RESEARCH ARTICLE

An Integrated View of Whole-Tree Hydraulic Architecture. Does Stomatal or Hydraulic Conductance Determine Whole Tree Transpiration?

Juan Rodríguez-Gamir, Eduardo Primo-Millo, María Ángeles Forner-Giner*

Department of Citriculture and Vegetal Production, Valencian Institute of Agrarias Research, Moncada, Valencia, Spain

* former_margin@gva.es

Abstract

Hydraulic conductance exerts a strong influence on many aspects of plant physiology, namely: transpiration, CO_2 assimilation, growth, productivity or stress response. However we lack full understanding of the contribution of root or shoot water transport capacity to the total water balance, something which is difficult to study in trees. Here we tested the hypothesis that whole plant hydraulic conductance modulates plant transpiration using two different seedlings of citrus rootstocks, *Poncirus trifoliata* (L.) Raf. and Cleopatra mandarin (*Citrus reshni* Hort ex Tan.). The two genotypes presented important differences in their root or shoot hydraulic conductance contribution to whole plant hydraulic conductance but, even so, water balance proved highly dependent on whole plant conductance. Further, we propose there is a possible equilibrium between root and shoot hydraulic conductance, similar to that between shoot and root biomass production, which could be related with xylem anatomy.

Introduction

Transpiration in plants plays a critical role in plant physiological processes, affecting carbon uptake by leaves, growth and productivity and depends on plant hydraulics, stomatal conductance and/or environment conditions [1, 2, 3, 4]. Plant hydraulics constrains ecosystem productivity by setting physical limits to water transport [5, 6], thus its study is essential to understanding plant water-use regulation and its associated impact on water balance.

Hydraulic conductance can be variable throughout the day [7] or in response to environmental conditions [8, 9]. Besides, maximum transpiration rates have been related to maximum values of whole plant hydraulic conductance [10]. Studies suggest that hydraulic conductance of different plant organs can regulate the opening and closing of stomata in response to vapor pressure deficits [11, 12], regulating, thus, plant water relations. In this respect, there are existing studies that link hydraulic conductance of leaf [13, 14, 15, 16, 17, 18, 19], shoot [20], stems [21, 22] or root [23, 24, 25] with transpiration or stomatal conductance. However, there is a

lack of understanding about the integration of whole woody plant hydraulics and its implica-
tion in water relations. As [26] state, few studies have examined all the organ components of
the hydraulic pathway to evaluate how they relate to whole plant conductance and most work
on hydraulic transport of woody plants has been conducted on lateral stems or branches.
Regarding different organ contribution, [11, 27, 28] propose that approximately 50–60% of the
whole-plant hydraulic resistances are located in the root system. [10] found higher values of
shoot hydraulic conductance compared with root hydraulic conductance values in sunflower.
However on kiwifruit plants, root hydraulic conductance was higher than shoot hydraulic con-
ductance [29]. It is evident that there is inconsistent information on the contribution of root or
shoot hydraulic conductance to total hydraulic conductance and, more so, to plant
transpiration.

Most studies on whole plant hydraulic architecture parameters with exchange parameters
use the evaporative flux method [30]. This method assumes the hydraulic conductance of the
soil/root/leaf pathway is $K = E/\Delta p$ (Kg s^{-1} MPa^{-1}), where Δp is the difference in the water poten-
tial between the two considered points (e.g. between soil water potential and leaf water poten-
tial), and E is the amount of water lost through transpiration at the time plant water potential
is determined. The method has been validated [31], however, it makes possible to establish bet-
ter relationships between gas exchange parameters and hydraulic conductance. In our study,
we measured hydraulic conductance with a high-pressure flow meter (HPFM), therefore
hydraulic trait measurements were totally independent of gas exchange measurements.

Furthermore, plant hydraulics is a complex system and its regulation is complex as well. It
can depend on root anatomy, interactions between water and solute flow or on aquaporin
activity in cell membranes [9, 32, 33, 34]. Many studies indicate stem or root hydraulic conduc-
tance in trees are related with xylem structure [35, 36]. And also, plants have the ability to
adjust their water uptake capacity to changing environmental conditions by regulating aqua-
porins in the plasma membrane [37].

Taking all this into account, we hypothesized that whole plant hydraulic conductance mod-
ulates plant transpiration. Accordingly, we tested whether plants that differ in their contribu-
tion of root and shoot hydraulic conductance to whole plant hydraulic conductance also differ
in their hydraulic conductance-transpiration relationship. We also studied the influence of
xylem anatomy on root and shoot hydraulic conductance, as well as its possible effect on differ-
ent plant-part contribution to plant hydraulic conductance.

To do so, we used seedlings of two different citrus rootstocks *Poncirus trifoliata* (L.) Raf.
(PT) and Cleopatra mandarin (*Citrus reshni* Hort ex Tan.) (CM). Citrus rootstocks present dif-
ferent hydraulic characteristics that determine tree behavior, water relations or response to dif-
ferent stresses. *Poncirus trifoliata* and CM differ greatly at both physiological and
morphological levels. Cleopatra mandarin is characterized by simple leaves versus PT that
presents trifoliate leaves, smaller than those of CM. In previous studies, PT showed higher tran-
spiration rates than CM, both in seedlings and in varieties grafted on them [23, 38, 39], and
higher root hydraulic conductance values than CM [23, 38, 40]. To date studies of shoot
hydraulic conductance of these genotypes are lacking.

Material and Methods

Plant Material and growth conditions

Ten-month-old seedlings of *Poncirus trifoliata* (PT) and Cleopatra mandarin (CM) were used
in the experiment. PT and CM seeds were harvested from the mother seed trees held in the
germplasm collection at IVIA, Valencia, Spain. Seeds were sown on 55x40 cm trays containing
a mixture of peat and siliceous sand (3:2 v:v) in an aphid-proof greenhouse with a cooling

system that kept temperatures between 15°C and 18°C and 80% relative humidity. Plants were grown with supplementary light (<50 μmol m^{-2} s^{-1}, 400–700 nm) to extend the photoperiod to 16 h. Five-month-old plants were transplanted into 3 L pots with a mixture of peat and siliceous sand (3:2 v:v) and were watered twice a week with the following nutrient solution: 3 mMCa(NO$_3$)$_2$, 3 mM KNO$_3$, 2 mM MgSO$_4$, 2.3 mM H$_3$PO$_4$, 17.9 mM Fe-EDDHA and micronutrients as described by [41]. The pH of the nutrient solution was adjusted to 6.0 with 1 M KOH or 1 M H$_2$SO$_4$.

At the beginning of the experiment, sixteen plants of each genotype with no branches, (i.e., all leaves were on the main stem) and with different levels of development, were selected. Plants were selected for the experiment according to individual whole plant transpiration (measured gravimetrically before to the experiment, as described below) as we wanted to have a wide range of whole plant transpiration values and wanted this range to be similar for both genotypes. Selected CM plants had between 17 and 77 leaves and PT plants had 15 to 80 leaves. The experiments were conducted at the IVIA, Valencia, Spain (39.28 N—0.22 W) during late summer, with a photoperiod of 14 daylight hours. The plants were outdoors where average temperature was 24 ± 1°C and relative humidity, 80%.

During the experiment, plants were contained individually in the aforementioned 3 L pots and irrigated with 1.5 L water daily. The experiment lasted 5 days and plants were randomly distributed and surrounded by a guard row (buffer) not included in the experiment.

Whole plant transpiration (T_p)

From day 1 to 3, to measure whole plant transpiration, after each irrigation pots were covered with a sheet of plastic with a hole of similar diameter to that of the plant stem, through which the plant protruded. This system prevented evaporation from the substrate. The daily transpiration of each plant (T_p) was calculated as the difference between the weight of the watered pot (after draining) and the weight of the pot before watering the following day. The mean of three determinations (one per day) on each plant was considered as representative of each individual plant

Gas exchange and vapor pressure deficit

Transpiration rate (E, $mmolH_2O\ m^{-2}\ s^{-1}$) and stomatal conductance (g_s, $mmolH_2Om^{-2}\ s^{-1}$) were measured on days 2 and 3, recording conditions of ambient light, temperature, relative humidity and CO$_2$ concentration with an LCi Portable Photosynthesis System (ADC, herts, UK). The measurements were taken from 8:00 am to 6:00 pm. Air and leaf temperatures and photosynthetic photon flux density, provided by the LCi ranged from 19.1 to 32.2°C and 101.5 to 1971.7 μmol m^{-2} s^{-1}, respectively. Both parameters reached their maximum values between 10:00 am and 3:00 pm. The E and g_s were measured in fully expanded leaves taken from the central part of each plant. At each sampling time, six different leaves were measured in six different plants for each type plant.

Leaf-to-air vapor pressure deficit (VPD) is given by $VPD = e_s - e_{air}$, where e_{air} is the partial pressure of water vapor in the air, while e_s is the saturation water vapour pressure as it is assumed that water vapor pressure in the substomatal cavity is close to 100% [42]. e_{air} was provided by the LCi. e_s was calculated by: $e_s = 6,1373\ 10^{-3}\ e^\wedge(T_{leaf}*(18,568-T_{leaf}/254)/(T_{leaf}+255,57))$, obtained by the numerical integration of Clausius-Clapeyron equation, where e_s is the saturation water vapour pressure (bar) and T_{leaf} is the leaf temperature (°C) measured with the LCi.

Root, Shoot and Plant hydraulic conductance

On day 4 and 5 of the experiment, root hydraulic conductance (K_r, $KgH_2O\ MPa^{-1}\ s^{-1}$) and shoot hydraulic conductance (K_s, $KgH_2O\ MPa^{-1}\ s^{-1}$) were measured in each experimental plant

with a High Pressure Flow Meter (HPFM) (Dynamax Inc., Houston, TX). We followed the method described by [43]. To minimize the potential impact of diurnal periodicity on hydraulic conductance [10], all measurements were taken between 10:00 am and 2:00 pm at ambient temperature ($24 \pm 1°C$).

The plants were cut 5 cm above the soil surface and the stumps of shoots and roots were connected to the HPFM with a water-tight seal, and the conductance of these plant parts (K_s and K_r) was determined using the transient measurement mode. Plant hydraulic conductance (K_p) was calculated by the equation $1/K_p = 1/K_r + 1/K_s$.

At the end of the measurements, the leaf areas of each plant were measured with a Li-Cor Li-3100 Area Meter (Li-Cor, inc. Lincoln, Nebraska, USA). All plant fractions were dried in a forced-draft oven at 60°C for 48 hours and weighed, and we calculated the Leaf dry weight/ Root dry weight ratio (L/R).

We calculated leaf-specific root hydraulic conductance (K_{r-l}) and leaf-specific shoot hydraulic conductance (K_{s-l}) by dividing K_r or K_s, respectively, by the total plant leaf area. Root biomass-specific root hydraulic conductance (K_{r-r}) was calculated by dividing K_r by the root dry weight.

Light microscopy

Samples of about 3 mm of pioneer roots and 3×2 mm of wood from basal stem and taproot wood from seedlings were fixed, dehydrated and embedded in LR White (London Resin Co., Woking, Surrey, UK) according to [44]. Semi-thin transverse sections (1 μm thickness) were cut with a Leica RM2165 Rotary Microtome (Leica Instruments, Heidelberg, Germany) and stained with toluidine blue 0 (CI 52040, Merck, Darmstad, Germany) according to O'Brien et al. (1964). Representative sections of two tissue samples per plant and organ part (basal stem and taproot wood) from six independent plants of each seedling were examined and photographed with an Olympus BX-51 microscope (Olympus Imaging Corp., Tokyo, Japan), with a digital camera DP-12 and the Analysis program (Soft Imaging System GmbH, Munster, Germany). The anatomical data correspond to the mean of six independent plants on each rootstock. Three samples per tissue and plant were studied, and the average values were considered as representative of each individual plant. For each sample, values are the mean of three visual fields from three sections.

Statistical Analyses

Although plant development differed, data for biomass, whole plant transpiration and hydraulic conductance showed a normal distribution. Therefore, in a first analysis, parameters were tested by analysis of variance (ANOVA) and comparisons of means were determined by the least significant differences (LSD) method, at 95% confidence level with Statgraphics Plus ver. 5.1 (Statistical Graphics, Englewood Cliffs, NJ, USA). Additionally, slopes and intercepts of the performed linear regressions were compared between genotypes using the same software application.

Results

Plant biomass and whole plant transpiration

We found morphological differences between the two types of plant (Table 1). This was due, in part, to the fact that Cleopatra mandarin (CM) is characterized by simple leaves while *P. trifoliata* (PT) presents smaller trifoliate leaves. Both genotypes were similar in terms of root system development. However, significant differences in foliar biomass values were found, being

Table 1. Dry weight of leaf (DW leaves) and roots (DW roots), leaf dry weight/root dry weight ratio (L/R), and whole plant transpiration (T_p) in *P. trifoliata* (PT) and Cleopatra mandarin (CM).

|	DW Leaves	DW Roots	L/R	T_p
	(g)	(g)		g d^{-1}
PT	1.55 ± 0.19 b	3.70 ± 0.31 a	0.44 ± 0.05 b	39.71 ± 1.20 a
CM	5.66 ± 0.68 a	3.68 ± 0.46 a	1.65 ± 0.15 a	38.07 ± 1.16 a

For each parameter, means (n = 16) with different letters show statistically significant differences (P<0.05).

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3.65-fold higher for CM than for PT plants, which increased the Leaf dry weight/Root dry weight ratio (L/R) for CM compared with PT (3.72-fold higher in CM). Although CM had a higher foliar biomass than PT, whole plant transpiration (T_p) values were similar in both plant groups. There was a positive and curvilinear correlation between leaf biomass and T_p for both genotypes (Fig 1).
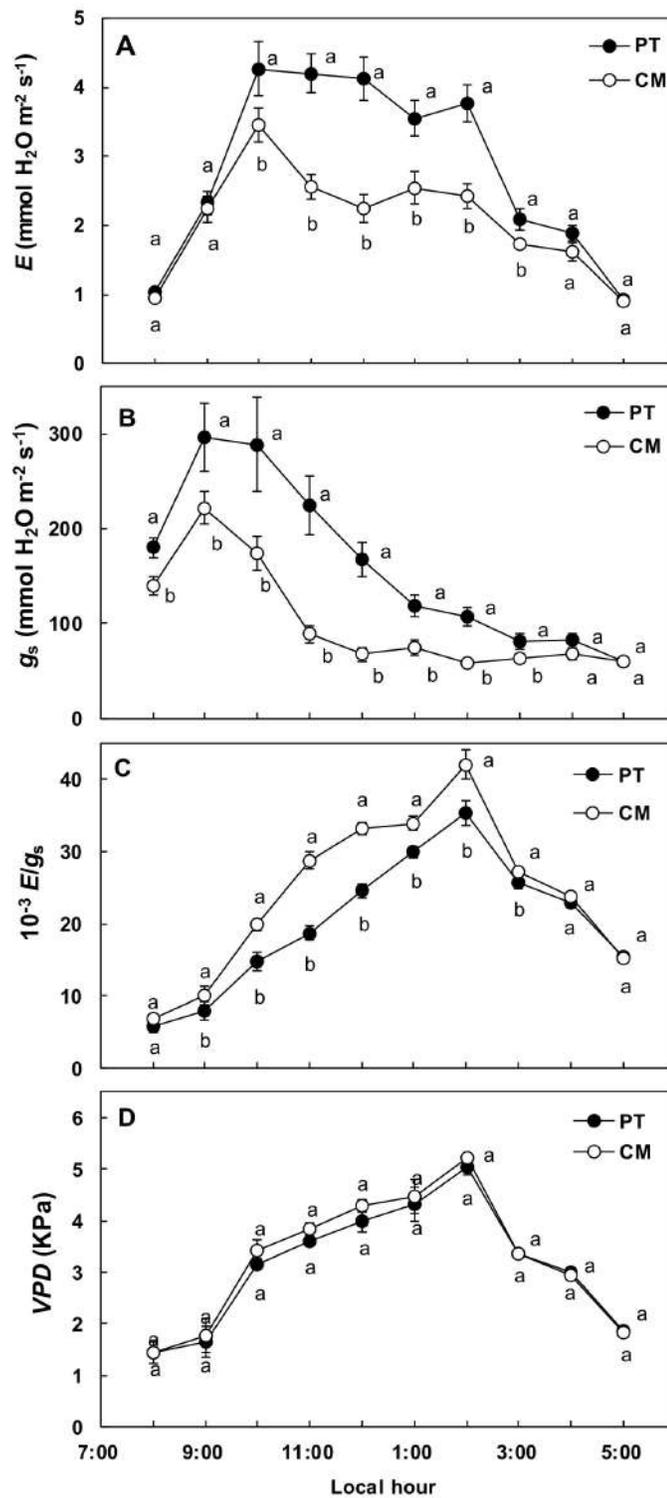
Gas exchange and vapor pressure deficit

Fig 2A shows the diurnal variations of transpiration rate (*E*) in leaves from plants of the two genotypes. Transpiration rate displayed typical behavior, increasing in the early morning (8:00 to 10:00 am) and then decreasing after 2:00 pm. Leaves from PT showed significantly higher *E*
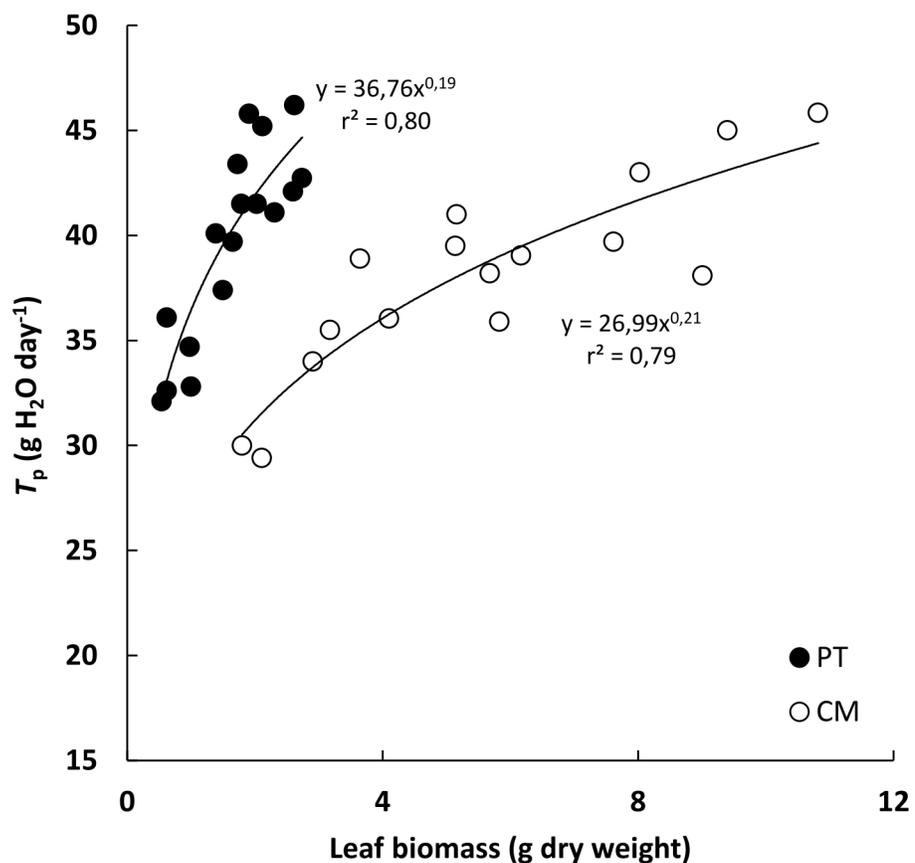
Fig 1. Relationship between foliar biomass and whole plant transpiration (T_p) in *P. trifoliata* (PT) and Cleopatra mandarin (CM). Each point represents the mean of three measurements of T_p.

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Fig 2. Diurnal time courses of stomatal conductance (g_s), transpiration (E), E/g_s ratio and vapor pressure deficit (VPD) in *P. trifoliata* (PT) and Cleopatra mandarin (CM). Values are means of six replicates ± SE (n = 6). For each time, different letters indicate statistically significant differences (P <0.05) (LSD test).

values throughout the day than leaves from CM (average E value between 10:00 am and 2:00 pm was 3.98 mmol H_2O m^{-2} s^{-1} for PT and 2.68 mmol H_2O m^{-2} s^{-1} for CM). Stomatal conductance (g_s) (Fig 2B) differed with respect to E trend. Maximum g_s values were reached at 9:00 am in both genotypes and stomatal closure occurred from 10:00 am. CM presented significantly lower g_s values than PT and there was not a constant relationship between E and g_s during the day (ratio E/g_s) (Fig 2C), which also differed between genotypes. CM had higher E/g_s values than PT between 9.00 am and 3.00 pm, despite the vapor pressure deficit (VPD), driving force for water loss by via transpiration, showed a similar pattern throughout the day for both genotypes with no statistical differences between them (Fig 2D). This suggests transpiration was not solely regulated by stomatal conductance.
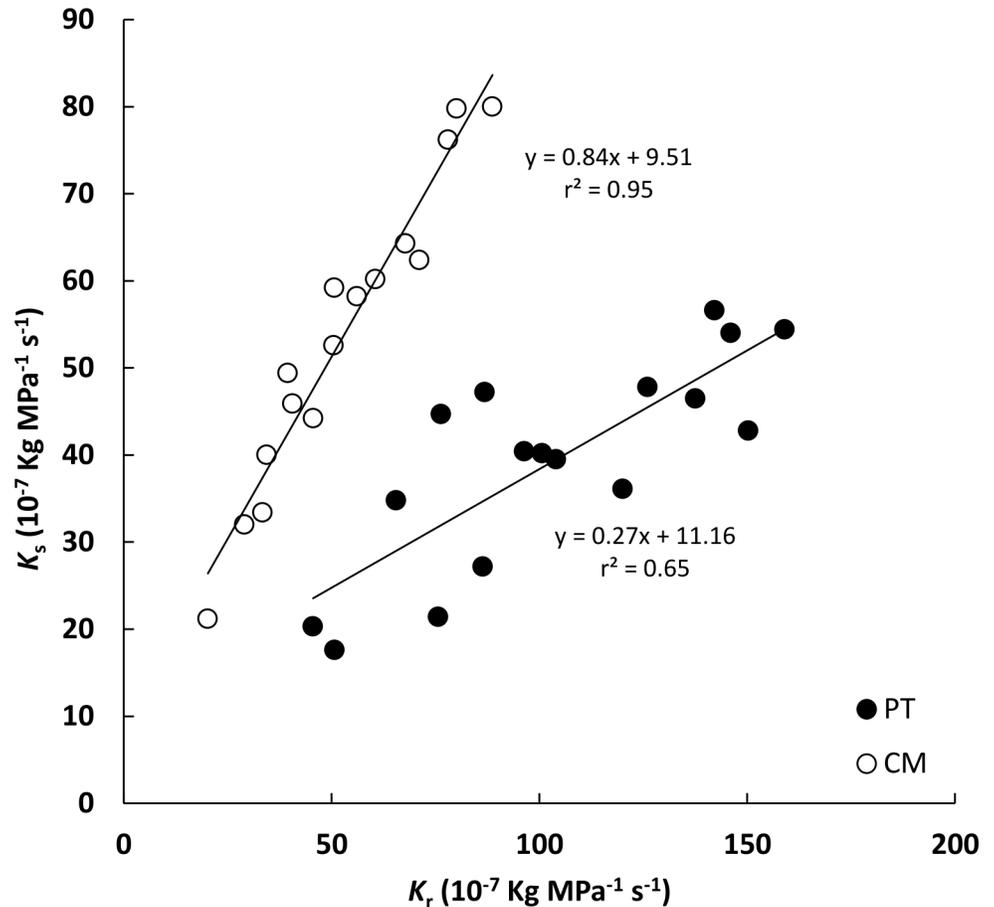
Root, shoot and plant hydraulic conductance

The average value of root hydraulic conductance (K_r) was significantly higher in PT than in CM (104.03 10^{-7} and 52.87 10^{-7} Kg MPa^{-1} s^{-1}, respectively) (Fig 3). This, together with the results presented in Table 1, which show a similar root-system biomass for both genotypes, demonstrates the higher water transport capacity of PT than CM at similar root biomass levels. However, shoot hydraulic conductance (K_s) was significantly higher in CM than in PT (53.69 10^{-7} and 39,50 10^{-7} Kg MPa^{-1} s^{-1}, respectively). Moreover, in PT seedlings, K_r was significantly higher than K_s, while CM presented no significant differences between K_r and K_s. Despite differences between K_r and K_s values between CM and PT, whole plant hydraulic conductance (K_p) was similar in both plant types.

When the K_s value of each individual plant was plotted versus its respective K_r value, these two parameters were linearly related in both genotypes ($r^2 = 0.95$ for CM, and $r^2 = 0.65$ for PT) (Fig 4). However this relationship differed in each plant type. For a given K_r value, K_s was higher in CM than in PT plants.
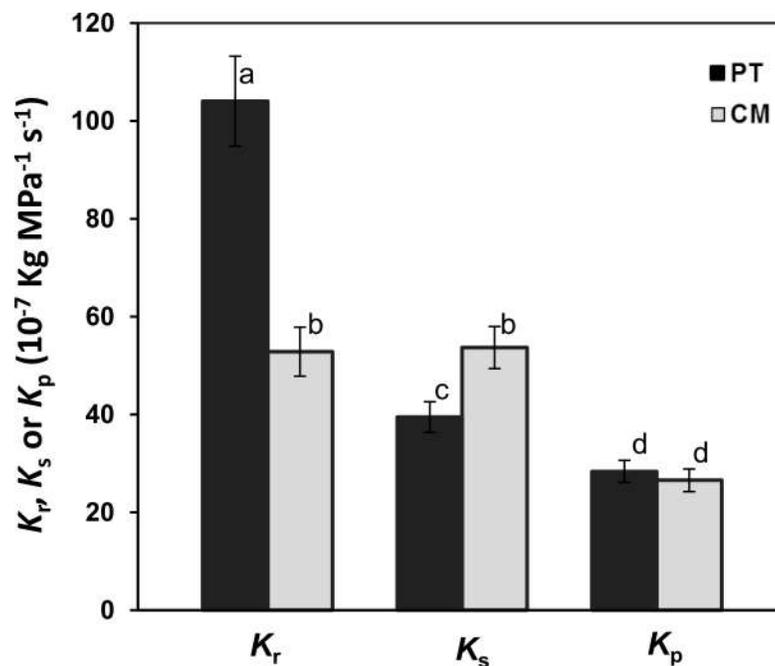
Fig 3. Root, shoot and plant hydraulic conductance (K_r, K_s and K_p, respectively), of *P. trifoliata* (PT) and Cleopatra mandarin (CM). The mean (n = 16) with different letters show statistically significant differences (P <0.05).

Fig 4. Relationship between root hydraulic conductance (K_r) and shoot hydraulic conductance (K_s) in *P. trifoliata* (PT) and Cleopatra mandarin (CM).
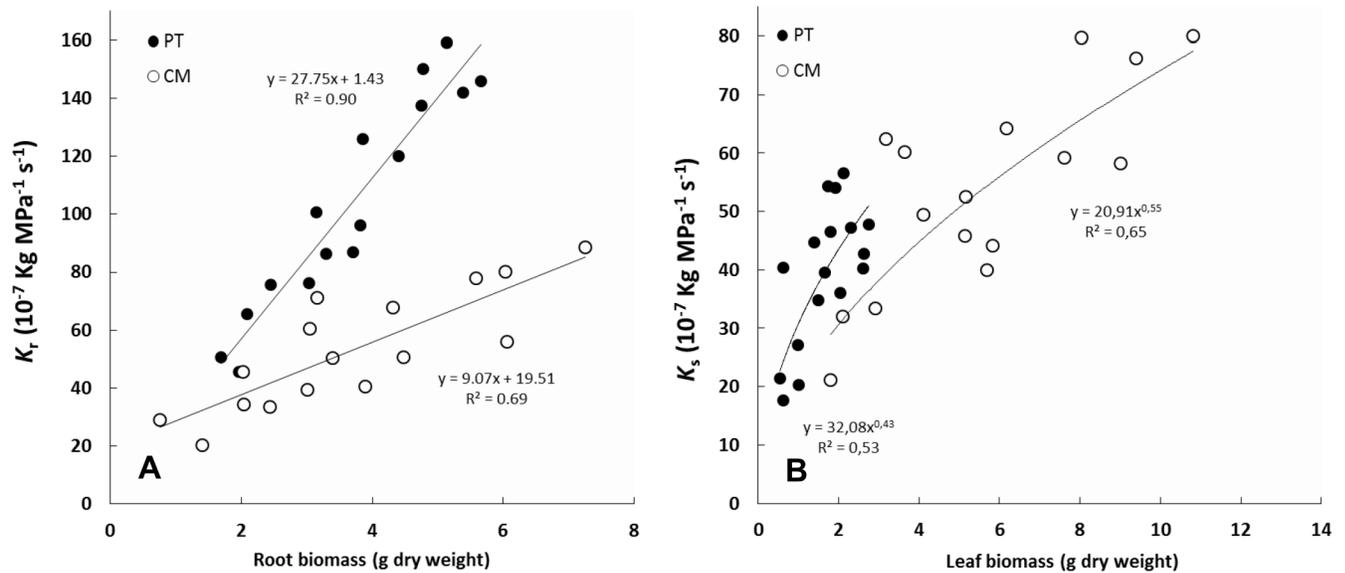
Relationship between hydraulic conductance and plant biomass

In PT, root hydraulic conductance values measured (K_r) ranged from $45.60 \cdot 10^{-7}$ to $159.00 \cdot 10^{-7}$ Kg MPa^{-1} s^{-1} for roots which their dry weight ranged between 1.69 and 5.67 g dry weight (Fig 5A). In CM, K_r values ranged from $20.20 \cdot 10^{-7}$ to $88.70 \cdot 10^{-7}$ kg MPa^{-1} s^{-1} for roots between 0.76 and 7.25 g dry weight. K_r increased linearly both for CM and for PT with increased root biomass, with r^2 values of 0.90 and 0.69 for PT and CM, respectively. For a given root biomass, K_r values were higher in PT than in CM.

Leaves of PT plants of the experiment showed values between 0.54 and 2.74 g dry weight (Fig 5B). K_s values of PT were between $17.60 \cdot 10^{-7}$ and $56.60 \cdot 10^{-7}$ Kg MPa^{-1} s^{-1}. In CM, whose foliar biomass ranged from 1.80 to 10.82 g dry weight, K_s values were in a range $21.21 \cdot 10^{-7}$ to $80.03 \cdot 10^{-7}$ kg MPa^{-1} s^{-1}. There was a curvilinear increase in K_s with increased foliar biomass presenting r^2 values of 0.53 and 0.65 for PT and CM, respectively. For similar foliar biomass, PT presented higher K_s values.

Relationship between whole plant transpiration and root, shoot and plant hydraulic conductance

Whole plant transpiration (T_p), measured gravimetrically, increased linearly with increasing K_r and K_s values in both genotypes (Fig 6). In CM, T_p was affected in a similar way by K_r and K_s

Fig 5. Relationship between root hydraulic conductance (K_r) and root biomass and between shoot hydraulic conductance (K_s) and the leave biomass expressed in g of dry weight in *P. trifoliata* (PT) and Cleopatra mandarin (CM).

([Fig 6B](#)) (there were no differences in the slope and the ordinate of the regression line between both regression lines). However, in PT plants, there was a greater T_p increase when K_s rose than when K_r rose ([Fig 6A](#)).

Moreover, the hydraulic conductance of the whole plant (K_p) was linearly related to T_p ([Fig 7](#)). This relationship was identical in both genotypes (slopes and intercepts were not significantly different) despite morphological, physiological and hydraulic traits these genotypes presented.

Xylem anatomy

Xylem anatomical differences existed between the two genotypes (Figs [8](#) and [9](#)). There were no observed changes in xylem vessel diameter in PT between taproot and basal stem while CM presented larger vessel diameter in basal stem than in taproot ([Fig 8A](#)). Despite this increasing diameter in the CM basal stem, xylem vessel diameter was larger in PT than in CM, both in taproot and basal stem.

On the other hand, xylem vessel density was higher in the basal stem than in the taproot in both genotypes although this difference was greater in CM ([Fig 8B](#)). In CM, the increased vessel diameter and vessel density in basal stem compared to their values in taproot resulted in a total vessel lumen area 2.17-fold higher in basal stem than in taproot. PT showed a 1.22-fold higher total vessel lumen area in basal stem compared to that of taproot ([Fig 8C](#)).

Discussion

The main objective of this work was to determine the influence of hydraulic conductance on transpiration in citrus. Apparently, transpiration rate (E) was not regulated solely by the degree of stomatal opening ([Fig 2](#)). However, results showed a linear relationship, identical for both studied genotypes (*P. trifoliata* [PT] and Cleopatra mandarin [CM]), between whole plant transpiration (T_p) and plant hydraulic conductance (K_p) ([Fig 7](#)).

Leaf biomass and Leaf dry weight/Root dry weight ratio (L/R) were higher in CM than PT. Both the transpiration rate (E) and the average root hydraulic conductance (K_r) were higher in
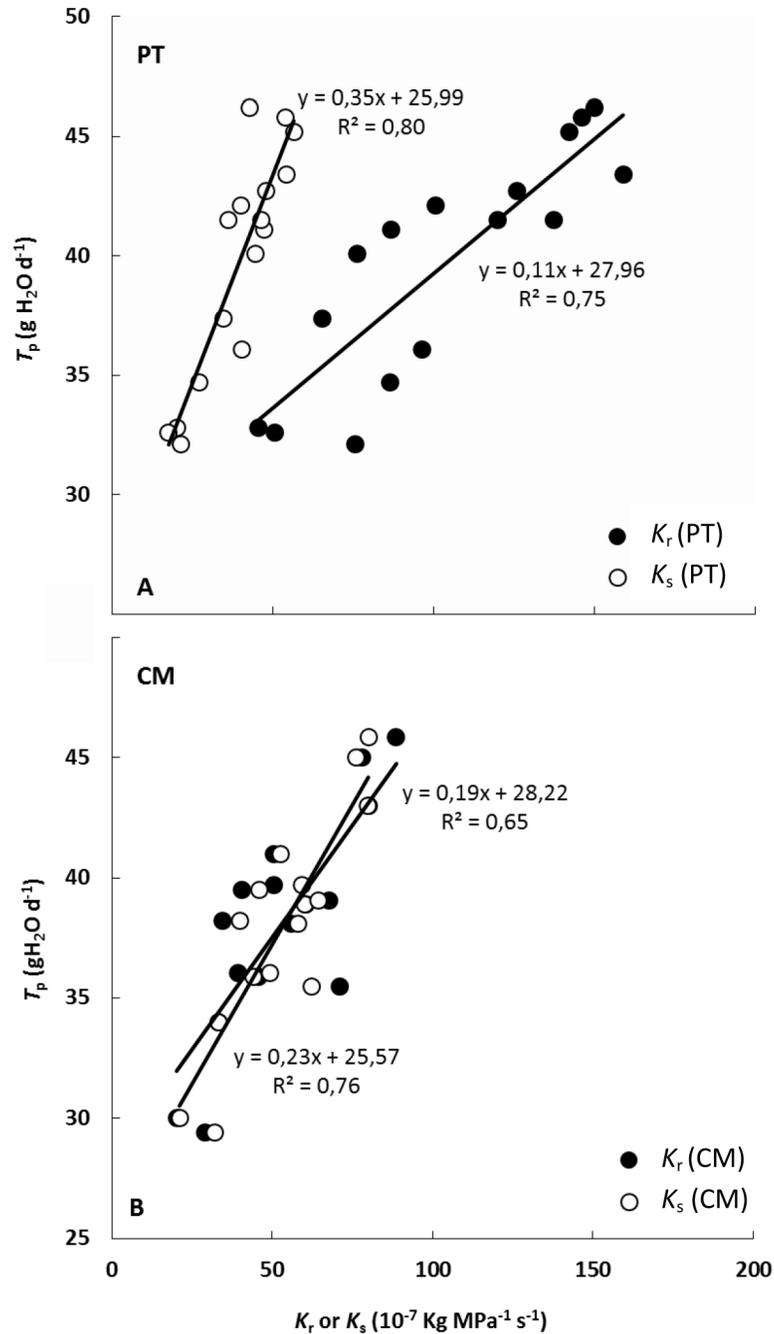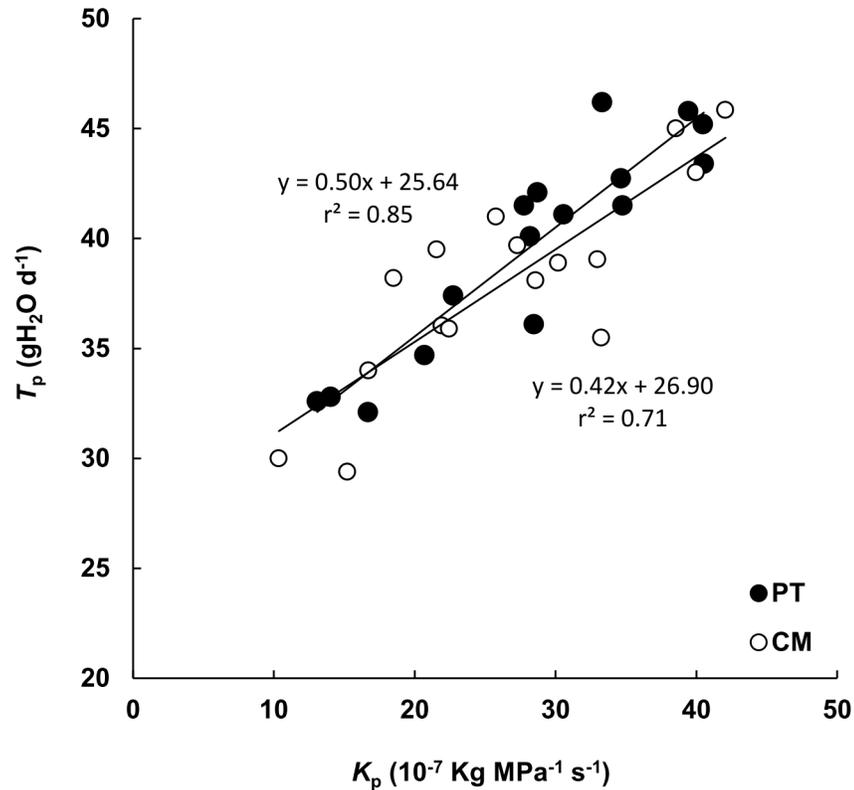
Fig 6. Relationship between root hydraulic conductance (K_r) and shoot hydraulic conductance (K_s) with whole plant transpiration (T_p) in *P. trifoliata* (PT) and Cleopatra mandarin (CM). Each point represents the mean of three measurements of T_p.

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PT than in CM (Figs 2 and 3). However, CM presented higher average shoot hydraulic conductance (K_s) value (Fig 3).

In both genotypes K_s and K_r were related positively with foliar and root biomass, respectively, as reported by other authors (e.g., [45]). When both parameters are scaled by dividing between leaf surface to obtain leaf-specific root hydraulic conductance (K_{r-l}) and leaf-specific

Fig 7. Relationship between plant hydraulic conductance (K_p) and whole plant transpiration (T_p) in *P. trifoliata* (PT) and Cleopatra mandarin (CM) in sixteen independent plants for each seedling. Each point represents the mean of three measurements of T_p for each plant.

shoot hydraulic conductance (K_{s-l}) (Table 2), K_{r-l} and K_{s-l} values were higher in PT. Both could determine the higher transpiration rate, stomatal conductance and net CO_2 assimilation that PT presents, compared with CM [39, 40, 46].

A linear relationship between K_r and K_s was found in both genotypes, although this relationship was not the same for both types of plants. For equal K_r values, CM presented higher K_s than PT (Fig 4). Similar relationships between different plant hydraulic parameters have been described by other authors. [47] showed a linear regression between leaf hydraulic conductance and whole plant hydraulic conductance while [21] reported relationships between whole-plant and shoot hydraulic conductance. In our study, interestingly, for an individual plant independent of its genotype, K_s was always similar or lower than K_r. Also [48] observed that whole leaf hydraulic conductance was lower than K_r in nineteen grass species. This could be an evolutionary trait to avoid cavitation. If K_s was greater than K_r and water flow in shoots was close to its possible peak flow, roots could not provide all the water claimed by the air component. This could result in increased water tension that would facilitate air bubble formation in the xylem. The lack of literature integrating the study of whole root and shoot systems in tree species does not enable us to pose this hypothesis. However, our results support the assumption that the design of plant hydraulic conductance and resistance to cavitation within a plant is optimized to deal with the conflicting balance between evaporative demand and protection from hydraulic failure [49].

The higher K_s/K_r ratio in CM could be attributed to the anatomical features of the xylem of the main root and stem base. In CM seedlings, values of lumen diameter and density of vessels
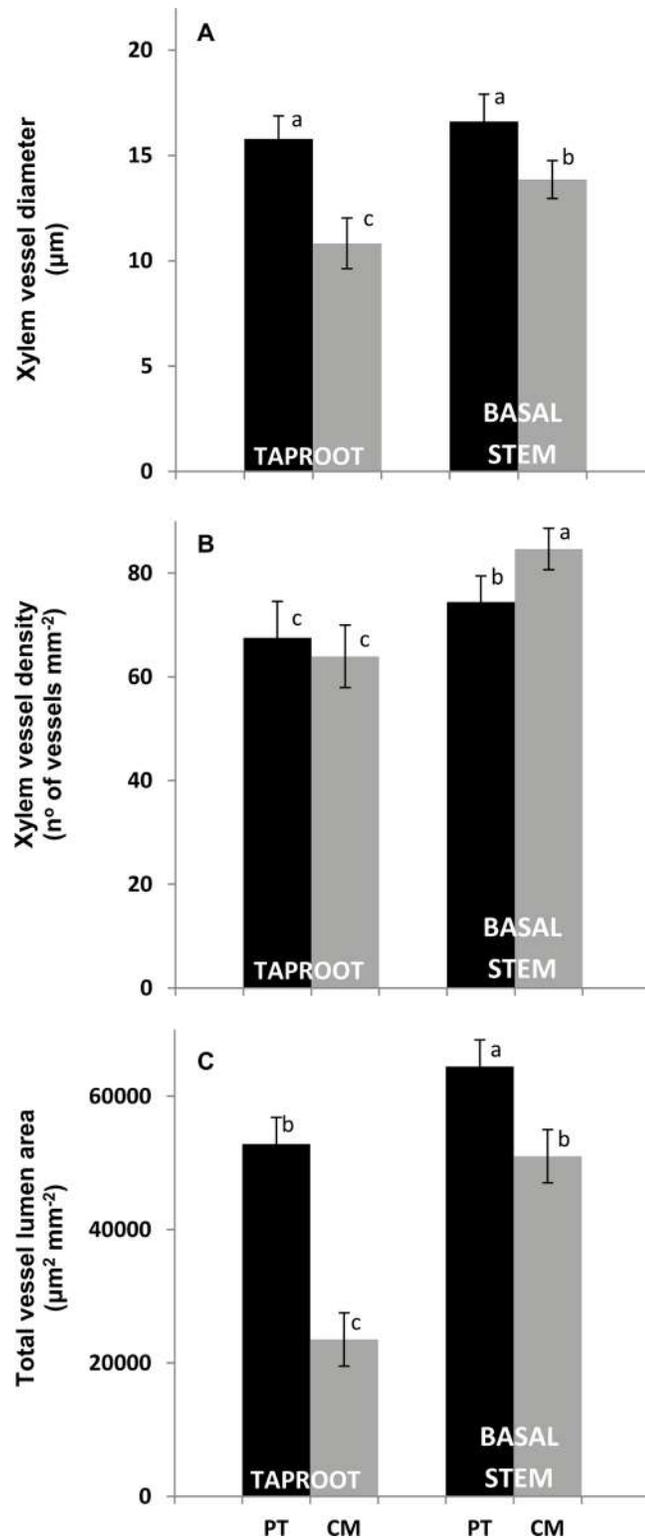
Fig 8. (A) Diameter, (B) density and (C) total lumen area of xylem vessels of taproot and basal stem in cross sections of *Poncirus trifoliata* (PT) and Cleopatra mandarin (CM) seedlings. Histological data correspond to the mean of six independent plants (n = 6) of each rootstock. The value for each plant is the mean of three visual fields of three sections from three samples per root and stem. Different letters indicate statistically significant differences (P <0.05) (LSD test).

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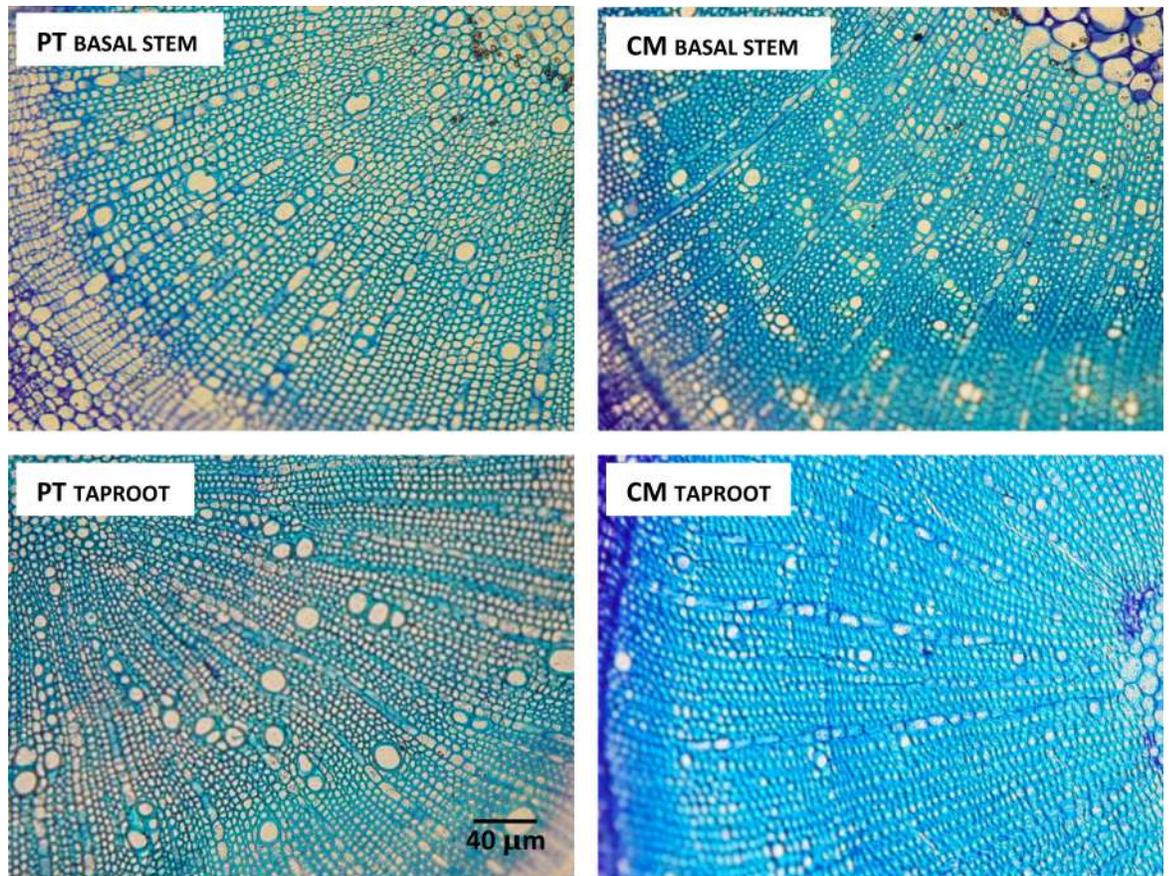
Fig 9. Light micrograph of secondary xylem from basal stem and taproot of *P. trifoliata* (PT) and Cleopatra mandarin (CM). Sections were cut at 20–25 mm under and above to soil surface.

in the base of the stem were 27.9% and 32.4% higher compared with the values of these parameters in the taproot, resulting in a two-fold increase in total lumen vessel area compared with this area in the taproot, while in PT this increase was much lower ([Fig 8]). Therefore, the same as with xylem traits and L/R ratio, the K_s/K_r ratio could be genetically determined. On the other hand, the higher diameter in xylem vessels in PT could be attributed to higher aquaporin activity. According to [50], aquaporins play a role in mediating water transport to support xylogenesis because plants with higher aquaporin expression achieve a greater final cell diameter in xylem vessels. In other studies, we have observed more aquaporin expression in roots of PT than in CM [24, 51], which could be partially responsible for the high root hydraulic conductance of PT plants, and also for their greater vessel diameter.

Table 2. Root-specific root hydraulic conductance (K_{r-r}), leaf-specific root hydraulic conductance (K_{r-l}) and leaf-specific shoot hydraulic conductance (K_{s-l}) in *P. trifoliata* (PT) and Cleopatra mandarin (CM).

	K_{r-r}	K_{r-l}	K_{s-l}
	(10^{-7} x Kg MPa^{-1} s^{-1} g^{-1})	10^{-5} x Kg MPa^{-1} s^{-1} cm^{-2}	10^{-5} x Kg MPa^{-1} s^{-1} cm^{-2}
PT	28.12 ± 2.23 a	177.58 ± 9.18 a	66.80 ± 7.10 a
CM	14.37 ± 2.06 b	23.93 ± 3.01 b	24.44 ± 2.31 b

For each parameter, means (n = 16) with different letters show statistically significant differences (P<0.05).

Clearly root and shoot growth must be coordinated somehow during the life of a plant [52]. The relationship between both K_r and K_s with plant biomass (Fig 5) and the relationship between both conductances (Fig 4) suggests that hydraulic traits should influence Shoot/Root ratio and plant growth, in accordance with other authors [36, 53, 54, 55]. It is known that growth of the aerial component in olive trees is related to K_r [53] and also that K_r is responsible for the vigor of grafted peach varieties [54]. However, although many studies associate high values of K_{r-l} or K_{r-r} (leaf or root-specific root hydraulic conductance) with strong shoot development, in our experiment CM showed a higher L/R ratio even though PT presented higher K_{r-l} and K_{r-r} (Table 2). Therefore, root hydraulic conductance may apparently determine the gas exchange features of leaves rather than shoot growth rate. [54] observed that non-vigorous peach varieties grafted on vigorous rootstocks presented lower K_s, and less vigorous development than vigorous varieties grafted on the same rootstocks, indicating that genetic factors probably determine the K_s and growth of these varieties. Therefore, these could explain the higher L/R ratio in CM. Accordingly, the effect of rootstocks hydraulic traits on shoot growth would be more evident when rootstocks were grafted with same variety. Taking all this into account, in our study, the highest L/R ratio in CM could be more closely related with the K_s/K_r ratio and xylem anatomy traits than with differences in water transport capacity of the root system.

On the other hand, E stayed practically constant between 10:00 am– 4:00 pm, even though stomatal closure was observed after 11:00 am. The reduction in g_s was probably due to the high temperature increase in late morning, which resulted in a high VPD (Fig 2D). By reducing g_s, plants minimize water loss and maintain plant cell hydration as VPD increases. Despite reduced stomatal conductance in response to increasing vapor pressure deficit, transpiration rate can increase [56, 57], as occurred in this experiment, since E/g_s ratio increased (Fig 2C). This response varies among species and genotypes [58, 59, 60]. Our results showed that at early morning (from 8:00 am to 10:00 am E was limited by VPD and not by g_s (Fig 2A, 2B and 2D), which presented its maximum values in this period and was not completely related to the increasing E. At mid-range values of VPD (approximately from 10:00 am to 12:00 am, stomata progressively closed as VPD increased. However, E remained constant as the increase in the driving force (VPD) compensated the reduction in g_s. It suggested that hydraulic limitation more than stomatal limitation were related to the maximum E values reached at midday. A number of studies have indicated a functional relationship between g_s and hydraulic conductance [18, 52]. Accordingly, as VPD increased, the rate of stomatal closure (which controls water losses) could be modulated by the plant hydraulic system (which controls water supply to the leaves). Therefore E would be the result of the balance between g_s, VPD and hydraulic conductance.

The relationship between whole plant transpiration (T_p) and K_r, presented in Fig 6, has also been reported in citrus by [38]. The latter authors found a significant correlation ($r^2 = 0.63$) between these two parameters, noting that rootstocks with low K_r values showed lower T_p values than rootstocks with higher K_r values. However, our study shows that apparently K_s also influences plant transpiration (Fig 6B). Previous studies on citrus rootstocks suggest that differences in the root size or in the crown volume of grafted varieties affect the plant-soil-water relationship [61, 62, 63, 64, 65]. Moreover, T_p in both genotypes were strongly associated with foliar biomass (Fig 1). This suggests that foliar biomass is probably an important factor determining transpiration, which in turn appears to be related to the fact that increases in foliar biomass are related to K_s increments (Fig 5B). Our results support the dependence of T_p on foliar biomass, in accordance with previous studies in citrus suggesting that the crown size influences the rate of water absorbed by trees grafted on different rootstocks [61] but also indicate that this is associated with the higher hydraulic conductance values reached with higher crown size.

The relationships between K_r and K_s with T_p differed between CM and PT (Fig 6), but the relationship between whole plant hydraulic conductance (K_p) and T_p was identical for both

genotypes ([Fig 7](#)). Shoot and root contribution to whole plant hydraulic conductance differed greatly between the two genotypes, even so, the relationship between K_p and T_p remained constant, suggesting that plant transpiration is regulated directly by K_p, which in turn depends on K_r and K_s [10, 66]. Several studies have highlighted the coordination between plant water transport capacity and leaf-level gas exchange or photosynthetic capacity [67, 68, 69, 70, 71]. It is therefore assumed that a high plant hydraulic efficiency (i.e. high leaf-specific hydraulic conductance) which modulates transpiration is an essential prerequisite for a high productivity in tree species [3]. However, a decrease in future precipitation and an increase in the temperature have been predicted for many *Citrus* growing regions, affecting the plant water relations and potentially constraining its productivity [72]. Both CM and PT are widely used as citrus rootstocks. The ability of the rootstock to supply water and nutrients to the plant is a main factor influencing fruit development in citrus trees, determining the strength of the grafted variety and tolerance to water stress [73, 74]. When comparing grafted varieties onto CM and PT rootstocks, grafted varieties on CM presented less net CO_2 assimilation, transpiration rate and reduced stomatal conductance than when are grafted in PT [23, 24]. However, grafted varieties on CM present higher tolerance to water stress probably attributed to the relatively low values of K_{r-r} and K_{r-l} that limits sap flow from roots to leaves promoting conservative water use and favoring better water balance in the plant [24, 75]. Therefore, in areas where water availability can be a limiting factor the use of CM could be a strategy for reducing water stress.

This work shows the need for integration of whole plant hydraulic system when attempting to understand the plant water relations and we conclude that transpiration in citrus is strongly determined by the hydraulic system of the whole plant and the differences in root and shoot contribution to K_p could be related to the leaf/root ratio, as well as to xylem anatomy characteristics.

Supporting Information

S1 Table. Number of leaves, dry weight of stem, leaves and root, leave surface and shoot, root and plant hydraulic conductance of the sixteen plants of Cleopatra Mandarin and Poncirus trifoliata used in the experiment.
(XLSX)

S2 Table. Stomatal conductance (g_s), transpiration (E), E/g_s ratio and vapor pressure deficit (*VPD*) in *P. trifoliata* (PT) and Cleopatra mandarin (CM). At each hour of the day, values are means of six replicates for each type of plant.
(XLSX)

S3 Table. Histological data correspond to the mean of six independent plants of *Poncirus trifoliata* and six independent plants of Cleopatra mandarin.
(XLSX)

Acknowledgments

Author Contributions

Conceived and designed the experiments: MÁFG. Performed the experiments: MÁFG JRG. Analyzed the data: EPM JRG. Contributed reagents/materials/analysis tools: MÁFG. Wrote the paper: JRG MÁFG.

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