

Gas exchange, root hydraulic conductivity, water use efficiency and the growth of *Toona ciliata* clones and seedlings

Trocas gasosas, condutividade hidráulica das raízes, eficiência do uso da água e crescimento de mudas clonais e seminíferas de *Toona ciliata*

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Abstract

Forest plantations established with seedlings have heterogeneity and are difficult to manage, so an alternative is the use of clones with high productivity. In addition, clonal plants differ in the structure of the root system that can influence the water and nutrient uptake efficiency and therefore productivity. We evaluated leaf gas exchange, root hydraulic conductivity, and water use efficiency of *Toona ciliata* clonal cuttings and the seedlings growth. The study was performed in a completely randomized design with four treatments: a) TC3 clone; b) TC9 clone; c) TC15 clone and d) seedlings with five replicates and ten plants per plot. On the 120th day, the net photosynthetic rate, transpiration and stomatal conductance were evaluated between 12:00 and 13:00 hours and the values calculated as efficient, instantaneous (A/E) and intrinsic (A/g_s) water use were calculated. Hydraulic root conductivity (k_{root}) was obtained by applying increasing pressures (0.1, 0.2, 0.3, and 0.4 MPa) using Scholander chamber. The height, stem diameter, leaf area, dry mass of shoot and root, length, diameter, surface area and root volume were also determined. The data were submitted to Pearson's correlation and analysis of variance, comparing by Tukey's test (5%). The genetic materials studied presented an equal capacity of water absorption and transport by the roots, even though they exhibited a visual anatomical differences of the root system. Although, the clones exhibited low transpiration and net photosynthetic rates, they were generally more efficient in water use, and the TC3 and TC9 clones were more efficient to convert the assimilated carbon to biomass.

Keywords: Photosynthesis; Root hydraulic conductivity; *Toona ciliata*, Cuttings

Resumo

As plantações florestais estabelecidas com mudas apresentam heterogeneidade e dificuldades de manejo. Assim, uma alternativa a estes obstáculos é o uso de clones com alta produtividade. Além disso, as plantas clonais diferem das plantas seminíferas na estrutura do sistema radicular, o que pode influenciar a eficiência na absorção de água e de nutrientes minerais e, portanto, a produtividade. Nesta pesquisa, avaliaram-se as trocas gasosas foliares, a condutividade hidráulica da raiz, a eficiência no uso da água e o crescimento de plântulas de estacas clonais e de plantas propagadas por semente da espécie *Toona ciliata*. O estudo foi realizado em delineamento inteiramente casualizado com quatro tratamentos: a) clone TC3; b) clone TC9; c) clone TC15 e d) mudas seminíferas, com cinco repetições e dez plantas por parcela. Aos 120 dias, a taxa fotossintética líquida, a transpiração e a condutância estomática foram avaliadas entre as 12h00 e as 13h00 horas, e a partir desses valores foram calculados as eficiências instantânea (A/E) e intrínseca (A/g_s) do uso da água. A condutividade hidráulica da raiz (K_{root}) foi obtida pela aplicação de pressões crescentes (0,1, 0,2, 0,3 e 0,4 MPa) neste órgão por meio de uma câmara de pressão. A altura, o diâmetro do caule, a área foliar, a massa seca da parte aérea e raiz, o comprimento, o diâmetro, a área superficial e o volume radicular foram determinados. Os dados foram submetidos à correlação de Pearson e à análise de variância, comparando-se pelo teste de Tukey (5%). Os materiais genéticos estudados tiveram igual capacidade de absorção e transporte de água através das raízes, apesar de terem diferenças anatômicas visuais do sistema radicular. Os clones tiveram taxas de transpiração e taxa fotossintética líquida reduzidas, e estes foram mais eficientes no uso da água. Os clones TC3 e TC9 foram mais eficientes na conversão do carbono assimilado em biomassa.

Palavras-chave: Fotossíntese; Condutividade hidráulica da raiz; *Toona ciliata*; Mudas

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Introduction

The success of timber plantations depends on their photosynthetic capacity and the water use efficiency, among other factors, considering the environmental conditions during the plant establishment. Plants can be subjected to different types of stress in these environments, including high light intensity, high temperature, and low water availability. In this context, it is very important to select genotypes which present higher photosynthetic rates and/or lower losses through the respiratory process under these conditions of environmental stress. However, the majority of researchers still use only biometric characteristics to select and to determine seedling qualities, since these characteristics are easy and inexpensive to measure.

For *Toona ciliata*, which has excellent quality wood and high economic value (PINHEIRO; LANI; COUTO, 2006), the Brazilian plantations are almost entirely planted from the seed. However, due to the difficulties of handling the heterogeneous population and in order to obtain high productivity and fast growing genotypes, the planting of clones has been a viable alternative and is gaining popularity among the producers.

Plant root systems serve numerous functions including mechanic support, water and soil nutrients uptake, nutrients storage and synthesis of growth regulators (FITTER, 1991; GREGORY, 1994). The efficiency of the root system in absorbing water and nutrients strongly affects the growth of the aerial part of the plants (KOZLOWSKI; PALLARDY, 1997). This growth is the result of the difference between the gain of carbon via photosynthesis and the loss via respiration (ATKIN; SCHEURWATER; PONS, 2007). Studies aimed at understanding the root system structure are of fundamental importance to explain the ecophysiological processes related to the tree mineral nutrition and water balance (GONÇALVES; MELO, 2005).

Vegetative propagated clones and plants established from seeds show differences between their root systems. In the plants originated from seeds, the root system is developed from the dominant primary root with ramifications or lateral roots (RAVEN; EICHHOM; EVERT, 2014). On the other hand, the root system formed from the clone seedlings is adventitious and it can be able to be formed from the stem or, in some cases, from the leaves. For *Toona ciliata* it is still unclear if these differences in the root origin result in differences in the root behavior.

Plant water transport from the soil, into the roots, through stems and leaves, and into the atmosphere is regulated by the hydraulic conductivity (k) of each part involved in the pathway. Therefore, changes that decrease k especially in the early portions of the pathway (e.g. root) will increase the tension in the plant (MAUREL; SIMONNEAU; SUTKA, 2010), possibly decreasing the leaf gas exchange (TYREE; EWERS, 1991; HUBBARD; BOND; RYAN, 1999), resulting in negative effects on growth and productivity.

Numerous factors influence the hydraulic conductivity of the root system (k_{root}), such as the anatomic characteristics (number and the diameter) of the xylem vessel (TOMBESI *et al.*, 2010), root surface area (SOLARI; PERNICE; DEJONG, 2006), the confinement and the deformation of the root (FIGUEIREDO *et al.*, 2014a), and abiotic stresses (BOURSIAC *et al.*, 2008).

Differences in k_{root} could potentially have large effects on the efficiency of the plant water use (WUE). For example, in soybeans, sorghum and millet plants, the capacity in conserving water is related to the transpiration restriction in an environment of high evaporative demand of the air [high values of air vapour pressure deficit (VPD_{air})] (GHOLIPOOR *et al.*, 2010; KHOLOVA *et al.*, 2010; GILBERT *et al.*, 2011). This capacity of water conservation in the aerial part is related to the low hydraulic conductivity in the plant that reduces the turgor pressure of the cells and promotes stomata closure.

In those genotypes with restricted transpiration rate at high VPD_{air} , it has been hypothesized (SINCLAIR; ZWIENIECKI; HOLBROOK, 2008) that low hydraulic conductance in the plant limits the flow of water to guard cells so that there is a loss of guard cell turgor and a decrease in stomatal conductance. The decrease in stomatal conductance results in a new balance of lower water flow into, and evaporation rate from, guard cells under higher VPD_{air} .

conditions. The source of the limiting hydraulic conductance under high VPD_{air} might be in roots and/or leaves. Sinclair, Zwieniecki and Holbrook (2008) demonstrated that the limiting hydraulic conductance in soybeans genotype was between the leaf xylem and the guard cells. According to Boyer (1985), the root hydraulic resistance to water flow through whole plants is higher than the shoot resistance.

It has been hypothesized that clone seedlings may have higher k_{root} possibly through larger root volume and quantity of fine roots. This higher k_{root} would increase gas exchange at midday and positively influence shoot growth and plant productivity. The goal of this study was to evaluate this hypothesis through measures of midday gas exchange, k_{root} , WUE, and the growth of *Toona ciliata* var. *australis*, from clonal and seminal seedlings.

Materials and methods

Cultivation conditions of *Toona ciliata*

The study was performed in a greenhouse at the Universidade Estadual do Norte Fluminense- UENF, Campos dos Goytacazes, Rio de Janeiro state (RJ) (21°44'47"S and 41°18'24"W), Brazil. The treatments consisted of either seedlings produced from minicutting plants for clones TC3, TC9 and TC15 and seedlings originated from seed.

The seed used in the experiment was acquired from Caiçara Comércio de Sementes Ltda., located in Brejo Alegre, SP state, derived from the seed production area, located in Venda Nova do Imigrante – ES state, Brazil (20°20'23"S and 41°08'05"W). Two seeds were put into polyvinyl chloride (PVC) pots of 280 cm³, containing commercial forest substrate made out of decomposed pine tree bark, and were maintained in a greenhouse with plastic coverage (plastic covering film 150 µm) with 30% photosynthetic photon flux interception. When the plants presented two pairs of leaves, the pots were thinned out, leaving only one plant per pot. On the sowing day, the minicuttings of the three clones (TC3, TC9 and TC15) were prepared with 5 cm of height, average diameter of 3.6 mm and a pair of leaves reduced in 50% to their leaf area. These minicuttings were cultivated in the same PVC pots types of 280 cm³ with the same substrate used to cultivate the seedlings and they remained in a nebulization chamber for thirty days until rooting. After this period, the mini plant cutting completed the production cycle in a greenhouse, with plastic coverage (plastic covering film 150 µm) with 30% photosynthetic photon flux interception. During the cultivation of the clone plants along with the seedlings in an environment without nebulization, the micrometeorological variables (temperature and relative humidity) of the greenhouse were monitored at each hour using automatic sensors of data collection (Data Logger RHT10 Model) (Extech Instruments, USA), recording in average temperature of 25°C and relative humidity of 74.5%. From these variables the VPD_{air} was calculated according to the equation proposed by Jones (1992), finding on average 1.1 kPa. The micrometeorological variables of the greenhouse during the gas exchange measurements between 12:00 HR and 13:00 HR were 36.6°C, 61% and 2.4 kPa on average.

On the 120th day, at the end of the seedling and cuttings formation period, the net photosynthetic rate, transpiration, stomatal conductance, instantaneous and intrinsic water use efficiency and the root hydraulic conductivity were evaluated. At this date, height, stem diameter, leaf area, shoot dry mass and root dry mass, length, diameter, area and root volume were also evaluated.

Gas exchange

The net photosynthetic rate (A), transpiration (E) and stomatal conductance (g_s), were determined using the infrared gas analyzer (IRGA), Li-6400 model (Li-Cor, Lincoln, NE, USA).

A $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity was used on a 6 cm^2 leaf surface, using an artificial lighting system composed of a mix of blue and red light-emitting diodes coupled to the equipment. The measurements were performed in a fully expanded leaf, in the third pair, from the apex. These evaluations started at 12:00 and extended until 13:00 HR, in the moment of higher atmospheric demand for water (FIGUEIREDO *et al.*, 2014b). The air temperature ($^{\circ}\text{C}$), relative humidity (%) and VPD_{air} (kPa) inside the infrared gas analyzer chamber, during the measures were $36.8 \pm 2^{\circ}\text{C}$, $26.1 \pm 2\%$ and $4.4 \pm 1 \text{ kPa}$ for the air temperature, relative humidity and air vapour pressure deficit.

Instantaneous water use efficiency (WUE) and intrinsic water use efficiency (iWUE)

The instantaneous water use efficiency (WUE) was obtained by the relation between the net photosynthetic rate and the transpiration (A/E) and the intrinsic water use efficiency (iWUE) was obtained by the ratio between the net photosynthetic rate and the stomatal conductance (A/g_s).

Root hydraulic conductivity (k_{root})

The hydrostatic root hydraulic conductivity (k_{root}) was determined by measuring the steady state flow at four pressures (0.1; 0.2; 0.3; and 0.4 MPa). Each plant was removed from the pot and the aerial part of the plant was cut off under water. The whole root system was put in another vessel containing water, and both were immediately inserted into a Scholander chamber (Soil Moisture model, California, USA). Positive pressures were applied at 0.1, 0.2, 0.3, and 0.4 MPa (proceeding from low to high pressure). At each pressure step the xylem sap in the stem was collected and weighed on a precision analytical balance (TYREE, 2003). Pressure flow curves were constructed and k_{root} was determined by determining the slope of the linear regression of this relationship (Figures 1 and 2).

Growth measurements

At 120 days (end of the experiment), the shoot height and the stem diameter of the clones and seedling were measured using a ruler and a digital caliper, respectively. The leaf area was determined using the leaf area meter (model LI-3000, LI-COR Inc, USA). To evaluate the root system, roots were washed with water using sieves to retain the roots. After the washing, the roots were placed in an acrylic tank of 20 cm of width and 30 cm of length, containing distilled water, and were analyzed for length, diameter, area and root volume, using the *WinRhizo Pro 2007a* software (Régent Instr. Tnc.), connected to a professional scanner Epson XL 10000 with a 400 dpi resolution.

To determine the dry mass, both the shoot and the root system were put, separately, in paper bags, inserted in an oven with forced air circulation at $65^{\circ}\text{C} \pm 2$ for 72 hours, to be weighed in an analytical balance later.

Experimental study design

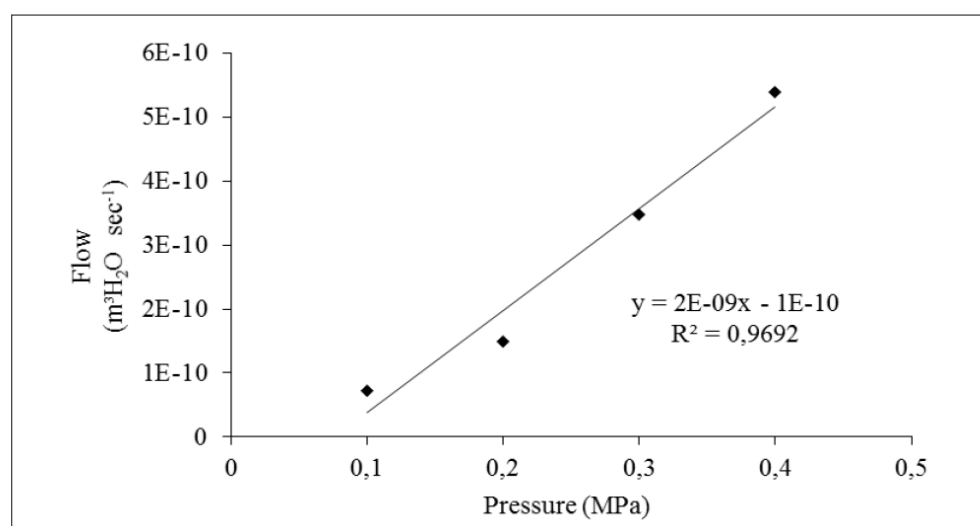
The study was implanted in completely randomized design, with five replications. The data presented a homoscedasticity and normal distribution, verified by the Cochran and Lilliefors tests. After the application of these tests, data were analyzed using Pearson's correlation and analysis of variance and the differences were compared by Tukey's Test (5%). The water flow quantitative data were submitted to a regression analysis as a function of applied pressure.

Results and discussion

Figure 1 shows a typically example of the pressure vs. flow relationship in the root system in *Toona ciliata* clones and seedlings. The flow increased linearly with the applied pressure linear consistent as described in Knipfer and Steudle (2008) and Knipfer and Fricke (2011).

Figure 1 – Example of a linear pressure flow relationship for a *Toona ciliata* root system.

Figura 1 – Exemplo de uma relação de fluxo linear de pressão para o sistema radicular de *Toona ciliata*.



Fonte: Oliveira, T. P. F. (2016)

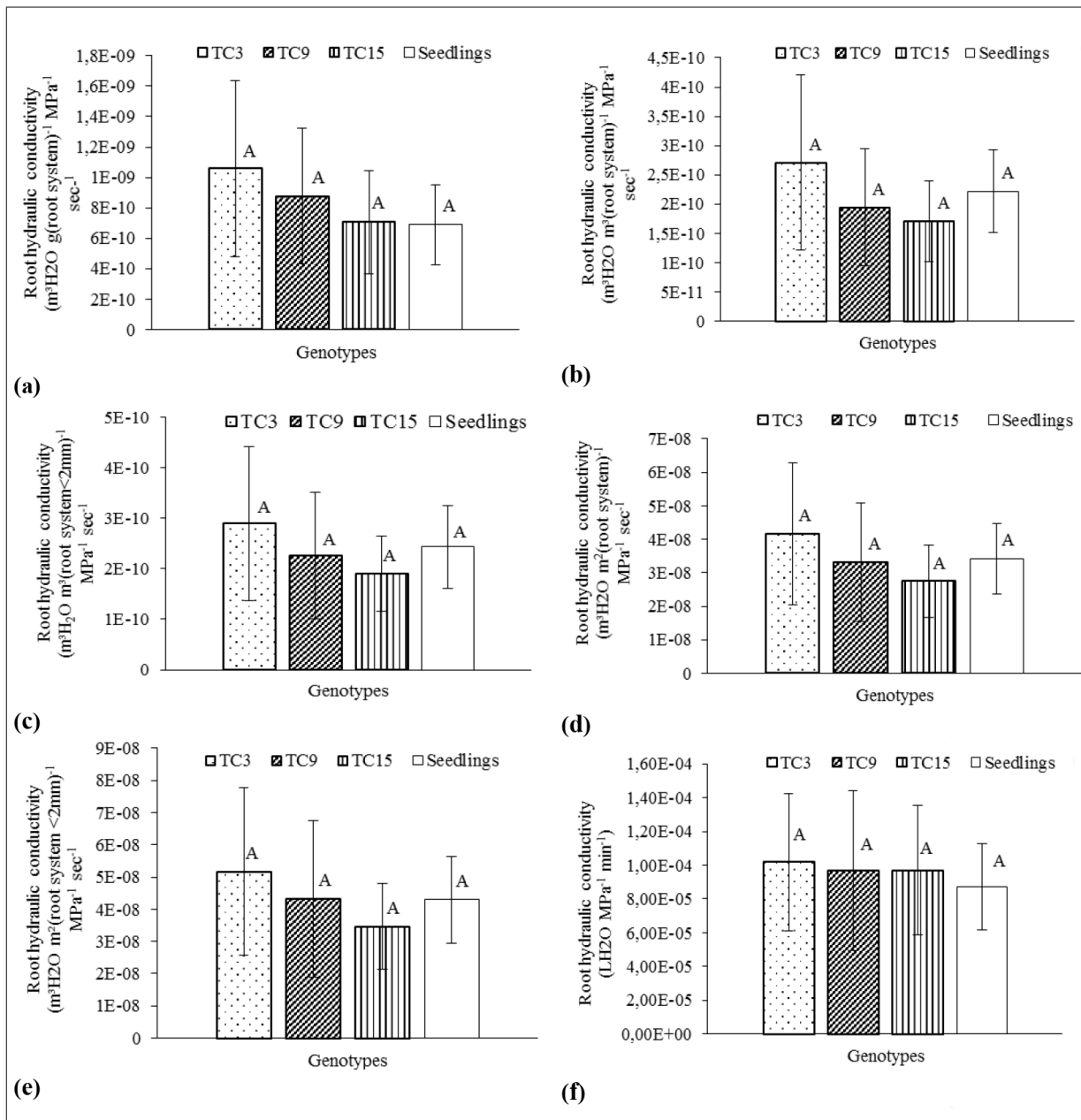
There was no difference between the clones (TC3, TC9 and TC15) and the seedlings for the hydraulic conductivity of the root (k_{root}) by the dry mass of the root system (Figure 2a), the volume of the root system (Figure 2b), the volume of thin roots (\varnothing 0 - 2mm) (Figure 2c), the root surface area (Figure 2d), thin root surface area (\varnothing 0 - 2mm) (Figure 2e) and non-normalized (Figure 2f).

The water absorption and transport by roots are influenced by several factors, such as the anatomic characteristics of the xylem (TOMBESI *et al.*, 2010), the confinement and the deformation of the roots (FIGUEIREDO *et al.*, 2014a), and the lower number of cells in the cortex (STEUDLE; PETERSON, 1998; RIEGER; LIVTIN, 1999). The evaluation of these variables in future studies with clones of *Toona ciliata* plants is relevant. Thus, in the *Toona ciliata* plants it will be important to incorporate measures of k_{root} in future studies aimed at understanding responses to water deficit and/or high VPD_{air} , since these studies were not found in the literature for *T. ciliata*.

Seedlings exhibited a higher net photosynthetic rate at when compared to the clones, with an average of $7.04 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, and this result was due to a higher stomatal conductance ($0.10 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and transpiration ($3.19 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) (Table 1). The direct relationship between the photosynthetic rate and the stomatal conductance was also observed by Murphy, Jordan and Brodribb (2012) in *Toona ciliata* seedlings, with the highest photosynthetic rate ($9 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) also showed greater stomatal conductance between 10:00 and 13:00 HR, when cultivated under high irradiation.

Figure 2 – Root Hydraulic Conductivity, by the dry mass of the root system (a), the volume of the root system (b), the volume of thin roots (Ø 0 - 2mm) (c), the root surface area (d), thin root surface area (Ø 0 - 2mm) (e) and non-normalized one (f), considering the three clones and seedlings of *Toona ciliata* at 120 days.

Figura 2 – Condutividade hidráulica das raízes pela massa seca do sistema radicular (a), pelo volume do sistema radicular (b), pelo volume das raízes finas (Ø 0-2mm) (c), pela área de superfície das raízes (d), pela área de superfície das raízes finas (Ø 0-2mm) (e) e não normalizada (f), considerando-se os três clones e as mudas propagadas por semente de *Toona ciliata* aos 120 dias.



Fonte: Oliveira, T. P. F. (2016)

Table 1 – Net photosynthetic rate ($\mu\text{molCO}_2\text{m}^{-2}\text{s}^{-1}$), Stomatal conductance ($\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$) and Transpiration ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) of three clones (TC3, TC9 e TC15) and seedlings *Toona ciliata* clones at 120 days, measured between 12:00 and 13:00.

Tabela 1 – Taxa fotossintética líquida, condutância estomática e transpiração de três clones (TC3, TC9 e TC15) e mudas propagadas por semente de *Toona ciliata* aos 120 dias, mensurados entre 12h00 e 13h00.

Variables	Genotypes									
	TC3 Clone		TC9 Clone		TC15 Clone		Seedlings	Average	CV (%)	
Net photosynthetic rate ($\mu\text{molCO}_2\text{m}^{-2}\text{s}^{-1}$)	2.81	BC	1.49	C	4.47	AB	7.04	A	3.95	38.6
Stomatal conductance ($\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}$)	0.03	B	0.01	B	0.03	B	0.10	A	0.04	62.0
Transpiration ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$)	1.09	BC	0.50	C	1.63	B	3.19	A	1.60	38.5

Averages followed by the same capital letter in the line do not differ among themselves by Tukey's test $P < 0.05$.

In the present study, it is not possible to establish a relationship between the gas exchange responses and the hydraulic conductivity of the roots, since there was no statistical difference between the genetic materials (Figure 2). In fact, k_{root} was not correlated with values of the gas exchange across genotypes (Table 3). This response can demonstrate that the k_{root} component may not significantly impact stomatal conductance under these conditions. The leaf hydraulic conductivity may be the most important component in limiting the plant water use.

Plants that have high photosynthetic carbon assimilation generally present higher water losses through transpiration and, generally, great consumption of water that implies in the productivity increment. In this current study, the results demonstrate that, although the net photosynthetic rates of the clone were inferior to the ones presented by the seedlings, the TC3 and TC9 clones were similar to the seedlings for leaf area, stem diameter, root length, root volume, root dry mass and root surface area (Table 2). According to the data presented in Table 2, no difference was observed between the genetic materials (TC3, TC9, TC15 and seedling) when considering the variables of shoot growth, dry mass of root, average diameter of the roots and volume of the thin roots (\varnothing 0- 2mm).

In the current study with *Toona ciliata*, it was observed reduced ratio (RSA/RDM) in the seedlings, but did not influence the kroot, since there was no difference between the genetic material (Table 2 and Figure 2). In rootstocks of peaches with reduced surface area of thin roots per unit of root dry mass (RSA/RDM), Solari, Pernice and Dejong (2006) verified lower kroot when compared to the more vigorous rootstock.

On the other hand, Rieger and Litvin (1999) studied two woody species and three herbaceous species which exhibit a range of root anatomical features: *Asparagus densiflorus* (Kunth) Jessop (asparagus), *Dendrobium superbum* Rchb. F (Dendrobium), *Glycine max* (L.) Merr. (Soybean), *Prunus persica* (L.) Batsch (Peach) and *Citrus aurantium* L. (sour orange). The hydraulic conductivity was inversely related to root diameter and cortex width suggesting that species with thinner roots or roots with a thin cortex had the highest root hydraulic conductivity.

Table 2 – Height (H), stem diameter (SD), leaves number (LN), Leaf area (LA), shoot dry mass (SDM), root dry mass (RDM), total root length (TRL) and length of thin root (LR Ø 0-2mm), average root diameter (RD), total volume (TV) and volume of thin root (VR Ø 0-2mm), thin root surface area (RSA Ø 0-2mm), surface area and roots dry mass ratio (RSA/RDM) of three clones (TC3, TC9 e TC15) and seminiferous *Toona ciliata* clones at 120 days.

Tabela 2 – Altura da parte aérea (H), diâmetro do colo (DAC), número de folhas (NF), área foliar (AF), massa seca da parte aérea (MSPA), massa seca (MSR), comprimento total (CRT) e até 2 mm de diâmetro (CR Ø 0-2 mm), diâmetro médio (DMR), volume total (VR) e até 2 mm de diâmetro (VR Ø 0-2 mm), área superficial de raiz (ASR) e até 2mm de diâmetro (ASR Ø 0-2 mm) e relação área superficial por massa seca de raiz (ASR/MSR) de três clones (TC3, TC9 e TC15) e mudas seminíferas de *Toona ciliata* aos 120 dias.

Variables	Genotypes									
	TC3 Clone		TC9 Clone		TC15 Clone		Seedlings		Average	CV (%)
H (m)	0.111	B	0.108	B	0.136	B	0.220	A	0.146	12.4
SD (m)	0.00600	B	0.00651	AB	0.0072	A	0.00601	B	0.0064	9.8
LN	7.6	B	5.80	B	6.80	B	10.0	A	7.6	17.1
LA (m ²)	0.07102	A	0.06154	A	0.06852	A	0.06207	A	0.06579	17.6
SDM (g)	5.11	AB	5.0	AB	5.83	A	4.21	B	5.04	11.3
RDM (g)	1.83	A	1.96	A	2.37	A	2.18	A	2.09	20.6
TRL (m)	22.64	B	24.77	AB	29.25	A	22.21	B	24.72	14.0
LR (m) Ø 0-2mm	22.192	B	24.073	AB	28.640	A	21.856	B	24.190	13.8
RD (m)	0.000613	A	0.000625	A	0.000632	A	0.000620	A	0.000622	4.7
TV (m ³)	0.0000072	AB	0.0000088	AB	0.0000095	A	0.0000067	B	0.0000081	18.2
VR (m ³) Ø 0-2mm	0.0000066	A	0.0000078	A	0.0000085	A	0.0000062	A	0.0000073	17.6
RSA (m ²)	0.04527	AB	0.05245	AB	0.05896	A	0.04332	B	0.05000	15.8
RSA (m ²) Ø 0-2mm	0.03622	AB	0.04064	AB	0.04658	A	0.03459	B	0.03951	15.5
RSA/ RDM	25.27	A	26.65	A	25.08	A	20.06	B	24.27	9.5

Fonte: Oliveira, T. P. F. (2016)

The difference between the architecture/anatomy of the root system of the seedlings may have contributed to the results in the present study. The tendency of the clones is to present a root system with more root volume, root hair and surface area, when compared to seedlings. The seedlings present higher amount of thick and lignified roots. Anatomically, an adventitious root may have anatomical differences from a primary root formed by the seedlings, which can affect root hydraulic conductivity and consequently gas exchange, growth, and productivity. Jesus *et al.* (2010) verified that there is an anatomical difference between adventitious roots and primary root for the *Coffea arabica* Rubi cultivar. The adventitious roots presented larger diameter of the vascular cylinder in relation to the primary root of the seedlings. These authors report that there may be an increase in the hydraulic conductivity of these adventitious roots, assuming the root hydraulic conductivity is directly related to the diameter of the vascular cylinder.

The results of the gas exchange shown in Table 1 corroborate the data presented in Table 3, in which a significant and positive correlation between net photosynthetic rate (A) and stomatal conductance, and between A and E , are shown (Table 3). There was a negative correlation between k_{root} and root dry mass (RDM), (Table 3). This result showed that more biomass production reduced k_{root} in 120 days after planting. We can hypothesize that the root system confinement in *Toona ciliata* clones and seedlings promoted root deformation, reducing k_{root} . Actually, root confinement in pots could negatively impact the formation of new roots, since there is limited physical space for the growth of fine roots, which are responsible for the higher uptake of water and mineral nutrients (RICHARDS; ROWE, 1977). This reduction in the physical space can result in the reduction in the k_{root} , since the quantity of thick and more suberized roots would be bigger and could contribute to the elevation of the root dry mass and root volume. Possibly, if the plants of the current study remained for less time in the greenhouse, the root restriction would be minimized and an increment in the hydraulic conductivity of the roots could be observed.

Another indicative that these plants may be under a root confinement are the negative correlations between the gas exchange and the root growth. However, in the current study, these correlations were negative and were not significant (Table 3). The studies developed by Ronchi *et al.* (2006) and Shi *et al.* (2008) have demonstrated that there was a reduction in the net photosynthetic rate when the root restriction in seedlings of *Coffea arabica* and *Lycopersicon esculentum* increased, due to stomatal or non-stomatal effects. Ronchi *et al.* (2006) reported that the severe reduction of photosynthesis in those seedlings grown under root restriction occurred due to the non-stomatal factors, such as decreased Rubisco activity. Shi *et al.* (2008) verified that the reduction of CO_2 assimilation rates was largely due stomatal limitation. Reduced values in leaf water potential, stomatal conductance, transpiration, intracellular CO_2 concentration and increased stomatal limitation and the xylem sap ABA concentration were also found in these seedlings.

Figueiredo *et al.* (2014b) evaluated the influence of the permanency time of the three eucalyptus clones under no coverage, at the nursery (clear sky), on the net photosynthetic rate and observed that the longest time of permanency (180 days) of the clones in pots of 53 cm^3 resulted in reductions in the net photosynthetic capacity, evidencing that in these conditions the clones were affected with the mechanical root restrictions.

There were significant positive correlations between stem diameter with total root length (TRL), root volume (RV), and root surface area (RSA). There were also positive correlations between the root dry mass (DMR) and the volume, the surface area of total roots, and the volume expressed in roots smaller than 2mm. The DMR was also positively correlated to the length of the roots. In addition, there were positive correlations with A versus g_s and A versus E (Table 3). The genotypes that exhibited higher $iWUE$ were the TC9 and TC15 clones. The seedlings exhibited a lower $iWUE$ and WUE ($A/g_s - 77.7\ \mu\text{mol CO}_2\ \text{mol H}_2\text{O}^{-1}$; $A/E - 2.18\ \mu\text{mol CO}_2\ \text{mmol H}_2\text{O}^{-1}$), although it did not differ from the TC3 clone ($A/E - 2.57\ \mu\text{mol CO}_2\ \text{mmol H}_2\text{O}^{-1}$; $A/g_s - 101.8\ \mu\text{mol CO}_2\ \text{mol H}_2\text{O}^{-1}$) (Table 4). The $iWUE$ and WUE of clone TC9 were 55% and 27% higher than the seedlings (Table 4).

Although seedlings exhibited elevated net photosynthetic rate, they showed reduced $iWUE$ (A/g_s) and WUE (A/E). This result contrasted with the TC9 clone which, besides the reduced value of A and E , presented a higher $iWUE$ among the genetic materials studied (Table 1 and 4). As g_s was very reduced in the seedlings of the TC9 clone, this reduction suggests these seedlings were submitted to reduced tensions of the xylem in relation to the other genotypes.

According to El-Sharkawy, Cock and Hernandez (1985), the reduction in the stomatal conductance can reduce the water loss of the leaves, causing an increase in the water use efficiency. Results obtained by Rocha and Moraes (1997) using young plants of *Stryphondendron adstringens* corroborate this current study with *Toona ciliata* seedlings, in which the increase in the water use efficiency occurred in the genotypes with lower values of stomatal conductance

and transpiration. The authors demonstrate that *Stryphondendron adstringens* is well adapted to the conditions of water limitation in the soil.

Table 3 – Pearson’s correlation between the variables: root hydraulic conductivity, stem diameter (SD), leaf area (LA), root dry mass (RDM), root diameter (RD), diameter of thin roots (DFR_{Ø 0-2mm}), total root length (TRL), root length of thin roots (RL_{Ø 0-2mm}), root volume (RV), root volume of thin roots (RV_{Ø 0-2mm}), root surface area (RSA), thin root surface area (RSA_{Ø 0-2mm}), net photosynthetic rate (A); stomatal conductance (g_s); transpiration (E) of *Toona ciliata* seedlings and clones.

Tabela 3 – Correlação de Pearson entre as variáveis, condutividade hidráulica das raízes (K_{raiz}) em quatro pressões (0,1; 0,2; 0,3 e 0,4 MPa), diâmetro do colo (DAC), área foliar (AF), massa seca das raízes (MSR), diâmetro médio das raízes (DMR), diâmetro médio das raízes até 2 mm de diâmetro (DMR_{Ø 0-2 mm}), comprimento das raízes (CR), comprimento de raízes até 2 mm de diâmetro (CR_{Ø 0-2 mm}), volume das raízes (V), volume das raízes finas (V Ø 0 – 2 mm), área superficial das raízes (ASR), área superficial de raízes finas (ASR_{Ø 0-2 mm}), fotossíntese (A); condutância (g_s); transpiração (E) de mudas seminíferas e clonais de *Toona ciliata*.

Va- ria- bles	k _{root}	SD	LA	RDM	RD	DMR Ø 0-2mm	TRL	RL Ø 0-2mm	RV	RV Ø 0-2mm	RSA	RSA Ø 0-2mm	A	g _s	E
k _{root}	1														
SD	0,16	1													
LA	-0,03	-0,23	1												
RDM	-0,25*	0,3	-0,07	1											
RD	-0,04	0,33	-0,01	0,25	1										
DMR Ø 0-2mm	-0,12	0,25	0,13	0,13	0,93*	1									
TRL	-0,24	0,44*	0,06	0,78*	0,37	0,29	1								
RL Ø 0-2mm	-0,24	0,44	0,06	0,79*	0,35	0,28	0,99*	1							
RV	-0,21	0,46*	0,04	0,68*	0,74*	0,65*	0,88*	0,87*	1						
RV Ø 0-2mm	-0,29	0,42	0,14	0,68*	0,66*	0,63*	0,90*	0,90*	0,96*	1					
RSA	-0,23	0,47*	0,05	0,73*	0,58*	0,50*	0,66*	0,67*	0,97*	0,97*	1				
RSA Ø 0-2mm	-0,25	0,46*	0,1	0,73*	0,55*	0,50*	0,69*	0,70*	0,95*	0,97*	0,99*	1			
A	0,03	0,01	-0,29	0,14	-0,25	-0,17	-0,13	-0,11	-0,24	-0,16	-0,19	-0,16	1		
g _s	0	-0,13	-0,37	-0,03	-0,3	-0,22	-0,33	-0,32	-0,4	-0,33	-0,36	-0,34	0,96*	1	
E	0,02	-0,09	-0,3	0,12	-0,31	-0,24	-0,21	-0,19	-0,32	-0,24	-0,28	-0,24	0,99*	0,98*	1

Fonte: Oliveira, T. P. F. (2016)

Table 4 – Intrinsic water use efficiency ($\mu\text{molCO}_2 \text{ mol H}_2\text{O}^{-1}$) and water use efficiency ($\mu\text{molCO}_2 \text{ mmol H}_2\text{O}^{-1}$) of three clones (TC3, TC9 e TC15) and seedlings *Toona ciliata* clones at 120 days.

Tabela 4 – Eficiência intrínseca e instantânea do uso da água de três clones (TC3, TC9 e TC15) e de mudas propagadas por sementes de *Toona ciliata* aos 120 dias.

Variables	Genotypes									
	TC3 Clone		TC9 Clone		TC15 Clone		Seedlings		Average	CV (%)
Intrinsic water use efficiency ($\mu\text{molCO}_2 \text{ mol H}_2\text{O}^{-1}$)	101.8	C	172.5	A	130.1	B	77.7	C	120.5	12.3
Water use efficiency ($\mu\text{molCO}_2 \text{ mmol H}_2\text{O}^{-1}$)	2.6	AB	3.0	A	2.7	A	2.2	B	2.6	10.1

Averages followed by the same capital letter in the line do not differ among themselves by Tukey's test $P < 0.05$.

The difference found between the genetic materials in the present study can use as a basis for future studies of *Toona ciliata* genotype selection. Among the clone studied, the TC3 showed to be a promising clone in the aspects related to the water conservation in shoot, since this clone has a higher k_{root} and an efficient control of the water loss through the leaves. However, new studies need to be done in order to obtain more conclusive information. The results can show a special attention to obtain more research related to possible relations between the root hydraulic conductivity and root restriction in genotypes of *Toona ciliata*, as well as further studies needed to verify the behavior of the clones studied when considering water deficit.

Conclusion

The genetic materials studied exhibited an equal capacity of water absorption and transport by the roots, even though they exhibited visual anatomical differences of the root system. Although the clones presented low transpiration and net photosynthetic rate they were generally more efficient in water use, and the TC3 and TC9 clones were more efficient to convert the assimilated carbon to biomass.

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