

## Physical-Anatomical and Initial Growth of *Tabebuia roseoalba* (Ridl.) under Different Water Regimes

Silvana de Paula Quintão Scalón<sup>1</sup>  0000-0003-2024-7695

Rosilda Mara Mussury<sup>1</sup>  0000-0002-8961-9146

### Abstract

The present work evaluated the physical-anatomical and initial growth of *Tabebuia roseoalba* seedlings exposed to different water regimes. The seedlings were grown under 100, 50, 25 and 12.5% of the water retention capacity (WRC). After 90 days, the following aspects were evaluated: height, diameter, fresh and dry weights of aerial and root, root length, and leaf area, Dickson quality index, liquid assimilation rate, relative growth rate, specific leaf area, stomatal index, and number of trichomes and leaf epidermal cells. The results indicated seedlings have greater initial growth and quality when cultivated at 100% WRC, and 25 and 12.5% WRC represented stress conditions for the plants. The species is hypostomatous with anomocytic stomata. The number of stomata, the stomatal index, and the number of epidermal cells did diminish with reduced water availability. The number of trichomes were larger on the adaxial face as water availability decreased.

**Keywords:** anatomy, seedling production, water stress.

### 1. Introduction and objectives

One of the greatest difficulties to cultivate native species is the available water in the soil, and there are few studies demonstrating this availability in the soil and its transfer to the plants, which can affect the establishment of individuals (Chirino et al., 2017).

Under conditions of low soil water availability, some metabolic processes are affected, with the partial or total closing of the stomata to limit water loss and consequent reduction in CO<sub>2</sub> acquisition, which can lead to negative growth affects – including reduction of leaf expansion. Moreover, it can also accelerate their senescence and abscission (which will directly affect photosynthesis), leaf abscission, inhibiting the internodes elongation and affecting root and shoot growth, the increased deposition of wax and increased trichome growth on the leaf surfaces (Jeromine et al., 2019; Scalón et al., 2011; Rosa et al., 2017; Silva et al., 2016).

According to a review by Abbade & Takaki (2014) the “Ipê-branco”, *Tabebuia roseoalba* (Ridl.) Sandwith, is a tree species widely used in urban forestry and recovery of degraded

areas with great ornamental, medicinal and commercial value. The recommended mixture for seedling production (soil + sand + semi-decomposed chicken-bed wastes) promotes good seedling heights, radical-hypocotyl transition zone diameters, chlorophyll indices, leaf areas, root lengths, and dry weights of both the aerial and root portions (Macedo et al., 2011).

Seedling quality is an extremely important factor for successful reforestation, and they must be produced in sufficient quantity and quality to overcome adversities they will inevitably experience under field conditions and to have high survival percentages (Farias et al., 2007).

As there is very little information available about the eco-physiology and leaf anatomical of this species, the present work sought to evaluate the physical-anatomical and initial growth of *Tabebuia roseoalba* (ipê-branco) seedlings under different soil-water regimes.

### 2. Materials and methods

The seeds were sown into expanded polystyrene (Isopor®) germination trays with 128 cells containing Bioplant® commercial

<sup>1</sup> Universidade Federal da Grande Dourados (UFGD), Dourados, MS, Brasil

germination substrate and subsequently maintained in an outdoor nursery covered with shade screen (50%).

After germination and initial growth, approximately 15 cm tall seedlings were transplanted to plastic bags (3 kg capacity) containing soil + sand + semi-decomposed chicken-bed wastes (1:1:1) substrate and maintained under 50% shading for 10 more days. At the end of this period the plants were exposed to the following water regimes: 100, 50, 25 and 12.5% water retention capacity (WRC), calculated based on the soil substrate density (Souza et al., 2000).

After 90 days of treatment, three seedlings from each treatment condition were evaluated in terms of stem height, radical-hypocotyl transition zone diameter, fresh and dry weights of the aerial and root portions (FWAP; FWRP; DWAP and DWRP respectively – g), average root length (ARL – cm), and leaf area (LA – cm<sup>2</sup>) using a Li Cor 3000 leaf area meter.

The Dickson quality index was calculated using the Equation 1 (Dickson et al., 1960).

$$DQI = [\text{total dry material} / (\text{RAD} + \text{RAPR})] \quad (1)$$

Where RAD: height/diameter ratio, RAPR: the ratio between the dry weight of the aerial portion/dry weight of the root.

We also analyzed the liquid assimilation rate (LAR – g cm<sup>-2</sup> day g<sup>-1</sup>), relative growth rate (RGR – g g<sup>-1</sup>), and specific leaf area (LRA – cm<sup>2</sup> g<sup>-1</sup>) expressed according to Equation 2, 3 and 4.

$$RGR = (\ln P_2 - \ln P_1) / (t_2 - t_1) \text{ g. g}^{-1} \cdot \text{day}^{-1} \quad (2)$$

$$LAR = P_2 - P_1 / A_2 - A_1 \times \ln A_2 - \ln A_1 / t_2 - t_1 \text{ g. dm}^2 \cdot \text{day}^{-1} \quad (3)$$

$$LRA = A / P \quad (4)$$

Where ln: Napierian logarithm; P: total mass of plant; t<sub>2</sub> - t<sub>1</sub>: interval between evaluations; A: leaf area of plant.

The experiment was conducted with three repetitions of 12 seedlings each. The results were analyzed for variance, and the averages compared using the Tukey's test at a 5% level of probability.

Ten totally expanded leaves from different plants subjected to each of the treatments were used for anatomical studies. Free-hand paradermal cuts were made in the median region

of the adaxial and abaxial portions of the leaf with a razor blade. Leaf surface impressions were made in the same areas with Super Bonder<sup>®</sup> glue to analyze the shape and number of common epidermal cells, the characteristics of the stomata on the abaxial and adaxial leaf faces, and the quantity of trichomes. Three optical fields were examined in the median region of the leaf blade, for a total of 30 fields per treatment. Stomatal counts were made using a Coleman light microscope with a camera lucida attachment, following the methodology described by Labouriau et al. (1961).

The stomatal index (SI) was calculated with the Equation 5 (Wilkinson, 1979 as cited in Hu et al., 2015).

$$SI(\%) = SD / (SD + ED) \times 100 \quad (5)$$

Where Sn: number of stomata; Em: number of epidermal cells.

The results were documented as drawings made with the aid of the camera lucida, with its appropriate scales.

### 3. Results

The evaluation of seedling growth of *Tabebuia roseoalba* after 90 days in each treatment regime indicated the plants achieved the greatest maximum height when exposed to conditions of high water availability, and were 50% smaller under restricted water conditions; height values did not vary significantly among the intermediate treatments. Stem diameters were greater at 100% WRC (Table 1).

The average root lengths of *T. roseoalba* were greater at 100% WRC and smaller at 12.5% WRC. The leaf area of *T. roseoalba* seedlings were likewise greater with higher water availability, and decreased under water-deficit conditions (Table 1).

The average values of the fresh and dry weights of the aerial portions and roots of *T. roseoalba* seedlings were lower under conditions of restricted water availability, with FWAP values being 73% and 92% lower under 25% and 12.5% WRC respectively, while FWRP, DWAP and DWRP values were 50% lower on the average (Table 1), indicating that the *T. roseoalba* aerial portion was more influenced by water deficit conditions than the root system.

The Dickson quality index (DQI) was higher in seedlings cultivated at 100% WRC, and lower with decreasing soil-water availability (although without significant differences between the 25 and 12.5% WRC conditions) (Table 2). This indicates that the best growing conditions were provided under the 100% WRC regime.

As the seedlings cultivated under 12.5% WRC conditions did not demonstrate satisfactory growth or healthy aspects,

they were not evaluated in terms of LRA, RGR, or LAR – which were significantly higher in seedlings grown at 100% WRC (Table 2), with larger leaf areas and presumed greater production of photoassimilates.

Ostiole openings and stomatal indices were greater at 100% WRC. The closing of the stomata under water-stress conditions was observed at 12.5% WRA (Table 3).

The paradermal sections showed that *T. roseoalba* is a hypostomatous species with anomocytic stomata and star-shaped trichomes on both leaf surfaces (Table 3 and Figure 1).

Stomata were sporadically observed on the adaxial leaf face near the central vein (Figure 1g). The cell walls on the abaxial face (Figure 1a, b, c and d) were more sinuous than those on the adaxial face (Figure 1e, f, g and h), although no modifications were observed in these structures in response to the deficit water regimes.

The number of stomata, the stomatal index, and the quantity of epidermal cells did diminish with reduced water availability; however, the trichomes on the abaxial face did not vary with different water regimes, although a lot of these structures were observed on the adaxial face as water availability decreased.

**Table 1.** Height, diameter, average root length (ARL), leaf area, fresh weight of the aerial portion (FWAP), fresh weight of the root (FWRP), dry weight of the aerial portion (DWAP) and dry weight of the roots (DWRP) of *Tabebuia roseoalba* seedlings after 90 days of growth under different soil water conditions.

Water regimes (% WRC)	Height (cm)	Diameter (mm)	ARL (cm)	Leaf area (cm) <sup>2</sup>
100	28.16 a	11.99 a	30.50 a	831.89 a
50	25.49 ab	9.31 b	24.66 ab	716.43 b
25	17.88 bc	8.54 b	24.33 ab	296.59 c
12.5	12.05 c	8.52 b	21.50 b	80.89 d
	FWAP(g)	FWRP(g)	DWAP(g)	DWRP(g)
100	17.96 a	23.02 a	6.60 a	8.72 a
50	14.60 a	18.26 b	5.93 b	6.07 b
25	4.80 b	14.25 c	4.01 c	4.68 c
12.5	1.30 b	12.44 d	3.38 d	3.05 d

Averages followed by the same lowercase letters in a column are statistically equivalent according to the Tukey's test at a 5% level of significance.

**Table 2.** Dickson quality index, liquid assimilation rate (LAR), relative growth rate (RGR), and specific leaf area (LRA) of *Tabebuia roseoalba* seedlings after 90 days of growth under different soil water conditions.

Water regimes (% WRC)	DQI	LAR (g cm <sup>2</sup> d <sup>-1</sup> )	RGR (g g <sup>-1</sup> d)	LRA (cm <sup>2</sup> g <sup>-1</sup> )	Opening ostiole (mm)
100	4.94a	0.2643 a	0.0168 a	72.41 a	31.5 a
50	3.23b	0.0007 b	0.0102 b	58.41 b	26.3 b
25	2.95c	0.0002 c	0.0027 c	29.94 c	22.0 c
12.5	2.90c	–	–	–	–

Averages followed by the same lowercase letters in a column are statistically equivalent according to the Tukey's test at a 5% level of significance.

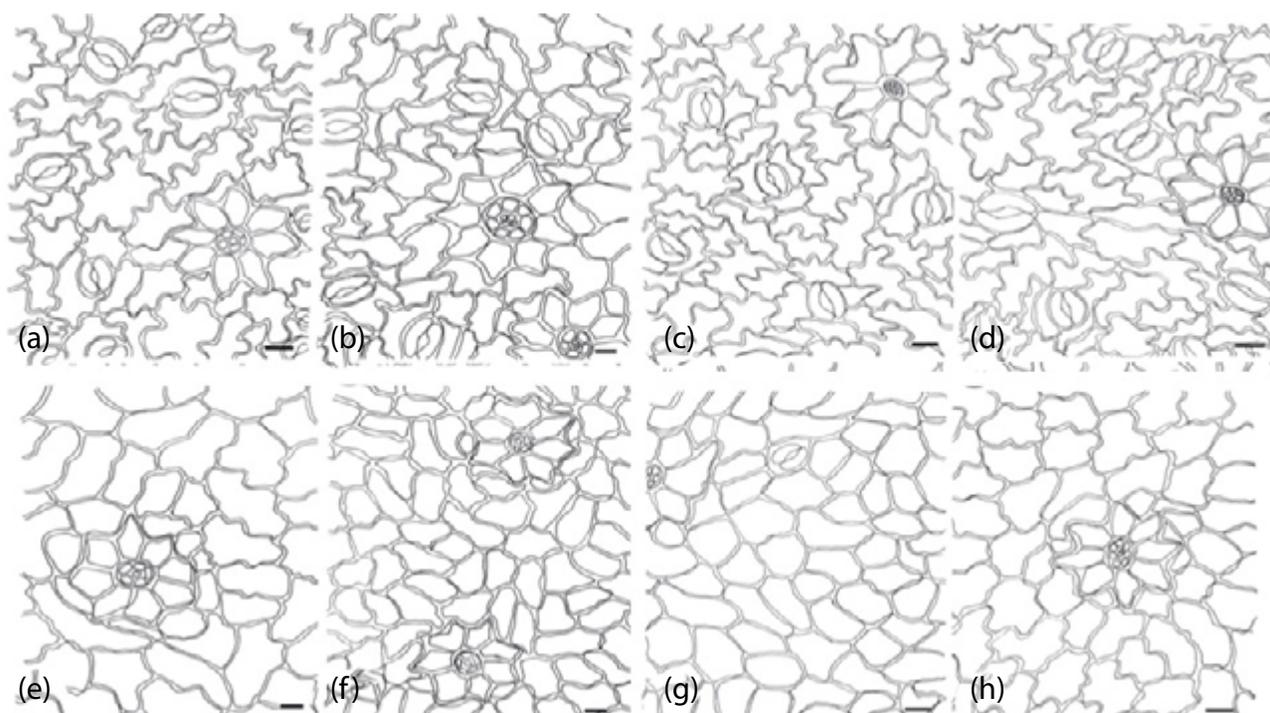
**Table 3.** Numbers of stomata, trichomes, epidermal cells, stomatal indices, and ostiole openings on the leaves of *Tabebuia roseoalba* seedlings after 90 days of exposure to different soil water regimes.

Face leaf / WRC	Stomata	Trichomes	Epidermal cells	Stomatal indices
	<b>Abaxial</b>			
12.5 %	14.2 c	15.8 a	2.71 b	78.19 c
25 %	24.9 c	15.53 a	3.96 b	83.75 c
50 %	33.0 b	11.61a	4.83 b	92.44 b

**Table 2.** Continued...

Face leaf / WRC	Stomata	Trichomes	Epidermal cells	Stomatal indices
100 %	48.2 a	16.46 a	10.81a	81.69 a
<b>Adaxial</b>				
12.5 %	0.0	18.81 a	9.23 a	0.0
25 %	0.0	13.66 b	3.91 b	0.0
50 %	0.0	13.61 b	1.51 c	0.0
100 %	0.0	8.46 c	1.46 c	0.0

Averages followed by the same lowercase letters in a column are statistically equivalent according to the Tukey’s test at a 5% level of significance.



**Figure 1.** Paradermal sections of *Tabebuia roseoalba* leaves exposed to different water retention capacity. Abaxial face: (a) 100% WRC, (b) 50% WRC, (c) 25% WRC and (d) 12.5% WRC. Adaxial face: (e) 100% WRC, (f) 50% WRC, (g) 25% WRC and (h) 12.5% WRC. Bar: 50µm.

#### 4. Discussion

Based on the results of this study, we concluded that all aspects of plant growth and development are affected by soil water deficiencies and the precise effects of these deficits on plant development will depend on the intensity and duration of the stress, on the growth stage of the plant and genetic responses – resulting in diverse morpho-physiological modifications or even plant death (Martins et al., 2010).

It is observed in the literature that, for overcoming the water deficit stress, plants invest in root biomass with reduction of leaf expansion and, consequently, reduced consumption of carbon, being observed reductions in the growth of shoots

(Carvalho et al., 2012.), leaf area (Jeromine et al., 2019; Mar et al., 2013; Scalón et al., 2011) and increased ratio of root biomass and shoot (Silva et al., 2016; Verma et al., 2012). However, this behavior was not observed in *T. roseoalba* seedlings, probably due to the assessed stage of development or the period in which they remained in culture conditions

Plant responses to soil water conditions are quite variable, but tree species tend to attain greater heights and diameter as soil-water becomes more available. Seedlings of *Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex S. Moore, *Alibertia edulis* (Rich) A. Rich. ex DC., *Guazuma ulmifolia* Lam., *Hymenaea coubaril* L. demonstrated greater heights when cultivated under conditions of greater water availability

(Cabral et al., 2004; Jeromine et al., 2019; Scalón et al., 2011; Silva et al., 2016, respectively).

*Qualea grandiflora* Mart. seedlings native from the Cerrado (Brazilian savanna) did not show significant differences in terms of stem diameter when grown at different water retention capacity (12.5; 50 and 100%), showing linear growth during 120 days of exposure to all treatments (Vieira & Gomes, 2011). These results indicate that the duration of exposure to deficit water conditions must be considered in growth evaluations. Increases in stem diameter depend on cambium layer activities, which are stimulated, in turn, by photosynthetic production and by hormones translocated from apical sites (Aguilar et al., 2011).

Jeromine et al. (2019) and Rosa et al. (2017) suggest that the balance between assimilate production and demand can be severely affected under water-deficit conditions due to reductions in photosynthetically productive leaf areas. The growth of the root system into the deepest soil levels allows plants to take advantage of the increased humidity and soil fertility at lower depths (depending on the morphological and genetic characteristics of the plant). Water-deficit conditions will diminish mitotic activity in plant cells and initiate the synthesis of abscisic acid in the roots, which is transported to different parts of the plant and can provoke (among other responses) precocious reproduction and leaf abscission (this helps to explain significant reductions of leaf area seen in the water-stressed *T. roseoalba* seedlings).

Similarly, *G. ulmifolia* seedlings did not have significant differences between the lengths of the largest roots after 35 days of cultivation under the same conditions as the present research, although the seedlings demonstrated higher root dry weights at 100 and 50% WRC; seedlings grown at 12.5% WRC died before the 83rd day of the experiment (Scalón et al., 2011).

Similar results were observed with *T. aurea* seedlings, which did not show any differences in relation to the ratio of the dry matter of the roots/dry mass aerial portions (DMRP/DMAP) after two days of treatment. However, starting at 90 days, the 25% WRC treatment seedlings were significantly smaller than the others (Cabral et al., 2004) indicating, once again, the importance of exposure time.

These results indicate that water deficits can severely affect growth and fresh and dry biomass accumulation in plants (Silva et al., 2016). Jeromine et al. (2019) observed that seedlings with greater root dry weights probably have greater chances of surviving water-stress during the dry season in the natural environment, and this characteristic is therefore extremely important for the successful growth of seedlings.

Another result that supported the above-mentioned analysis was the observation that the greatest water availability (100% WRC) yielded the greatest increase in plant biomass;

severe water deficiencies (12.5% WRC) resulted in reduced growth, indicating *T. roseoalba* seedlings could not tolerate water deficit conditions for 90-day periods.

In the review by Gordin et al. (2016), we observed that leaf area is an important factor in plant photosynthetic production and will determine plant water-use, considering productivity will be severely inhibited under deficit water conditions. As such, under water-stress conditions, when there is a decrease in cell turgidity, there will be reductions in leaf expansion and size.

There are many references in the literature reporting the leaf area tends to be lower under conditions of lower water availability, including Cabral et al. (2004) for *T. aurea*, Scalón et al. (2011) for *G. ulmifolia*, Vieira & Gomes (2011) for *Q. grandiflora*, Silva et al. (2016) for *Hymeneae coubaril*, Gordin et al. (2016) for *Hancornia speciosa*, and Jeromine et al. (2019) for *A. edulis* seedlings.

Dickson quality index is an appropriate parameter to measure the quality of seedlings, because it defines the strength and balance of biomass distribution in the change (Freitas et al., 2018; Gordin et al., 2016; Jeromine et al., 2019) – the higher the value, the better the quality changes (César et al., 2014)

Ostiole openings and stomatal indices were greater at 100% WRC, indicating that the plants could better absorb atmospheric CO<sub>2</sub> and would have higher photosynthetic rates under these conditions. The closing of the stomata under water-stress compromises CO<sub>2</sub> acquisition and reduces biomass production and plant productivity – yielding smaller plants with reduced dry weight accumulations, as reported by Rosa et al. (2017) for *Copaifera langsdorffii* Desf., as well as in the study presented here.

Other factors such as changes in plant hormonal balances or reductions in soil nutrient uptake, translocation, respiration or protein metabolism will also reduce plant growth and help to explain the reductions in the dry weight of the aerial and root portions of plants and diminished root lengths as water-stress became more severe, according to the results found for *C. langsdorffii* (Rosa et al., 2017), *Hymeneae coubaril* L. (Freitas et al., 2018) and *A. edulis* (Jeromine et al., 2019).

The water deficit caused stomatal changes (density, index, opening, size, diameters) related to the regulation of gas exchange under stress conditions. Therefore, leaves with fewer stomatal openings show higher efficiency under water-deficit conditions, because they have smaller stomatal pores, which reduces water loss through transpiration (Rosa et al., 2017). This tendency was observed in young plants exposed to 25% WRC in relation to 50% WRC in the present work. Decreases in the number of stomata were also observed in young *T. aurea* – plants experiencing water deficits (25% WRC) showed decreases in the number of

stomata after 90 to 120 days as compared to 100% WRC treatments (Cabral et al., 2004).

Plants can change their phenotype in response to abiotic stress, a phenomenon known as phenotypic plasticity. This plasticity can result in anatomical changes of the leaves to mitigate the effects of abiotic extremes. Silva et al. (2005) observed in their review that responses involve changes in leaf area, leaf orientation and position foliage, trichomes and waxy cuticles, which affects even the growth of plants under low soil-water conditions.

## 5. Conclusions

*Tabebuia roseoalba* seedlings had greater initial growth and quality when cultivated at 100% water retention capacity, while seedlings grown under 25 and 12.5% WRC demonstrated stress characteristics.

The species is hypostomatous with anomocytic stomata. The numbers of stomata, the stomatal index, and the numbers of epidermal cells did diminish with reduced water availability. The trichomes on the abaxial face did not vary among the different water regimes, although many of these structures were observed on the adaxial face as water availability decreased.

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## Correspondence to

**Silvana de Paula Quintão Scalón**

Rod. Dourados Itahum, km 12, CEP 79825-070, Dourados, MS, Brasil  
e-mail: silvanascalon@ufgd.edu.br

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