

## RESISTANCE TO WATER FLOW, HYDRAULIC CONDUCTIVITY AND LEAF NUTRIENT CONCENTRATION AMONG *Theobroma cacao* L. GRAFTS

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*Theobroma cacao* L. is a perennial species of worldwide economic importance. In Brazil, in the last 25 year there was a decrease in the production of dried cacao beans caused by biotic and abiotic factors, especially cacao witches' broom disease. This malady is considered one of the most important diseases of cacao due to the enormous economic damage it causes to the crop. Installed in the Cacao Region of South Bahia, Brazil, for over two decades, cacao production decreased about 75%. Aiming to minimize the damage caused by this pathogen, production of seedlings with genetic tolerance/resistance is the most efficient and economical alternative to control the disease. Especially grafting resistant genotypes onto rootstocks is of wide use in the field. However, there are no studies specifically addressing the effect(s) of the interaction between scion and rootstock in cacao. The objective of this study was to verify and determine the interactions between scion and rootstock of cacao clones using resistance to water flow and leaf mineral concentration as diagnostic parameters. The evaluated seedlings were cleft grafted individuals. The clones used were CCN51, Eucalipto, UP and their respective combinations and three rooted stocks, with four replications. After grafting, the plants were grown in 5.0 L pots in a greenhouse and maintained under a drip automatic irrigation system. The experimental design was completely randomized. It was evaluated resistance to water flow and leaf mineral concentrations three months after grafting. The results showed a significant difference within and between grafted plants and control plants for resistance to water flow and concentration of minerals at the leaf level, suggesting that the grafting process changes these parameters, as a function of the genotype used. It was found that CCN51 showed to be an adequate rootstock.

**Key words:** cacao, interaction, scion, rootstock, nutrients.

**Resistência ao fluxo de água, condutividade hidráulica e concentração de minerais foliares entre enxertos de *Theobroma cacao* L.** *Theobroma cacao* L. é uma espécie perene de importância econômica mundial. No Brasil, nos últimos 25 anos houve uma diminuição na produção de amêndoas de cacau causada por fatores bióticos e abióticos, especialmente a vassoura de bruxa do cacau. Esta doença é considerada uma das mais importantes do cacau devido ao enorme dano econômico que causa à cultura. Instalada na Região Cacaueira do Sul da Bahia, Brasil, há mais de duas décadas, a produção de cacau diminuiu cerca de 75%. Visando minimizar os danos causados por este patógeno, a produção de mudas com tolerância/resistência genética é a alternativa mais eficiente e econômica para o controle da enfermidade. Especialmente a enxertia de genótipos resistentes sobre portaenxertos é de vasta utilização no campo. No entanto, não há estudos especificamente abordando o(s) efeito(s) da interação entre enxerto e portaenxerto em cacau. O objetivo deste estudo foi verificar e determinar as interações entre o enxerto e o portaenxerto de clones de cacau usando resistência ao fluxo de água e concentração de minerais foliares como parâmetros de diagnóstico. As mudas avaliadas foram indivíduos enxertados em fenda cheia. Os clones utilizados foram CCN51, Eucalipto, UP e respectivas combinações e três portaenxertos enraizados, com quatro repetições. Após a enxertia, as plantas foram cultivadas em vasos de 5,0 L numa estufa e mantidas sob um sistema de irrigação automático por gotejamento. O delineamento experimental foi inteiramente casualizado. Avaliou-se a resistência ao fluxo de água e às concentrações de minerais foliares três meses após o enxerto. Os resultados mostraram uma diferença significativa dentro e entre plantas enxertadas e plantas controle para resistência ao fluxo de água e concentração de minerais em nível foliar, sugerindo que o processo de enxerto altera esses parâmetros, em função do genótipo utilizado. Verificou-se que CCN51 mostrou ser um portaenxerto adequado.

**Palavras-chave:** cacau, interação, enxerto, portaenxerto, nutrientes.

## Introduction

The strong decrease of cacao production in the southern region of Bahia in the last 30 years resulted mainly by damages caused to cacao (*Theobroma cacao* L.) by *Moniliophthora perniciosa* (Aime and Phillips-Mora, 2005), causal agent of witches' broom (WB). The impact of the spread of the disease in this region is demonstrated by the production drop at the end of the 1990s compared to the 1980s. In the former decade, the production was near 400 thousand tons on average of dried cacao beans, while in the latter the yield decrease was about 75%, essentially due to the disease. Therefore, the control of WB has become a challenge for researchers working to minimize or eliminate the injuries caused by this pathogen.

Among the control strategies, one is the deployment of cacao genotypes with tolerance/resistance to the disease. The propagation of these materials is made through the use of plant propagules (cuttings and/or buds) rooted or grafted directly on adult plants or rootstocks. In cacao, the rootstock must include characteristics such as vigor, resistance or tolerance to root diseases and same physiological age of the graft (Bir et al., 2010), premises that are well accepted for crops as diverse as apples (Cummins and Aldwinckle, 1983) and grapes (Silva et al., 2007)

According Martinez-Ballesta et al. (2010), the vascular connection at the interface rootstock/scion determines the efficiency of water and nutrients translocation, affecting other physiological characteristics. Thus, the incompatibility between scion and rootstock can induce excessive growth or poor canopy, which may lead to decreased water and nutrients flow causing wilting and plant death (Davis et al., 2008). To date there are no published studies that specifically address the effect of interaction between scion and rootstock in the cacao crop.

Therefore, hypothesizing that the rootstock may influence physiological characteristics of the scion, a study was conducted to determine interactions among grafted clonal cacao seedlings using resistance to water flow, hydraulic conductivity and foliar nutrient concentrations as discriminatory variables to propose parameters to choose rootstocks for production of seedlings tolerant to witches' broom.

## Materials and Methods

The experiment was conducted in a greenhouse of the Cacao Research Center (CEPEC), main research unit of the Executive Commission for the Cacao Farming Plan (CEPLAC), located in Ilhéus, Bahia, Brazil (14°47'S, 39°16'W, 56 m asl). The regional climate is tropical *Af* type according to Köppen-Geiger classification (Köppen, 1936), with a well distributed annual rainfall between 1500–1750 mm, relative humidity around 80% and temperature ranging from 21.5 to 25.5°C (CEPEC/Climatology section).

The experiment was arranged in a completely randomized design. Combinations of scions and rootstocks of three cacao genotypes [*CCN-51*, *UP* and *Eucalypto* (EUC)] forming nine combinations, as well as controls without grafting, totaling 12 treatments with four replications. However, some combinations could not be analyzed due to plants losses. CCN-51, UP and EUC were obtained by rooted cuttings, *Cacao Comum* through seeds. CCN-51 and UP are clones of Trinitarian origin, EUC is a mutation with leaves similar as that of *Eucalyptus* ssp., *Cacao Comum* belongs to the Forastero group.

Four months-old seedlings were top wedge grafted and covered with plastic bags for 15 d or until the graft union was functional, observed through the emergence of the first leaves. After budding the plants were transplanted to 4.0 L plastic pots containing Plantmax® and coconut fiber (2:1) as substrate. Fertilization was performed with 25 g of Osmocot® (15-9-12), 25 g of PGmix® (14-16-18; 7.0% S, 0.03% B, 0.12% Cu, 0.16% Mn, 0.20% Mo) and 100 g of superphosphate (18% P<sub>2</sub>O<sub>5</sub>) for 60 kg of substrate. The plants were maintained in a greenhouse for 365 d under an automatic drip irrigation system. Irrigation consisted of daily applications of tap water to maintain the substrate water at around 70% of its maximum retention capacity.

Soil water potential was estimated by correlation between electrical conductivity and osmotic potential of sucrose (Brunini and Angelocci, 1998). Briefly, the electrical conductivity ( $\bar{n}$ ) of sucrose solutions in the molar concentrations of 0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50 was measured using a conductivity meter. The osmotic potential was obtained by applying van't Hoff's equation ( $\Psi_o = -R \times T \times C$ , where:  $\Psi_o$  is the osmotic potential,  $R$  the universal gas

constant,  $T$  the temperature and  $C$  solute concentration). The resulting equation ( $\Psi_o = -0.0817 - 0.0678 \bar{n}$ ;  $r^2 = 0.999$ ) was used to determine the water potential of the substrate as a function of its electrical conductivity. Briefly, pots were well watered for three days. At the end of the third day dripping water was collected, electrical conductivity measured and transformed to substrate  $\Psi$  using the above equation. The substrate  $\Psi$  values were subtracted from  $\Psi$  values of leaves of the scion obtaining a  $\Delta\Psi$ . Leaf  $\Psi$  was determined with a Scholander pressure chamber. Resistance to water flow was calculated using the equation  $Rw = (\Psi_s - \Psi_L)/E$ , where  $Rw$  denotes resistance to water flow,  $E$  transpiration rates,  $\Psi_s$  substrate water potential and  $\Psi_L$  leaf water potential (Brunini and Angelocci 1998). Transpiration rates,  $E$ , were measured with a LI-6400 (Li-cor Inc. Lincoln, NE, USA) portable photosynthetic system.

Determinations of leaf nutrient concentrations were made at the end of the experimental period. Scion leaf samples (second and third leaf from the top) of each treatment were collected, dried in a forced air circulation oven at 70°C for 72h, ground in a Wiley mill and stored in identified capped glass bottles. From the dried samples, 200 mg were weighed and submitted to nitroperchloric (2:1) digestion for Mg, Fe,

Zn, Cu and Mn determinations (atomic absorption spectrophotometry), P by colorimetry using the vitamin C method (Jackson, 1958), and K by flame emission photometry. Nitrogen content was determined by the Kjeldahl method, after sulphosalicylic digestion of leaf samples (Jackson, 1958), standard procedures at CEPEC's Physiology Laboratory. All the parameters were subjected to analysis of variance and discriminated by the Tukey test at 5% probability using the Statistical Analysis System (SAS, 1987).

## Results

**Resistance to water flow and hydraulic conductivity:** The methodology used to estimate soil water potential showed that the plants were in a well-watered environment with  $\Psi_s$  near to zero (Table 1). In general, higher values in the water potential differences ( $\Delta\Psi$ ) corresponded to higher resistances to water flow (Table 1;  $r = 0.71$ ,  $p < 0.01$ ). Although differences in resistance and  $\Delta\Psi$  values were found among well-watered cacao plants this may be due to the anatomical structure linked to the hydraulic part and of the varied dimensions of the root system and leaf area, which could be different among plants.

Table 1. Leaf ( $\Psi_L$ ) and substrate ( $\Psi_s$ ) water potentials, water potential differences ( $\Delta\Psi$ ) and resistance to water flow ( $Rw$ ) of *Theobroma cacao* L

Treatments	$\Psi_L$	$\Psi_s$	$\Delta\Psi$	$Rw$
	MPa			MPa mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup>
COMUM Seminal	-0.31b <sup>1</sup>	-0.0118a	0.257e	0.15f
CCN51 (rooted cutting)	-0.38h	-0.0136b	0.299b	0.19d
CCN51 X CCN51	-0.36f	-0.0181f	0.213l	0.12g
CCN51 X COMUM <sup>2</sup>	-0.36f	-0.0154c	0.253g	0.15f
CCN51 X EUC	-0.38h	-0.0166e	0.256f	0.15f
CCN51 X UP	-0.35e	-0.0163d	0.229i	0.15f
EUC (rooted cutting)	-0.34d	-0.0189g	0.182m	0.14f
EUC X CCN51	-0.46m	-0.0146c	0.365a	0.27a
EUC X EUC	-0.41l	-0.0174e	0.274c	0.18e
EUC X UP	-0.33c	-0.0142b	0.241h	0.18e
UP (rooted cutting)	-0.39i	-0.0146c	0.295b	0.30a
UP X CCN51	-0.40j	-0.0170e	0.269d	0.25b
UP X COMUM	-0.37g	-0.0169e	0.241h	0.22c
UP X EUC	-0.29a	-0.0178f	0.148n	0.12g
UP X UP	-0.39i	-0.0189g	0.222j	0.20d

<sup>1</sup> Means followed by the same letter in the same column do not differ according to Tukey's test ( $p < 0.05$ );

<sup>2</sup> Rootstock x scion

Among the control plants (rooted cuttings are considered control in this study), UP had one of the highest value of  $\Delta\Psi$  and hence higher  $R_w$  value (Table 1), confirming results found by Brunini and Angelocci (1998). Interestingly, lower  $R_w$  values were also found in UP combinations, probably showing scion:rootstock interaction. Same behavior was shown by the CCN-51 group. Within the CCN group, the control (CCN51 rooted cutting) showed the higher  $R_w$  value while in its combinations  $R_w$  values were lower. On the other hand, the EUC control showed the lower  $R_w$  values, while its combinations higher. In fact, among grafted seedlings, the combination EUC x CCN51 was the scion that presented the highest  $R_w$ .

**Foliar nutrients:** In general the leaf nutrient concentrations determined in this study are within the ranges found in productive adult cacao trees (Chepote et al., 2012). Concentrations of N, P, K, Mg, Fe and Cu were within the ranges accepted as adequate for cacao production; however, Zn and Mn were, according to ranges presented by Chepote et al. (2012), excessively high.

Significant differences among treatments were found for all the determined nutrients (Table 2) according to Tukey test ( $p < 0.05$ ), probably due to the influence of rootstocks in the nutrient absorption. Furthermore, significant differences were found for all nutrients in all combinations within groups (Tables 2-5).

Leaf N concentration showed statistical differences being the combination CCN51 x COMUM the highest (29.8 g N kg<sup>-1</sup> DW) and CCN51 x UP (24.1 g N kg<sup>-1</sup> DW) the lowest. Differences were also found in leaf P concentration for which only part of the crosses showed high P concentrations. The highest (2.67 g P kg<sup>-1</sup> DW) and lowest (1.64 g P kg<sup>-1</sup> DW) values were found in the CCN51 group (Table 2). Same behavior was also found in the UP group (Table 2). Regarding leaf K concentration values the CCN51 group presented the highest and the lower values in their combinations, being the control (CCN51 rooted cutting) the lowest among treatments (Table 2). Same performance was also found in the EUC and UP groups (Table 2).

The CCN51 group also showed variability with the highest and lowest leaf Mg concentration values among their combinations. The EUC x UP combination showed the highest Fe concentration values, while the UP x UP, CCN51 x UP and CCN51 x COMUM combinations the lowest. Same behavior was also found regarding leaf Zn concentration, that is, the CCN51 group showed higher and lower values within CCN51 combinations and the UP x UP, CCN51 x UP and CCN51 x COMUM combination the lowest leaf Zn concentration values. The lower values of leaf Cu concentrations were found in rooted cutting of EUC and in EUC x CCN51 and UP x CCN51 combinations. The highest values were found in the UP group (Table 2).

Table 2. Mean values of mineral nutrients in leaves of grafts of *Theobroma cacao* L.

Treatments	N	P	K	Mg	Fe	Zn	Cu	Mn
	g kg <sup>-1</sup> DW				mg kg <sup>-1</sup> DW			
COMUM Seminal	25.9g <sup>1</sup>	1.90j	17.3f	6.67g	106f	231c	13.1b	1862a
CCN51 (rooted cutting)	28.0e	1.79l	14.2j	7.27e	111e	230d	13.1b	862g
CCN51 X CCN51	28.0e	1.64n	15.3h	8.60c	111e	299b	10.7c	1010d
CCN51 X EUC <sup>2</sup>	28.3d	2.67a	20.3a	12.2a	151b	317a	13.1b	6286j
CCN51 X UP	24.2i	2.07d	18.4d	3.80n	40j	46m	10.7c	79n
CCN51 X COMUM	29.8a	2.31c	17.8e	3.77o	45i	44n	10.7c	381m
EUC (rooted cutting)	25.5h	1.98g	16.1g	6.58h	116d	135l	8.28d	948e
EUC X CCN51	28.0e	1.95i	14.7i	7.64d	111e	214f	8.28d	1638b
EUC X EUC	28.7c	1.96h	20.3a	6.42i	126c	144j	10.7c	887f
EUC X UP	29.7b	2.01f	16.1g	5.94l	177a	206g	13.1b	726i
UP X UP	28.3d	2.04e	19.8c	3.81m	40j	43o	13.1b	76o
UP X EUC	25.9g	1.64n	14.2j	9.05b	85g	218e	17.9a	443l
UP X COMUM	27.3f	2.32b	14.7i	6.16j	80h	193h	17.9a	1448c
UP X CCN51	28.0e	1.77m	20.0b	7.08f	106f	174i	8.28d	751h

<sup>1</sup> Means followed by the same letter in the same column do not differ according to Tukey's test ( $p < 0.05$ );

<sup>2</sup> Rootstock x scion

Regarding leaf Mn all values were high within the accepted concentrations for well-nourished plants. However, significantly lower Mn values were found in the CCN51 x UP and UP x UP combinations.

Statistical differences were also found for all the determined nutrients in all combinations (Tables 3-5). Within the CCN51 group the combination CCN51 x UP showed the lowest leaf N concentration. In fact, this combination showed the lowest concentration values for Mg, Fe, Zn, Cu and Mn. On the other hand, the combinations CCN51 x EUC showed the highest values of all the determined nutrients. In the other combinations, some nutrients showed high or low values reflecting the effect of the interaction rootstock x scion (Table 3).

Similarly, in the EUC group (Table 4), the combination EUC x UP showed the highest values for almost all nutrients (except Mg and Mn). In contrast, the combination EUC x CCN51 showed lower values for all nutrients except Mg, Zn and Mn (Table 4). Interestingly, in the UP group, all combinations and the control show higher and lower values for determined nutrients within each combination. For example, the combination UP x COMUM shows higher values of P, Zn, Cu and Mn but lower values of N, K, Mg and Fe. Contrary to this combination the treatment UP x

CCN51 shows N, K, Mg and Fe with high values and lower values for P, Zn, Cu and Mn (Table 5).

## Discussion

Resistance to water flow and hydraulic conductivity: Hydraulic conductivity is the property that describes the affluence with which a fluid (usually water) can move through pores. According to Taiz and Zeiger (2004), hydraulic conductivity describes how readily water can move through a membrane. The magnitude of the resistance to water flow in the soil-plant-atmosphere continuum, along with the difference in water potential, are the factors that govern the movement of water within a system.

According to Lacerda (2007), the highest resistance coincides with the higher water potential difference that exists between the walls of mesophyll cells and the outside air. He concludes that the limiting factor for the movement of water through the plant is the resistance to water movement from the cell walls into the intercellular spaces, substomatic chamber, stomata and the water vapor layer adjacent to the leaf. Therefore, transpiration is fundamental to water movement through the soil-plant-atmosphere system (Lacerda, 2007).

Table 3. Mean values of mineral nutrients in leaves of the grafts of the CCN51 group

Treatments	N	P	K	Mg	Fe	Zn	Cu	Mn
	g kg <sup>-1</sup> DW				mg kg <sup>-1</sup> DW			
COMUM Seminal	25.9d <sup>1</sup>	1.90d	17.3d	6.67d	106c	231c	13.1a	1862b
CCN51 (rooted cutting)	28.0c	1.79e	14.2f	7.27c	111b	231d	13.1a	862d
CCN51 X CCN51	28.0c	1.64f	15.3e	8.6b	111b	299b	10.7b	1010c
CCN51 X COMUM <sup>2</sup>	29.8a	2.31b	17.8c	3.77f	45d	44f	10.7b	381m
CCN51 X EUC	28.3b	2.67a	20.3a	12.2a	151a	317a	13.1a	6286a
CCN51 X UP	24.2e	2.07c	18.4b	3.80e	40e	46e	10.7b	79e

<sup>1</sup> Means followed by the same letter in the same column do not differ according to Tukey's test ( $p < 0.05$ );

<sup>2</sup> Rootstock x scion

Table 4. Mean values of mineral nutrients in leaves of the grafts of the EUC group

Treatments	N	P	K	Mg	Fe	Zn	Cu	Mn
	g kg <sup>-1</sup> DW				mg kg <sup>-1</sup> DW			
EUC (rooted cutting)	25.5d <sup>1</sup>	1.98b	16.1b	6.58b	116c	1351d	8.28c	948b
EUC X CCN51 <sup>2</sup>	28.0c	1.95d	14.7c	7.64a	111d	214a	8.28c	1638a
EUC X EUC	28.7b	1.96c	20.3a	6.42c	126b	144c	10.7b	887c
EUC X UP	29.7a	2.01a	16.1b	5.94d	177a	206b	13.1a	726d

<sup>1</sup> Means followed by the same letter in the same column do not differ according to Tukey's test ( $p < 0.05$ );

<sup>2</sup> Rootstock x scion

Table 5. Mean values of mineral nutrients in leaves of the grafts of the UP group

Treatments	N	P	K	Mg	Fe	Zn	Cu	Mn
	g kg <sup>-1</sup> DW				mg kg <sup>-1</sup> DW			
UP X CCN51 <sup>2</sup>	28.0b <sup>1</sup>	1.77c	20.0a	7.08b	106a	174c	8.28c	751c
UP X COMUM	27.3c	2.32a	14.7c	6.16c	80c	193b	17.9a	1448b
UP X EUC	25.9d	1.64d	14.2d	9.05a	85b	218a	17.9a	4431a
UP X UP	28.3a	2.04b	19.8b	3.81d	40d	43d	13.1b	76d

<sup>1</sup>. Means followed by the same letter in the same column do not differ according to Tukey's test ( $p < 0.05$ );

<sup>2</sup>. Rootstock x scion

On the other hand, Brunini and Angelocci (1998) report that the difference in resistance to water flow depends on the anatomical structure connected to the hydraulic part and the size of the root system and leaf area. This information gives grounds to conclude that the grafting technique can give different values of resistance to water flow due to anatomical differences between scion and rootstock of *T. cacao* genotypes. That is, different sizes of the conducting vessels of the selected genotypes as scion and as rootstock may result in different values of resistance to water flow.

The good connection between graft and rootstock is essential for the best plant growth and the absorption and transport of water and nutrients. Insufficient connections decrease the water flow (Torii et al., 1992).

When the water uptake by roots is suppressed in the scion:rootstock interface, stomatal conductance and canopy growth decrease (Atkinson and Else, 2001; Oda, Maruyana and Mori, 2005). Thus, hydraulic architecture becomes of fundamental importance, since the constant water flow controls many processes such as growth, mineral nutrition, photosynthesis and transpiration (Atkinson et al., 2003).

Within groups, CCN51 as a rootstock seems to adequately interact with the genotypes uses as scion in this study. Overall, this group shows the lower  $R_w$  value. Furthermore, the combinations studied actually showed lower values of  $R_w$  than the control plant without graft. Therefore, CCN51 is a suitable rootstock forming compatible interactions with the scion. On the other hand, differences in compatibility, inferred by the magnitude of the  $R_w$  values, which in turn shows that interactions rootstock:scion are presented in the EUC group. In this group relatively lower  $R_w$  values are shown by all combinations but EUC x CCN51. Contrary to the EUC group in the UP group all combinations but UP x EUC show high  $R_w$  values, which shows that this clone may

not be suitable for use as a rootstock. On the other hand, it can be inferred that CCN51, used as scion, should be combined with a compatible rootstock, probably with similar diameter of the conducting vessels. Kawaguchi et al. (2008) observed that the growth inhibition and high mortality of tomato/peppers and peppers/tomato grafts were due to discontinuities in the vascular bundles at the graft union. Measurements of hydraulic root conductance made below and above the grafting region indicated that the union between rootstock:scion in compatible grafts do not form barriers to the passage of water (Kawaguchi et al., 2008) as may be the case for the CCN51 X CCN51 and UP x EUC combinations. These combinations showed the lower resistance to water flow (Table 1).

As a rootstock CCN51 showed compatible interaction (low  $R_w$  values) with all combinations tested. On the other hand, by our results, UP should not be used as rootstock due to its low performance (high  $R_w$  values) in almost all combinations tested. Or, before use of this clone as a rootstock preliminary test should be done to assess compatibility as demonstrated by the combination UP x EUC, which showed low  $R_w$  values.

It was expected that control plants, that is, plants not grafted would show similar values of resistance ( $R_w$ ) than plants grafted onto them. In general, this was not the case. The results show that in two of the three full comparisons the grafted plants presented lower  $R_w$  values than plants without grafts (Table 1), demonstrating the interaction scion x rootstock. The high  $R_w$  value of the combination EUC x EUC in relation to EUC without graft can be justified by a provable lack of alignment among vessels. Several explanations for the lower  $R_w$  values of CCN51 x CCN51 and UP x UP compared to their controls (without grafts) can be given, however, without prove

in this paper. One of such proves should be anatomical studies of the scion:rootstock interface.

**Foliar nutrients:** The leaf nutrient concentrations found in this study are within the ranges reported in the literature (Chepote et al., 2012) for most nutrients except Zn and Mn. Although Zn and Mn in excess can cause growth reduction, visually our plants did not show any sign of toxicity. Cruz Neto et al. (2015) reported concentrations of Zn as high as the values found in this study with growth reduction however.

Although, the unusually high or low concentrations of Mn (Table 2) found in this work, values that could represent toxicity or deficiency, neither symptom was visually shown by the plants. Manganese is an important mineral cofactor and has important role in water splitting in photosystem II of photosynthesis (McEvoy and Brudvig, 2006), as well as, plant defense mechanisms (Dordas, 2008). The influence that rootstocks has on the mineral content in the shoots has been attributed to physical characteristics of the root system, such as lateral and vertical development, which results in higher absorption of water and minerals (Heo, 1991; Jang, 1992). This is one of the main reasons for the widespread use of rootstocks. In our study the influence of scion:rootstock can be seen by the different leaf nutrient concentrations as a function of the combination within each rootstock group.

Tagliavani, Bassi and Maragoni (1993) have suggested that the strength of both scion and rootstock have an important role in the absorption and translocation of nutrients in grafted fruit trees. However, in other grafted plants the rootstock influences the leaf content of certain essential minerals (Brown, Zhang and Ferguson, 1994). In this sense, the effect of three different rootstocks on the leaf contents of melon plant macronutrients (*Cucumis melo* L) were tested by Ruiz et al. (1997). They concluded that, the rootstocks and the interaction effect between rootstocks and scions determined fruit yield, showing differences between control and grafted plants and within grafted plants themselves. In addition to the scion:rootstock interaction, the N content depends on the environmental conditions in which the plants grow (Martínez-Ballesta et al., 2010). Furthermore, it has been reported that rootstocks may improve some morphological and physiological traits or increase the absorption and translocation of phosphorus (P) to the leaf canopy (Ruiz, Balakbir and Romero,

1996). It was also demonstrated by Ruiz et al. (1997) that the concentration of P in grafted melon plants may be affected similarly by the scion and the scion:rootstock interaction. However, Kawaguchi et al. (2008) concluded that the rootstocks genotypes were the main factor affecting the uptake and translocation of this element in Solanaceae graft combinations. According to Martínez-Ballesta (2010) the rootstock exerts significant influence on nutrient content, corroborating Kawaguchi et al. (2008) results. In our study either rootstock (CCN51 X UP vs UP X CCN51 or UP x EUC vs EUC x UP) or scion (most combinations) affect the translocations of this most important element. Even though, according to Chepote et al. (2012) the concentrations are within the accepted reported ranges for this element (Table 2 to 5).

It should be notice that Cacau Comum is also presented in all tables as an illustration. This variety and its selections were until the 1990's the most cropped varieties given their grain quality, resistance to *Ceratocystis* wilt and widespread use as rootstock. After the entrance and dispersion of witches' broom (Pereira et al., 1989) the cropping area of these genotypes decreased given their susceptibility to the fungus *M. perniciosa* causal agent of the disease. However, it can be observed that it shows low resistance to water flow and adequate leaf nutrient concentrations, which made it the most widespread genotype used as rootstock or for seed production.

According Tomaz et al. (2003), absorption, transportation and redistribution of nutrients show genetic control. Studies in several fruit species, in which the grafting process has been studied for some time, have demonstrated the positive influence of grafting on the absorption and mineral composition of plants (Smith, 1975; Economides, 1976; Lima, Michan and Salibe, 1980; Genú, 1985). This is the case on most combinations tested in this study.

## Conclusions

Our results show that depending upon the genotype used as rootstock the absorption and translocation of nutrients are increased or decreased. In this work CCN51 showed to be, for most combinations, a better rootstock than the other genotypes tested. The UP genotype, as a rootstock, showed higher resistant to

water movement and lower concentrations of nutrients for most combinations, therefore, is not recommended as a rootstock.

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