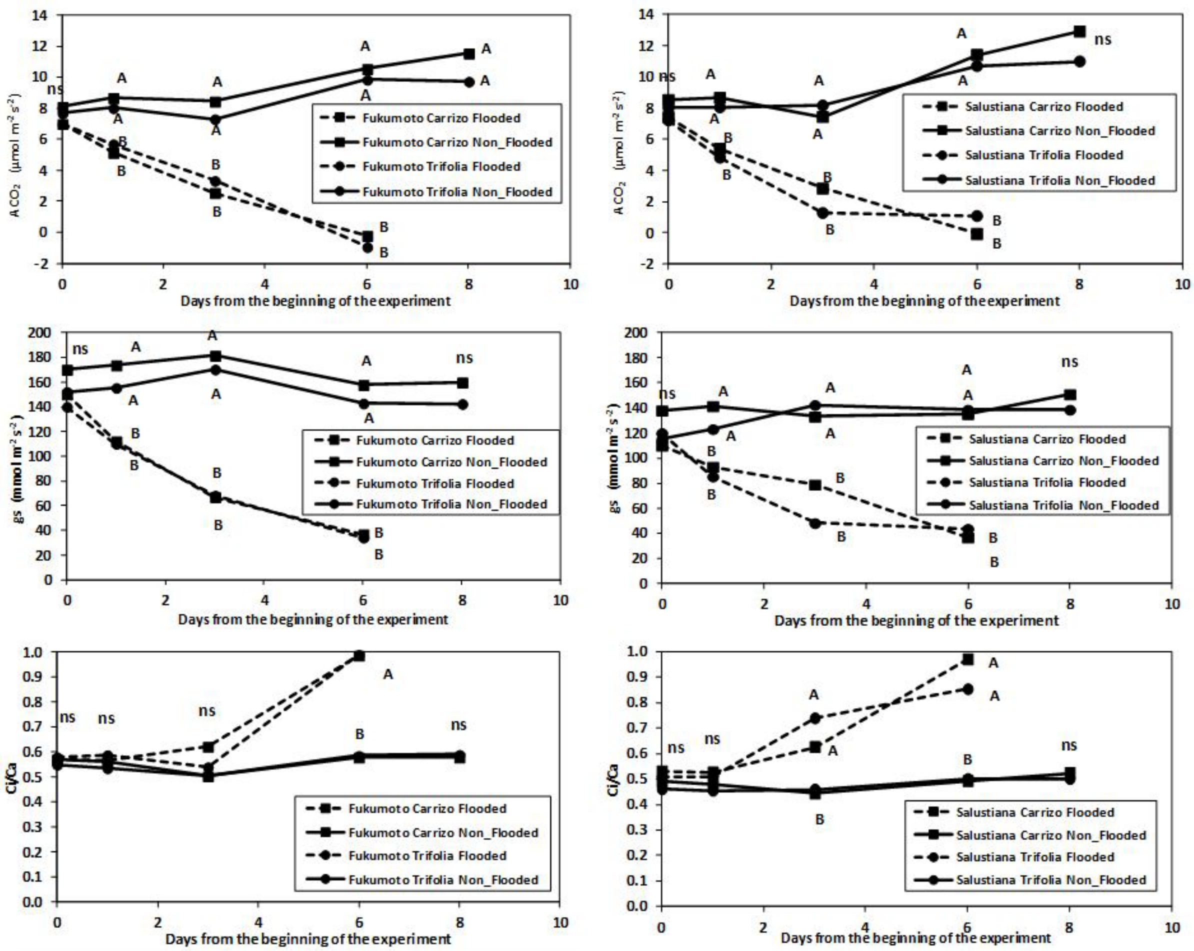

Figure 3. ACO_2, gs and Ci/Ca ratio from the beginning of the experiment. Similar letters for each day are not different by Tukey test ($p<0.05$).

(Otero et al. 2015) and rootstock (Arbona et al., 2009). Hypoxia and soil temperature can discriminate the physiological behavior between Trifoliate and Carrizo seedling, especially at lower (15 °C) soil temperature (Otero et al. 2015). Soil hypoxia reduces leaf gas exchange parameters by biochemical impairment of photosynthesis activity in seedling rootstock (Arbona et al., 2009), and in scion grafted cultivars irrespective of the the rootstock (Figure 3).

Short-term hypoxia reduced leaf relative water content, chlorophyll content and maximum quantum efficiency in both scion cultivars: Salustiana orange and Fukumoto navel orange grafted in Trifoliate and Carrizo after 6 days of lowering the oxygen in the pots (Tables 1, 2; Figure 2), in agreement with previous works (Syvertsen et al., 1983; Arbona et al., 2009; Otero et al., 2015). However, leaf gas exchange is immediately reduced after the soil oxygen shortage began, possibly by fast oxygen consumption by the roots and by soil microorganisms, in addition to the reduction of the oxygen renewal into the soil by fresh water (Reichardt et al., 2000).

On the other hand, previous seedling experiments reported that rootstocks maintained alive after 10 days of oxygen shortage began (Syvertsen et al., 1983; Arbona et al., 2009; Martínez-Alcántara et al., 2015; Otero et al. 2015). Scion canopy of young grafted rootstocks completely wilt after 6 days of flooding at 25 °C of soil temperature (Figure 3). During the first 2 days of flooding scion leaf ACO_2 and gs is reduced about 40%, but not the Ci/Ca ratio. This reduction in ACO_2 and gs can be associated with non-stomatal limitations due to the unchanged Ci/Ca ratio (Farquhar & Sharkey, 1982) and induced by reducing the rootstock conductivity (Syvertsen et al., 1983) or increasing root respiration (Reichardt et al., 2000). Scion canopy of young grafted rootstocks has bigger and more leaves than the seedling canopy of the rootstock without scion,

Table 2. Maximum quantum efficiency (Fv/Fm) of PSII throughout the experiment

Scion	Rootstock	Flooding	Days from the beginning of the experiment 1		3		6		8	
Fukumoto			0.831	ns [1]	0.796	ns	0.731	ns	0.694	ns
Salustiana			0.822		0.816		0.785		0.709	
	Carrizo		0.832	ns	0.806	ns	0.758	ns	0.728	ns
	Trifoliate		0.822		0.807		0.757		0.677	
		Flooded	0.834	ns	0.807	ns	0.696	b	0.578	b
		Non Flooded	0.820		0.805		0.818	a	0.816	a
Scion [2]			0.2510		0.0033		0.0527		0.4762	
Rootstock			0.2191		0.8758		0.8676		0.1469	
Flooding			0.0700		0.7351		<.0001		<.0001	
Scion*Rootstock			0.5388		0.9239		0.0636		0.4981	
Scion*Flooding			0.7837		0.4668		0.0960		0.5415	
Rootstock*Flooding			0.7346		0.5213		0.6279		0.0788	
Scion*Rootstock*Flooding			0.3221		0.2878		0.0866		0.8553	

[1] Column means followed with different letter are significant different by Tukey test ($p<0.05$); [2] Main effects and interaction significance (p< value); ns= non significant.

then the total canopy transpiration should also be higher than seedling rootstock without grafting. Therefore the increment in the total leaf area of the grafted rootstocks speeds up the wilt decay (Figure 3).

Based on the fast reduction of leaf net gas exchange, chlorophyll fluorescence and water relations, the difference in flooding tolerance between the seedlings of Citrange Carrizo and Trifoliate found in previous experiments (Arbona et al., 2009; Otero et al. 2015), was less important when the same rootstocks were grafted. Greenhouse hypoxia tolerance comparisons between seedling of citrus rootstocks do not make by themselves evidence to previously highlighted ranking tolerance when the same rootstocks were grafted.

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Received: March 29, 2017
Accepted: August 18, 2017