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Biochemical components and dry matter of lemon and mandarin hybrids under salt stress

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ABSTRACT
The objective was to study the biochemical changes and dry matter content in lemon and mandarin hybrids under salt stress during rootstock formation. For this, a study was conducted in randomized complete block, using a 2 x 5 factorial scheme, with two salinity levels (0.3 and 4.0 dS m^{-1}) applied in five citrus rootstock genotypes (1. TSKC x CTARG - 019; 2. LRF; 3. TSKC x (LCR x TR) - 040; 4. LCRSTC and 5. LVK), with three replicates and four plants per plot. At 90 days after sowing, saline treatments started to be applied and continued until 120 days after sowing, the moment in which the plants were collected for evaluation of biochemical characteristics and phytomass accumulation. The increase in water salinity negatively affected the biochemical components and dry matter accumulation of citrus genotypes. The genotypes TSKC x (LCR x TR) - 040, LCRSTC and LVK were the least affected by salt stress, standing out as the materials most tolerant to salinity.

Palavras-chave:
Citrus spp.
salinidade
clorofila e carotenoides
status hídrico

Componentes bioquímicos e fitomassa de híbridos de limoeiro e de tangerineira sob estresse salino

RESUMO
Objetivou-se, neste trabalho, estudar as alterações nos componentes bioquímicos e o acúmulo de fitomassa de limoeiros e de híbridos de tangerineira sob estresse salino na fase de formação de porta-enxertos. Para isto, um estudo foi realizado em delineamento experimental de blocos casualizados usando-se um esquema fatorial (2 x 5), com dois níveis de salinidade (0,3 e 4,0 dS m^{-1}) aplicados em cinco genótipos de porta-enxertos de citros (1. TSKC x CTARG - 019; 2. LRF; 3. TSKC x (LCR x TR) - 040; 4. LCRSTC e 5. LVK), com três repetições e quatro plantas por repetição. Aos 90 dias após a semeadura iniciou-se a aplicação dos tratamentos salinos que prosseguiu até os 120 dias após a semeadura, momento em que se coletaram as plantas para avaliação de características bioquímicas e o acúmulo de fitomassa das mesmas. O aumento da salinidade da água afetou negativamente os componentes bioquímicos e o acúmulo de fitomassa dos genótipos de citros. Os genótipos TSKC x (LCR x TR) – 040, LCRSTC e LVK foram os menos afetados pelo estresse salino destacando-se como os materiais mais tolerantes à salinidade.

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Introduction

Citric fruits are of great importance in the Brazilian agricultural scene, especially in the Southeast and Northeast regions of Brazil. However, in the Northeast region, the citrus production is low, approximately 17 t ha^{-1} (IBGE, 2014), compared with the potential of the crop, which can reach 40 t ha^{-1}. Such deficit in production is related to the water deficiency and to the high concentration of salts present in the waters available for irrigation (Fernandes et al., 2011; Silva et al., 2012; 2014; Brito et al., 2014).

Salinity and, especially, sodicity cause negative effects on plant growth and physiology (Mesquita et al., 2015) and, considering citrus plants, this problem is even worse, because of their sensitivity to the effects of salts (Ayers & Westcot et al., 1985). Thus, strategies that make viable the management of saline water in the irrigation of citrus orchards in semi-arid regions appear as an alternative for the expansion of the crop and increase in yield (Silva et al., 2012; Brito et al., 2014).

For that, the identification of materials tolerant to salt stress has been focused by studies for the expansion of the citrus cultivation in the semi-arid region of Northeast Brazil. However, the identification of materials tolerant to salinity is hampered by the large variations between species and inside the same species, since the tolerance to salinity is controlled by more than one gene and influenced by environmental factors (Syvertsen & García-Sánchez, 2014). Therefore, more-accurate evaluations that explain the physiological behavior and the expression of tolerance mechanisms of the studied materials are necessary (Habibi & Amiri, 2013; Silva et al., 2014). Thus, this study aimed to evaluate the biochemical components and phytomass accumulation of citrus genotypes under salt stress, applied during the rootstock formation stage.

Material and Methods

The experiment was carried out in a protected environment (greenhouse) of the Center of Sciences and Agri-food Technology - CCTA of the Federal University of Campina Grande - UFCG, Pombal-PB, Brazil (6º 47' 20" S, 37º 48' 01" W and altitude of 194 m).

The treatments were arranged in a 2 x 5 factorial scheme and distributed in a randomized block design, with the following factors: two levels of water salinity used to prepare the nutrient solutions (0.3 and 4.0 dS m^{-1}) and five citrus rootstock genotypes: 'Common Sunki' mandarin [*Citrus sunki* (Hayata) hort. ex Tanaka] x Citrange [*C. sinensis* (L.) Osbeck x *Poncirus trifoliata* (L) Raf.] 'Argentina'- 019 (TSKC x CTARG - 019); Florida Rough lemon (LRF) (*Citrus jambhiri* Lush.); 'Common Sunki' mandarin x ('Rangpur' lime *C. limonia* Osbeck x *P. trifoliata*) - 040 [TSKC x (LCR x TR) - 040]; 'Rangpur Santa Cruz' lime (LCRSTC) (*Citrus limonia* Osbeck); and 'Volkamer' lemon (LVK) (*C. volkameriana* V. Ten.; Pasq.) with three replications, totaling 30 plots, each one composed of four pots with one plant in each, totaling 120 experimental plants.

The growth of the seedlings of the hybrids occurred in the period of April to July 2013, in containers with capacity for 1.5 dm^3 and, as substrate, coconut fiber, which was washed to avoid the interference of salts present in the material, as well as the availability of nutrients, since it is an inert material. The utilized nutrient solutions followed the recommendations of Hoagland & Arnon (1950), but adding 25% of EDTA iron to supply the nutritional requirements of the genotypes (Table 1), observed in a preliminary test.

The irrigation waters were prepared in such a way to obtain a proportion equivalent to 7:2:1, between Na:Ca:Mg, respectively, using the salts of NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$. After preparation, the waters were stored in 60-L plastic containers, one for each ECw level, properly protected to avoid evaporation and contamination with materials that could compromise their quality. The nutrient solution was prepared with distilled water and, before preparing the solution of application in the system, it showed electrical conductivity of 2.3 dS m^{-1}. This solution was mixed with waters of 0.3 dS m^{-1} (S_0) (Table 2) and 4.0 dS m^{-1} (S_1), reaching salinity levels of 2.6 and 6.3 at the end of the preparation, respectively.

The alternative hydroponic system for the production of citrus seedlings was made using 2-L PET bottles, adopting the basic principles of the Leonard pots system proposed by Silva et al. (2014), interconnected in a system of communicating pots, through pipes. Each block was composed of 40 pots, of which 20 were interconnected to a tank of storage and distribution of the nutrient solution, for each salinity level under study.

The seeds, properly selected and treated with thiram sulfate fungicide (4 g kg^{-1} of seeds), were planted at the ratio of three per container and covered with the substrate. After seed germination, when the seedlings (ungrafted or plants from seeds) showed three or more pairs of true leaves, only one individual was left to develop, with caution to maintain only those that represented the standard of the plant of each genotype, in order to select seedlings of nucellar origin, thus representative of their respective mother plants. All necessary cultivation practices described in Mattos Júnior et al. (2005), relative to the production of citrus seedlings, were adopted.

Table 1. Concentration of nutrients in the nutrient solution for hydroponic cultivation of citrus

Nutrients	N	P	K	Ca	Mg	S	Fe	Mn	B	Cu	Zn	Mo
	(mmol L^{-1})											
Concentration	15	1	6	5	2	2	0.0625	0.01	0.05	0.003	0.0008	0.001

Adapted from Hoagland & Arnon (1950)

Table 2. Chemical analysis of the supply water used in the experiment

ECw dS m^{-1}	pH	K^+	Ca^{2+}	Mg^{2+}	Na^+	SO_4^{2-}	CO_3^{2-}	HCO_3^-	Cl^-	SAR[1] (mmol L^{-1})$^{0.5}$
		(mmol$_c$ L^{-1})								
0.3	7.0	0.3	0.2	0.6	1.4	0.2	0.0	0.8	1.3	2.21

[1]SAR -Sodium adsorption ratio

Plants were conducted until 120 days after sowing (DAS), when they reached stem diameter close to 4.0 mm. The beginning of application of salinized waters occurred at 90 DAS and continued up to 120 DAS, to allow total establishment of the stress on the rootstocks.

The relative water content (RWC) was determined at 120 DAS and consisted essentially in the comparison of water content in the tissue of one recently collected leaf (green weight = GW) with the water content of the same tissue when turgid, obtained by the immersion of the leaves in distilled water for 12 h (turgid weight = TW), expressing the result in percentage, making essential the determination of dry weight (DW), as follows: RWC = (GW - DW) / (TW - DW).

At 120 days, leaf discs were collected for the determination of contents of chlorophyll a and b, total chlorophyll and total carotenoids. The contents of chlorophyll a and b, and carotenoids (mg L^{-1}) were determined in the Laboratory of Plant Physiology of the UFCG, Campus of Pombal. Chlorophyll was extracted in 80% acetone and quantified through spectrophotometry, with results expressed in mg L^{-1}, using the methodology proposed by Lichtenthaler (1987).

Still at the end of the experiment, the dry matter accumulations in leaves (LDM) (g), stem (SDM) (g) and roots (RDM) (g) were determined through the collection and fractionation of the plants, when leaves, stem and roots were separated, placed in paper bags, dried in a forced-air oven at 65 ºC until constant weight, and then weighed on analytical scale (0.0001 g).

The obtained data were evaluated through analysis of variance by F test. In cases of significance, the test of means (Student's t-test at 0.05 probability level) was applied for the factor levels of irrigation water salinity, while means grouping test (Scott-Knott, $p \leq 0.05$) was applied for the factor rootstocks, using the statistical program Sisvar (Ferreira, 2011).

Table 3. Chlorophyll a (Chl a), b (Chl b), total (Chl total), carotenoids (Car), chlorophyll a/chlorophyll b ratio (Chl a/Chl b), total chlorophyll/carotenoids ratio (Chl/Car), leaf dry matter (LDM), stem dry matter (SDM), root dry matter (RDM), relative water content (RWC) of citrus genotypes under salt stress in hydroponic cultivation

Genotypes	Water salinity (dS m^{-1})			
	0.3	4.0	0.3	4.0
	Chl a*		Chl b*	
TSKC x CTARG - 019	5.6bA	4.2bB	2.3aA	1.6bB
LRF	5.6bA	5.6bA	2.1aA	1.9bA
TSKC x (LCR x TR) - 040	3.5cA	4.1bA	1.5bA	1.8bA
LCRSTC	4.3cA	4.6bA	1.5bA	1.8bA
LVK	7.1aB	9.2aA	2.6aB	4.1aA
	Chl total*		Car**	
TSKC x CTARG - 019	7.9bA	5.9bB	1.24 c	
LRF	7.7bA	7.6bA	1.81 b	
TSKC x (LCR x TR) - 040	5.0cA	5.9bA	1.07 c	
LCRSTC	5.8cA	6.4bA	1.44 c	
LVK	9.7 aB	13.3aA	2.20 a	
	Chl a / Chl b*		Chl / Car**	
TSKC x CTARG - 019	2.4bA	2.6aA	5.6a	
LRF	2.7aA	2.9aA	4.2b	
TSKC x (LCR x TR) - 040	2.4bA	2.3bA	5.1a	
LCRSTC	2.9aA	2.7aA	4.3b	
LVK	2.7aA	2.2bB	5.3a	
	LDM (g)*		SDM (g)*	
TSKC x CTARG – 019	1.3cA	0.9cB	1.7bA	0.5bA
LRF	1.1cA	1.1cA	3.5aA	0.2bB
TSKC x (LCR x TR) – 040	0.5dA	0.6dA	0.2cA	0.2bA
LCRSTC	2.3bA	2.1bA	2.6aA	1.4bA
LVK	4.3aA	3.0aB	4.4aA	3.2aA
	RDM (g)*		RWC***	
TSKC x CTARG – 019	0.8dA	0.3bB		
LRF	1.2cA	0.4bB		
TSKC x (LCR x TR) – 040	0.3eA	0.3bA	0.9A	0.8B
LCRSTC	2.6bA	1.0aB		
LVK	3.1aA	1.3aB		

*,**and***Interaction of genotype x water salinity, factor genotype and factor water salinity significant at 0.05 probability level, respectively; Equal lowercase letters in the column do not differ by Scott-Knott test at 0.05 probability level and equal uppercase letters in the row do not differ by Student's t-test at 0.05 probability level

Results and Discussion

For the contents of chlorophyll a, b and total, the genotype LVK showed the highest values, regardless of the applied levels of salinity, and these contents increased as a function of the application of the stress (Table 3). This fact indicates that the increase in salinity did not stimulate the increase in the enzyme chlorophyllase, responsible for the degradation of chlorophyll or, possible, due to the higher efficiency of the carotenoids of this genotype to protect the chlorophyll molecules from the action of the chlorophyllase (Taiz & Zaiger, 2013), since the LVK also showed the highest contents of carotenoids among the genotypes. This behavior can be explained by the tolerance of the genotype, which expresses a mechanism that stimulates the increase in photosynthetic rate in order to escape from the effects of the salt stress (Silva et al., 2014). Therefore, for the increase in photosynthesis to occur, there must be an increment in the number of electron acceptors (chlorophyll) combined with the increase in their activity (Taiz & Zaiger, 2013).

The genotypes LRF, LCRSTC and TSKC x (LCR x TR) - 040 did not suffer alterations in the chloroplast pigments or in their ratios, denoting that the deleterious effects of salinity on these genotypes are not related to the degradation of the contents of chlorophyll and carotenoids (Table 3). However, the genotype TSKC x CTARG – 019 showed reductions in the contents of chlorophyll a, b and total with the increase in salinity (Table 3). For Garcia et al. (1997), the high concentrations of sodium or chloride accumulated in the leaf tissues accelerate the process of leaf senescence and increase the synthesis of endoproteinases, which are responsible for the degradation of Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO), besides stimulating the synthesis of proteins responsible for the degradation and loss of chlorophyll, such as chlorophyllase.

Thus, the reduction of chlorophyll contents in the genotype TSKC x CTARG – 019 evidences the increase in the activity of endoproteinases and enzymes responsible for the degradation of photosynthesizing pigments in this genotype. This behavior is an indication that the effect of salt stress was higher than the capacity of the carotenoids to protect chlorophyll molecules in this genotype (Table 3).

Since carotenoids can act as antioxidant agents, Hernández et al. (2000) obtained the lipid membranes of the oxidative stress generated in plants exposed to salinity (Falk & Munné-Bosch, 2010). In addition, the contents of carotenoids in this genotype were lower than those in LVK,

which showed increase in chlorophyll contents under salinity conditions (Table 3). High contents of carotenoids in plants under conditions of high salinity may be a characteristic of genotypes tolerant to salinity.

For the chlorophyll a/chlorophyll b ratio, there were lower values in the genotypes LVK and TSKC x (LCR x TR) – 040 when subjected to the highest level of salinity (4.0 dS m^{-1}) (Table 3). According to Silveira et al. (2010), the reduction in the biosynthesis of chlorophylls can also be an adaptive response to a new stress condition, for the saving of energy and lower capture of light energy to avoid photo-oxidative stress. The reduction of chlorophyll a, combined with the increase in the contents of chlorophyll b, is due to the mechanism of its adjustment as accessory pigment, which captures light energy under atypical conditions regarding chlorophyll functioning. Hence, the reduction in the chloropyll a/chlorophyll b ratio can be a strategy to maintain the photosynthetic activity and send energy to the biochemical sites of the plant.

For the variable total chlorophyll/carotenoids, there was no significant effect of the salinity levels; however, the lowest ratios of total chlorophyll/carotenoids were observed in the genotypes LRF and LCRSTC, and it demonstrates that, in these genotypes, there is a greater proportion of carotenoids in relation to chlorophyll contents, in comparison to the others, which may have contributed to the absence of deleterious effects of salinity on the degradation of chlorophyll in these genotypes (Table 3).

Based on evaluation of leaf dry matter, there were reductions of phytomass accumulation in the genotypes TSKC x CTARG – 019 and LVK due to the increase in salinity from 0.3 to 4.0 dS m^{-1} (Table 3). This reduction is probably related to the decrease in leaf area and number of leaves under salt stress. This mechanism aims to decrease the transpiration rate and the absorption of water and salts by the plants, thus avoiding toxicity by specific ions (Syvertsen & García-Sánchez, 2014). Reductions of dry matter accumulations in citrus rootstocks under saline conditions were also reported by Fernandes et al. (2011), who also emphasize that the reduction is associated with the decrease in leaf production.

According to the data of stem dry matter (SDM), the increase in salinity reduced the dry matter accumulation in the genotype LRF by 93%, in the comparison between plants cultivated at the highest salinity level (4.0 dS m^{-1}) and those cultivated at the lowest level (0.3 dS m^{-1}) (Table 3). This fact can be related to the reduction in the formation of organic compounds, because, although no effect of salinity was observed on the contents of pigments in this genotype, there might have been inhibition in gas exchanges, limiting the formation and redistribution of photoassimilates to other plant parts, as observed by García-Sánchez & Syvertsen (2006), studying the growth of 'Carrizo' citrange and 'Cleopatra' mandarin under salinity.

The root dry matter of the genotypes was drastically reduced with the increase in irrigation water salinity from 0.3 to 4.0 dS m^{-1}, except for the genotype TSKC x (LCR x TR) – 040 (Table 3), which did not suffer effect of water salinity, confirming its potential. For Sá et al. (2013), the reduction in the root system with the increase in salinity can be related to the mechanisms of defense of the plant, such as the reduction in the absorption of toxic ions to allow the plant to tolerate the salt stress for a longer time, considering that the increase in the absorption of salts can cause, besides toxicity by specific ions, nutritional disorders in the plant (Epstein & Bloom, 2006).

Materials that can maintain root system growth even under saline conditions can, therefore, have higher efficiency in the exclusion of toxic ions by the roots. Hence, it is not necessary to reduce the root system under saline conditions (Rodriguez-Gamir et al. 2012; González et al. 2012), as observed in the genotype TSKC x (LCR x TR) – 040. Reinforcing this theory, Brito et al. (2015), studying the balance of salts in substrates of citrus seedlings under water salinity levels applied during the stages of rootstock formation until the seedling was ready for planting, observed that the highest concentration of salts, approximately 8.7 dS m^{-1}, in the electrical conductivity of the saturation extract of the substrate, did not limit the root system development of the more tolerant genotypes.

Regarding the relative water content, there was a reduction of 3.5% as a function of the increase in salinity, regardless of the studied rootstocks, which evidences a decrease in cell turgor caused by the reduction in the osmotic potential of the substrate. For Brito et al. (2008), this behavior is due to the decrease in transpiration and, therefore, in the formation of new tissues, having effects on the water potential of the cells and their turgor, affecting the formation of phytomass in the plants. However, although this reduction of phytomass was evident, it was more expressive in some genotypes, such as TSKC x (LCR x TR) - 040, which showed a slight variation in phytomass. This observation can reveal that the previously mentioned material is tolerant to salinity, a fact reinforced by its growth potential even under water salinity and reduction of turgor.

Conclusions

1. The increase in water salinity negatively affected the biochemical components and phytomass accumulation of the citrus genotypes.

2. The genotypes TSKC x (LCR x TR) – 040, LCRSTC and LVK were the least affected by the salt stress, standing out as the materials most tolerant to salinity.

3. The genotypes LRF and TSKC x CTARG – 019 suffered the greatest reductions in phytomass accumulation under salt stress and were more sensitive than the other genotypes studied.

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