



## Sap flow and water use in African baobab (*Adansonia digitata* L.) seedlings in response to drought stress

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### ABSTRACT

The African baobab (*Adansonia digitata* L.) is an important multi-purpose fruit tree with high potential for domestication in drier Africa. Although adult individuals are well-known to be drought resistant, only little has been reported on how young baobab trees can survive drought. Therefore, the aim of this study was to examine short-term soil drought effects on water relations of baobab seedlings. Baobab seedlings used a limited amount of stored water to buffer daily water deficits ( $\sim 8.5 \text{ g d}^{-1}$ ), which contributed up to only  $\sim 17.5\%$  of daily water use and  $\sim 6\%$  of total plant water. Under drought, a strong reduction in stomatal conductance ( $\sim 85\%$ ) resulted in a midday leaf water potential of  $-1 \text{ MPa}$  and zero stem sap flow followed by significant leaf loss. Plant anatomy evidenced the presence of water storage tissues and the vulnerability to xylem embolism. The taproot was the most important plant part for water storage (68% of total plant water), suggesting root-succulence rather than stem-succulence. When drought intensified, limitation of leaf transpiration and/or root water uptake led to drought-enforced dormancy. Despite the large amounts of water stored in the taproot ( $\sim 90\%$ ) and the stem ( $\sim 75\%$ ), only a limited amount of stored water appeared to be used to sustain upper leaves and plant metabolism during the dormant period, and to facilitate recovery following water supply. Drought avoidance, conservative water use and the presence of internal stored water allow baobab seedlings to survive drought.

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### 1. Introduction

The baobab tree (*Adansonia digitata* L.; family Malvaceae) is native to (semi-) arid Sub-Saharan Africa (Sidibé and Williams, 2002) and now appears in most of Sub-Saharan Africa's semi-arid and sub-humid regions as well as in western Madagascar (Diop et al., 2006). Baobab is considered as a multi-purpose tree species, because it has multiple applications ranging from food and fodder uses, to traditional medicine, cultural purposes, and trading (De Caluwé et al., 2009; De Caluwé et al., 2010; Kamatou et al., 2011). A full description of the species and its current and potential uses can be found in Wickens and Lowe (2008).

**Abbreviations:** D, stem diameter fluctuations (mm); DG, daily stem growth ( $\text{mm d}^{-1}$ ); DN, day number; DW, oven-dry mass (g) with subscript l = leaves, s = stems, r = roots; DWSC, total daily stem water storage capacity ( $\text{g d}^{-1}$ ); E, transpiration rate ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ); F, sap flow rate ( $\text{g h}^{-1}$ );  $F_b$ , sap flow rate at the stem base ( $\text{mg m}^{-2} \text{ s}^{-1}$ );  $F_t$ , sap flow rate at the stem top ( $\text{mg m}^{-2} \text{ s}^{-1}$ ); FW, fresh mass (g) with subscript l = leaves, s = stems, r = roots;  $g_s$ , stomatal conductance to water vapor ( $\text{mol m}^{-2} \text{ s}^{-1}$ ); MDS, maximum daily stem shrinkage (mm); PAR, photosynthetic active radiation ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ); RD, root density ( $\text{g cm}^{-3}$ ); RH, relative humidity (%); T, air temperature ( $^{\circ}\text{C}$ ); VPD, vapor pressure deficit (kPa); WC, water content (%) with subscript l = leaves, s = stems r = roots; WD, wood density ( $\text{g cm}^{-3}$ );  $\Psi_l$ , leaf water potential (MPa).

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Baobab is commonly integrated in agroforestry parkland systems (Boffa, 1999) and is identified by local West African communities as priority species for domestication (Kalinganire et al., 2008). Despite its importance, it has been recognized that there is a lack of natural regeneration in most baobab populations which has been related to (i) severe drought events, (ii) over-exploitation (extensive leaf pruning and fruit harvesting reducing fruit production and seed dispersal), (iii) grazing and (iv) rapid shifts in land use patterns and deforestation (Dhillon and Gustad, 2004; Assogbadjo et al., 2008; Buchmann et al., 2010; Schumann et al., 2010; Venter and Witkowski, 2013). For this reason, awareness is growing that cultivating and planting baobabs will be very important in satisfying future local and international demand, thereby reducing the pressure on natural baobab populations.

Tree planting for domestication and reforestation requires access to water which is often a limited resource. Although baobab is well-known for its drought resistance, only few studies have examined the water relations of the adult trees during the dry season (Fenner, 1980; Chapotin et al., 2006a,b) and the mechanisms seedlings use to withstand drought periods (De Smedt et al., 2012; Randriamanana et al., 2012). The published studies on baobab seedlings, in contrast with the studies on adult baobab trees, did not assess the dynamics of the transpiration stream, which reflects the species' water use strategy to cope with drought (Chirino et al., 2011) nor did they determine whether these seedlings relied on internal stored water to buffer water deficits. In this study, we therefore examined sap flow and stem diameter fluctuations of baobab seedlings in response to drought stress, by shortly

withholding water from the soil. In addition, we also investigated leaf stomatal behavior (stomatal conductance), turgor maintenance (leaf water potential and leaf phenology), wood/root properties (density) and plant anatomy to learn more about its water use and storage.

Based on the general postulate that baobab is a deciduous stem-succulent tree species, we hypothesized that baobab seedlings also use a water conservative strategy to survive drought stress by:

- (1) regulating stomatal closure and maintaining turgor;
- (2) minimizing water use and growth;
- (3) shedding its leaves; and
- (4) possessing a large amount of stored water in the stem.

## 2. Materials and methods

### 2.1. Plant material and experimental design

A batch of baobab seedlings (*A. digitata* L.) ( $n = 27$ ) were grown in a growth chamber (Snijders Scientific B.V., Economic Delux, Tilburg, The Netherlands) at the University of Antwerp in containers of 3.5 dm<sup>3</sup> (12 cm diameter × 40 cm height). The containers were filled with riverine sand (grain size ~0.2 mm) and a controlled release fertilizer (Scotts International B.V., Osmocote Exact Standard 5–6 M, Geldermalsen, The Netherlands; 15% N, 3.9% P and 9.9% K) was added. Before the start of the experiment, soil water content was maintained close to field capacity by watering seedlings twice a week. The experiment started in October 2010 and ended in January 2011.

Plant material (i.e. seeds) originated from Tatakarak, a northern province in the Sudano-Sahelian zone of Mali (15°4'N, 0°53'W, West-Africa), which is, according to the WorldClim database of Hijmans et al. (2005), characterized by a mean annual precipitation of 336 mm. More information on seed collection and provenance characteristics can be found in De Smedt et al. (2011). In order to improve germination, seeds were pre-treated with concentrated (96%) sulfuric acid for 6 h, after which they were soaked in water for 24 h (Danthu et al., 1995; Sidibé and Williams, 2002; Razanameharizaka et al., 2006).

The growth chamber allowed for control of radiation and air temperature regime. The daytime period was selected to last from 6 am until 6 pm. Photosynthetic active radiation (PAR) was measured with a quantum sensor (PAR-logger, Li-190 Quantum Sensor, Li-Cor, Lincoln, NE, USA) and yielded 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during the daytime period (the maximum light intensity of the growth chamber). The imposed PAR value was very low compared to midday tropical light intensity but similar to light conditions that seedlings receive in excessive shade in nurseries or in early morning and late evening when grown under baobab trees. Air temperature ( $T$ , °C) and relative humidity (RH, %) were continuously recorded using a mini data logger (Helios mini, Skye Instrument Ltd., Powys, UK). Vapor pressure deficit of the air (VPD, kPa) was calculated, based on measurements of  $T$  and RH, as the difference between saturation vapor pressure at air temperature ( $e_v^0$ , kPa) and ambient vapor pressure ( $e_v$ , kPa). During daytime, the average  $T$  and RH were 27.5 °C and 63%, respectively, which corresponded with a VPD of circa 1 kPa. During night time, the average  $T$  and RH were 20.5 °C and 68%, which corresponded with a VPD of circa 0.5 kPa.

At the start of the experiment (day number 1, DN 1), seedlings were six months old. They were approximately 30–100 cm high and stem diameters at soil surface ranged from 4.5 to 5.5 up to 14 mm. The experiment was subdivided into two parts:

- (1) Three seedlings were selected for dynamic water flow measurements at stem level. Two of the three seedlings were **continuously** monitored from DN 1–52 (see Sections 2.2.3 and 2.2.4). Drought stress was applied by withholding water completely from the soil from DN 4–27 (seedling 1) and from DN 4–39 (seedling 2) until significant leaf loss occurred. After the drought period both seedlings were re-watered and soil water content was maintained close to field capacity until DN 52. One of the

three seedlings was monitored for one week (DN 53–60) and used for the estimation of stem water storage capacity (see Section 2.2.3.).

- (2) 24 baobab seedlings were used to follow up the effects of drought and recovery on a number of physiological and morphological parameters (see Section 2.2.2; Section 2.2.5; Sections 2.2 and 2.3.). The seedlings were divided into three groups which were subjected to three different treatments: eight seedlings remained well-watered, i.e. close to field capacity (referred to as control seedlings), eight seedlings were subjected to drought by interrupting water supply from DN 4–27 (i.e. drought-stressed seedlings) and eight seedlings were first subjected to drought from DN 4–27 and then re-watered from DN 27–52 to allow for recovery (i.e. recovered seedlings). **Discontinuous** measurements on control and drought-stressed seedlings were performed between DN 22–24, and on recovered seedlings at the end of the experiment (DN 50–52). During the course of the experiment, soil moisture content in the containers was monitored gravimetrically (Fig. 1) by weighing the filled containers and correcting for seedling biomass to obtain mass of the wet soil. At the end of the experiment the oven-dry mass of the soil was weighed and soil moisture content calculated.

### 2.2. Ecophysiological measurements

#### 2.2.1. Stomatal conductance

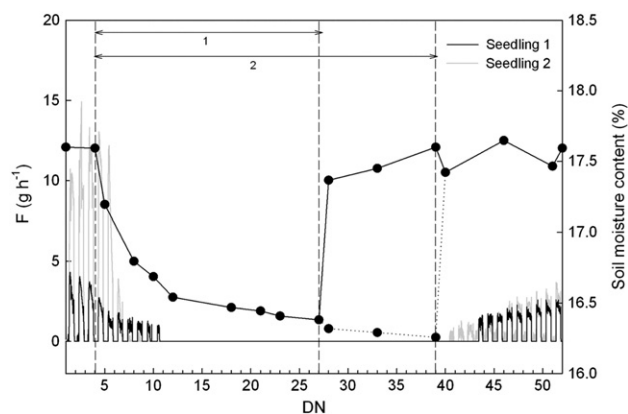
Stomatal conductance to water vapor ( $g_s$ , mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) and transpiration rate ( $E$ , mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) were measured using a portable infrared gas analyzer (LI-6400, Li-Cor, Lincoln, NE, USA) with a closed leaf chamber. PAR was set at 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , leaf temperature at 28 °C and RH of the reference air was kept around 60% (VPD around 1 kPa). The CO<sub>2</sub> concentration of the reference air was adjusted to 400 ppm with a CO<sub>2</sub> mixer and the air flow was kept at 500 mL min<sup>-1</sup>. Measurements were performed between 8 and 10 am on four leaves of three individuals each of the control, drought-stressed and recovered seedlings.

#### 2.2.2. Leaf water potential

Midday leaf water potential ( $\Psi_l$ , MPa) was measured with a pressure chamber (PMS Instrument Co., Corvallis, OR, USA). Measurements were conducted between 12 and 14 pm on three selected leaves of nine seedlings (three per treatment).

#### 2.2.3. Sap flow rate

Sap flow rates ( $F$ , g h<sup>-1</sup>) of two seedlings were continuously measured with sap flow sensors (Model SGA5, Dynamax Inc., Houston, TX,



**Fig. 1.** Sap flow rate ( $F$ , g h<sup>-1</sup>) and soil moisture content (%) as a function of day number (DN). Vertical dashed lines and horizontal arrows correspond with the beginning and the end of the drought period for seedling 1 and seedling 2.

USA), based on the constant power heat balance principle (Sakuratani, 1981; Baker and Van Bavel, 1987). The sap flow sensors were installed at ~15 cm and ~40 cm above soil surface on seedling 1 and seedling 2, respectively. Detailed information about the installation of sap flow sensors and the calculation of  $F$  can be found in the operation manual of Dynamax Inc. (2005) and Steppe and Lemeur (2004).  $F$  was corrected for early morning spikes, which were induced by heat storage effects, following the approach of Grime and Sinclair (1999) and Steppe and Lemeur (2004). The sheath conductance of the gauge was estimated daily from averaging the apparent sheath conductance values at night, when no sap flow occurred (Grime et al., 1995). The value for thermal conductance of woody stems of  $0.42 \text{ W m}^{-1} \text{ K}^{-1}$  from Steinberg et al. (1989) was used in our sap flow calculations.

Sap flow sensors were also installed at the base and top (~10 cm and ~30 cm above the soil surface, respectively) of the stem of one seedling to investigate the presence of stem water storage pools from which water could be withdrawn. Sap flow at the stem base ( $F_b$ ,  $\text{mg m}^{-2} \text{ s}^{-1}$ ) and stem top ( $F_t$ ,  $\text{mg m}^{-2} \text{ s}^{-1}$ ) were normalized by dividing values by estimated total leaf area above each sensor, to allow comparison. A relationship between both flows was established to detect possible time lags (i.e. hysteresis), interpreted as water storage pools (Goldstein et al., 1998; Samson, 2001; Meinzer et al., 2004; Steppe and Lemeur, 2004; Meinzer et al., 2008). Daily use of stem water storage was calculated as the difference between  $F_b$  and  $F_t$  and presented the process of water depletion (negative ordinates) and replenishment (positive ordinates) of internal storage pools (Steppe and Lemeur, 2004). The total daily stem water-storage capacity (DWSC,  $\text{g d}^{-1}$ ), i.e. the total amount of water that can be withdrawn from these storage pools during the day to replenish the water lost by transpiration (Goldstein et al., 1998), was estimated by integrating the absolute values of the negative 5-min differences  $|F_b - F_t|$  (Steppe and Lemeur, 2004).

#### 2.2.4. Stem diameter fluctuations

Stem diameter fluctuations ( $D$ , mm) were continuously measured with Linear Variable Displacement Transducers (LVDT, Model DC-SE 250, Schaevitz, Hampton, VA, USA), installed at the stem base ~10 cm above the soil surface on the same baobab seedlings (seedling 1 and seedling 2) where sap flow sensors were attached. The LVDT sensors were fixed on each tree by a custom-made stainless steel holder. Changes in diameter can be divided in an irreversible component related to tissue growth and a reversible component related to changes in hydration of the extensible tissues (i.e. the depletion of water storage pools during the day and the replenishment of water storage pools during the night) (Kozłowski, 1972). Thus, measurements of  $D$  were performed to evaluate the daily depletion and replenishment of internal water storage pools and to calculate daily stem growth (DG,  $\text{mm d}^{-1}$ ) and maximum daily stem shrinkage (MDS, mm). DG corresponded to the difference in maximum stem diameter between two successive days. MDS was defined as the difference between the daily maximum and minimum stem diameter.

#### 2.2.5. Plant water content

Fresh mass (FW, g) and oven-dry mass (DW, g) of leaves, stem (including branches) and taproot (including fine roots) of 15 seedlings (five per treatment) were weighed to determine the water content of each plant part (WC, %), an indicator for plant water storage:

$$WC = 100 \cdot (1 - DW \cdot FW^{-1}) \quad (2.1)$$

### 2.3. Morphological measurements

#### 2.3.1. Leaf phenology

Leaf shedding and formation of new leaves were monitored on a daily basis by counting the number of leaves (nL). Observations were made on the two baobab seedlings (seedling 1 and seedling 2), selected

for continuous measurements, and on nine baobab seedlings (three per treatment), selected for discontinuous measurements.

#### 2.3.2. Wood and root density

Wood and root density were estimated for 15 seedlings (five per treatment). Wood density (WD,  $\text{g cm}^{-3}$ ) was calculated as the ratio between  $DW_s$  and fresh volume (measured by the displacement of water). WD was considered as a proxy of the vulnerability to xylem cavitation (Pineda-García et al., 2011). Lower WD should lead to higher vulnerability to embolism (Hacke et al., 2001; Choat et al., 2005). Root density (RD,  $\text{g cm}^{-3}$ ) was calculated as the ratio between root DW ( $DW_r$ ) per unit taproot volume, assuming the shape of a cone.

### 2.4. Anatomical measurements

#### 2.4.1. Light microscopy

Transverse sections of fresh leaves, stem and root of three control seedlings were made by hand or by using a sliding microtome. Samples were dehydrated through graded ethanol series, resin-embedded and stained with astra blue and safranin. Some leaf samples were not dehydrated and stained with thionine for the detection of mucilage, a polysaccharide mixture with a high water-binding capacity which allows to hydrate and store water (Ghanem et al., 2010). Transverse sections were viewed by light microscopy images at different magnifications. From the stem and root sections mean vessel diameter ( $\mu\text{m}$ ), maximum vessel diameter ( $\mu\text{m}$ ), vessel lumen area ( $\mu\text{m}^2$ ), vessel densities ( $\text{mm}^{-2}$ ) and percent of parenchyma were determined using Cell<sup>D</sup> software (Olympus Soft Imaging Solutions, Münster, Germany). Percentage of parenchyma was calculated by subtracting vessel lumen area and area of lignified tissue from the area of each section as described by Chapotin et al. (2006c).

#### 2.4.2. Scanning electron microscopy

Specimens of fresh stems of three control seedlings were cut into small pieces and preserved in FAA-solution (formaldehyde–acetic acid–alcohol) until they were dehydrated through ethanol series and critical point dried (Leica EM CPD030, Wetzlar, Germany). The pieces were mounted on aluminum stubs, sputter coated with gold (Cressington 108 auto, Watford, UK) and transferred to the cold stage of the scanning electron microscope (SEM 515, Fei Company, Eindhoven, the Netherlands). The stem surface structure was visualized by SEM photographs at different magnifications.

### 2.5. Data acquisition and analysis

Signals from sap flow sensors, LVDTs and thermocouples were logged at 10s intervals and averaged every 5 min using a data logger (DL2e, Delta-T Devices Ltd., Cambridge, UK).

Normality of the data and homogeneity of the variances was tested with the Shapiro–Wilk normality and Bartlett test, respectively. Differences between treatments were analyzed using linear effects mixed models in case of nested data (nlme package, Pinheiro et al., 2009; Zuur et al., 2009), with treatment as fixed factor and leaf samples as random factor nested within a plant. One way ANOVA with factor treatment was assessed on non-hierarchical data. If significant differences between treatments were demonstrated, multiple comparisons were performed, using a Tukey Honestly Significant Difference (HSD) approach. All statistical tests were performed with R statistical software (R Development Core Team, 2009).

## 3. Results

### 3.1. Ecophysiology

After about three weeks of drought (DN 25),  $g_s$  and  $E$  of the seedlings were significantly reduced by 85% compared with the control seedlings.

At the same time  $\Psi_1$  of the seedlings was significantly reduced (from  $-0.57$  to  $-1.06$  MPa). The recovered seedlings had a significantly higher  $g_s$  and  $E$  compared to the control and the drought-stressed seedlings ( $p < 0.001$ ) (Table 1). Re-watering significantly increased  $\Psi_1$  on DN 51 compared with the drought-stressed seedlings ( $p < 0.001$ ).

Fig. 1 shows the evolution of sap flow for both seedlings in relation with soil moisture content during the drought-stress experiment. Mean daily sap flow rate remained more or less constant throughout the irrigation period (i.e. before the onset of the drought period), when soil moisture content was about 17.5%.  $F$  was negligible during night time. During daytime, mean  $F$  values were  $2.9 \pm 0.8$  and  $9.0 \pm 2.7$  g h<sup>-1</sup> for the well-watered seedling 1 and seedling 2, respectively.  $F$  gradually decreased after the onset of the drought period, and stopped in both seedlings after about one week of water withholding (Fig. 1), when soil moisture content was 16.5%. In seedling 1, sap flow started with the development of new leaves (about 14 days after re-watering), while in seedling 2 (which maintained its upper leaves as stated above)  $F$  started to increase the day after re-watering. In the recovery period we observed night-time flow rates in seedling 2.

$F_t$  (near the leaves) was only slightly higher than  $F_b$ . No hysteresis phenomenon (i.e. no time lag) was observed between  $F_b$  and  $F_t$  ( $F_t = 1.21 \times F_b - 0.34$ ;  $R^2 = 0.99$ ;  $p < 0.0001$ ) and thus time response of both flows was similar. DWSC during the irrigation period was  $8.59 \pm 1.32$  g d<sup>-1</sup>.

The baobab seedlings clearly showed daily stem diameter fluctuations, with a maximum diameter in early morning and a minimum diameter occurring in the afternoon (Fig. 2). DG and MDS exhibited an increasing trend during the irrigation period (Figs. 3 and 4). In seedling 1 no daily stem diameter fluctuations were found as drought intensified, while in seedling 2, an overall decrease in diameter fluctuations was observed (Fig. 2). Both DG and MDS decreased in response to drought (Figs. 3 and 4). Note that during drought in seedling 2, first a negative DG and a maximum shrinkage occurred, after which DG remained close to zero and MDS slightly decreased to zero (Figs. 3 and 4). In the recovery period, DG of seedling 1 showed oscillations (positive and negative values) which coincided with leaf development and MDS reached maximum values at points of water application. Seedling 2 reached a maximum DG and swelling when re-watered after which DG and MDS remained positive (Figs. 3 and 4).

Drought had no significant influence on  $WC_1$  of the seedlings. However,  $WC_1$  of newly developed leaves of the recovered seedlings was significantly lower than  $WC_1$  of the control and the drought-stressed seedlings (Table 1).  $WC_s$  and  $WC_r$  remained equally high for the different treatments (Table 1).

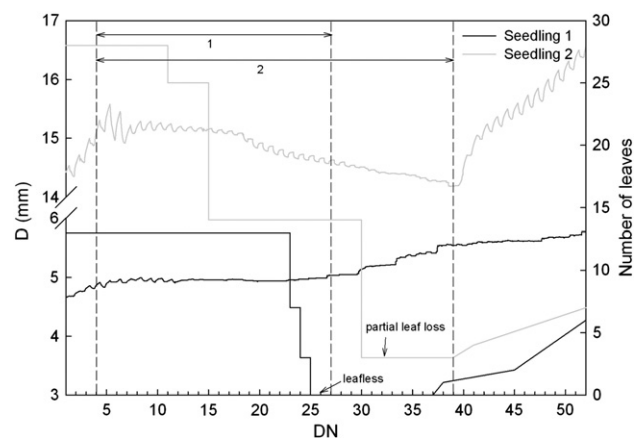


Fig. 2. Stem diameter variations ( $D$ , mm) and number of leaves as a function of day number (DN). Vertical dashed lines and horizontal arrows correspond with the beginning and the end of the drought period for seedling 1 and seedling 2.

### 3.2. Morphology

Leaf shedding started one to two weeks after water withholding (Fig. 2) at the bottom of the stem and progressed upwards. After three weeks of drought (DN 25), seedling 2 still maintained upper leaves (~50%) while seedling 1 lost all leaves (100%) (Fig. 2). Most of the seedlings dropped ~80–100% of their leaves (Table 1). No new leaf formation was observed during the drought period. Leaf development started shortly (one day to one week) after re-watering the seedlings (Fig. 2).

RD was generally lower than WD. RD and WD did not change during the experimental period (Table 1). Note that the results of RD are rough estimations (Table 1).

### 3.3. Anatomy

Leaf lamina of the seedlings showed an abundant presence of mucilage in adaxial and abaxial epidermis cells, and in the peripheral cortex of the veins. The bark consisted of a broad layer of parenchyma cells and fibers. Between the outer and inner bark a green layer was observed (Fig. 5A). The stem surface (periderm) of the seedlings was mainly characterized by lenticels and/or cracks and a few stomata (Fig. 5B,C,D). The stem contained  $79.0 \pm 4.3\%$  of parenchyma cells (including broad layers of ray parenchyma). Vessel density and lumen area ranged from 5 to 25 vessels mm<sup>-2</sup> and from 0.9 to 7%, respectively. Mean vessel

Table 1

Mean values ( $\pm$  standard deviation) of the leaf, stem and root variables of the seedlings subjected to different treatments (control, drought, recovery). Measurements on the control and stressed seedlings were performed between DN 22 and DN 24, and on the recovered seedlings between DN 50 and DN 52. Different characters indicate significant differences between treatments and  $P$ -values in bold are statistically significant ( $P < 0.05$ ) using one way ANOVA and Tukey HSD.

Measured variables	Symbol	Unit	Control	Drought	Recovery	$P$ -value
<i>Leaf</i>						
Number of leaves	nL	–	22.00 $\pm$ 3.60 <sup>c</sup>	5.00 $\pm$ 1.00 <sup>a</sup>	11.67 $\pm$ 2.31 <sup>b</sup>	<0.001
Stomatal conductance	$g_s$	mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup>	0.11 $\pm$ 0.06 <sup>b</sup>	0.02 $\pm$ 0.00 <sup>a</sup>	0.76 $\pm$ 0.22 <sup>c</sup>	<0.001
Transpiration	$E$	mmol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup>	1.34 $\pm$ 0.72 <sup>b</sup>	0.19 $\pm$ 0.03 <sup>a</sup>	7.83 $\pm$ 2.28 <sup>c</sup>	<0.001
Leaf water potential	$\Psi_1$	MPa	$-0.57 \pm 0.06^c$	$-1.06 \pm 0.10^a$	$-0.73 \pm 0.10^b$	<0.001
Leaf water content	$WC_1$	%	84.53 $\pm$ 0.77 <sup>b</sup>	82.91 $\pm$ 1.02 <sup>b</sup>	76.98 $\pm$ 3.51 <sup>a</sup>	<b>0.030</b>
Leaf dry weight	DW <sub>l</sub>	g	5.47 $\pm$ 0.41 <sup>b</sup>	0.188 $\pm$ 0.174 <sup>a</sup>	0.953 $\pm$ 0.735 <sup>a</sup>	<0.001
<i>Stem</i>						
Stem water content	$WC_s$	%	77.19 $\pm$ 0.30 <sup>a</sup>	75.01 $\pm$ 2.27 <sup>a</sup>	77.19 $\pm$ 0.75 <sup>a</sup>	0.155
Stem dry weight	DW <sub>s</sub>	g	6.22 $\pm$ 0.91 <sup>b</sup>	2.80 $\pm$ 0.40 <sup>a</sup>	3.29 $\pm$ 0.50 <sup>a</sup>	<b>0.002</b>
Wood density	WD	g cm <sup>-3</sup>	0.268 $\pm$ 0.041 <sup>a</sup>	0.212 $\pm$ 0.033 <sup>a</sup>	0.214 $\pm$ 0.058 <sup>a</sup>	0.204
<i>Root</i>						
Root water content	$WC_r$	%	89.30 $\pm$ 2.62 <sup>a</sup>	87.32 $\pm$ 1.82 <sup>a</sup>	88.68 $\pm$ 1.26 <sup>a</sup>	0.845
Root dry weight	DW <sub>r</sub>	g	11.91 $\pm$ 1.36 <sup>a</sup>	6.85 $\pm$ 0.91 <sup>a</sup>	8.41 $\pm$ 3.84 <sup>a</sup>	0.124
Root density	RD	g cm <sup>-3</sup>	0.095 $\pm$ 0.041 <sup>a</sup>	0.242 $\pm$ 0.139 <sup>a</sup>	0.148 $\pm$ 0.035 <sup>a</sup>	0.103

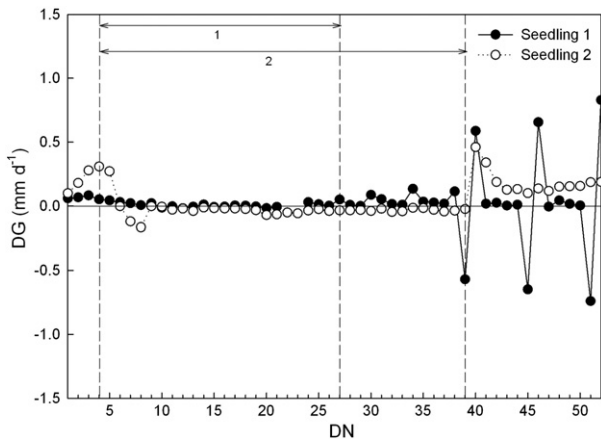


Fig. 3. Daily growth (DG, mm d<sup>-1</sup>) as a function of day number (DN). Vertical dashed lines and horizontal arrows correspond with the beginning and the end of the drought period for seedling 1 and seedling 2.

diameter amounted to  $57.8 \pm 12.8 \mu\text{m}$  and mean maximum diameter to  $76.7 \pm 5.7 \mu\text{m}$ . The root consisted of  $93.9 \pm 2.2\%$  parenchyma (including large parenchyma cells and rays), which was permeated by  $0.2 \pm 0.1\%$  of vascular tissue (xylem vessels). Vessel density was  $0.9 \pm 0.4 \text{ mm}^{-2}$ , mean vessel diameter  $54.5 \pm 11.9 \mu\text{m}$  and mean maximum diameter  $70.9 \pm 6.3 \mu\text{m}$ .

## 4. Discussion

### 4.1. Stomatal control limits water use

The baobab seedlings rapidly closed their stomata when already confronted with only a limited soil moisture deficit (1%). They sharply reduced  $g_s$  and  $E$  by about 85% at the end of the drought period, while minimum  $\Psi_1$  remained near  $-1 \text{ MPa}$ , which confirms the first hypothesis that baobab seedlings use a water conservative strategy to survive drought stress by regulating stomatal closure and maintaining turgor. Therefore, the seedlings can be considered as isohydric (Chapotin et al., 2006a). In general, isohydric species reduce  $g_s$  as soil water potential decreases and/or atmospheric conditions become dry, thereby maintaining a more or less constant midday  $\Psi_1$  (Tardieu and Simonneau, 1998; Franks et al.; 2007, McDowell et al., 2008).

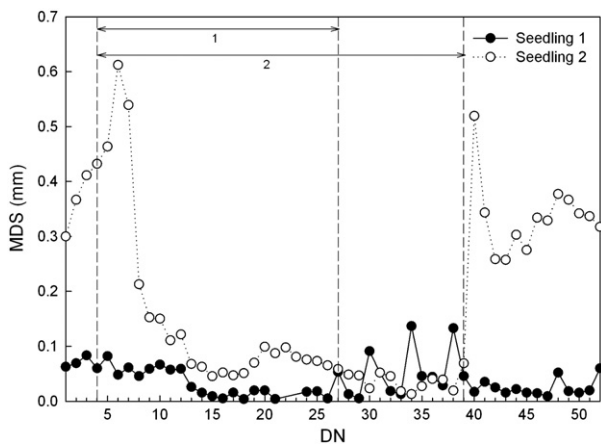


Fig. 4. Maximum daily shrinkage (MDS, mm) as a function of day number (DN). Vertical dashed lines and horizontal arrows correspond with the beginning and the end of the drought period for seedling 1 and seedling 2.

In contrast with other tree species growing in the same environment, the baobab seedlings were more effective in reducing  $g_s$  and consequently in minimizing  $E$  under drought. For example, *Jatropha curcas* seedlings (another stem succulent species) reduced  $g_s$  by ~65% (Díaz-López et al., 2012), while in *Parkia biglobosa* (a deciduous hardwood species) seedlings,  $g_s$  was lowered by ~50% and in *Ziziphus mauritiana* (an evergreen hardwood species) seedlings  $E$  was lowered by ~50% in response to drought stress (Osonubi and Fasehun, 1987; Teklehaimanot et al., 1998; Kulkarni et al., 2010).

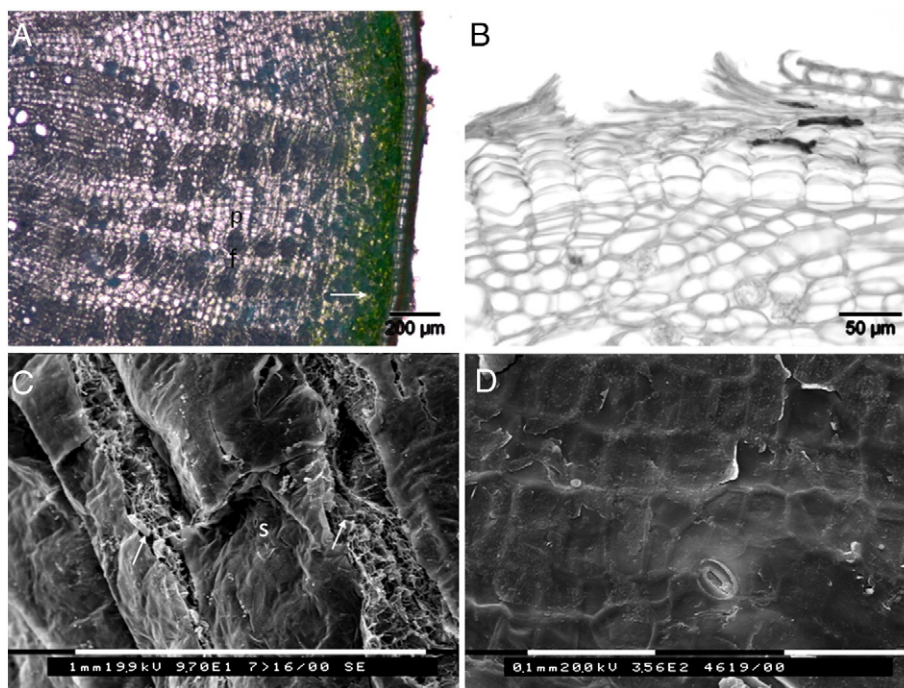
The reduction in  $g_s$  was not caused by impaired water uptake as soil moisture content was still close to field capacity (16.5% vs 17.5%), but the effective stomatal control prevented baobab seedlings from dropping  $\Psi_1$  lower than  $-1 \text{ MPa}$ , which agrees with previous findings of Chapotin et al. (2006b) for adult Malagasy baobab trees (*A. robustipa* and *A. za*). *P. biglobosa* and *Z. mauritiana* seedlings might decline  $\Psi_1$  to less than  $-2.5 \text{ MPa}$  (Osonubi and Fasehun, 1987; Kulkarni et al., 2010) as they could be classified as anisohydric species (they maintain a higher  $g_s$  but allow  $\Psi_1$  to decline as soil water potential decreases, McDowell et al., 2008). The reason why baobab seedlings try to maintain turgor, is to avoid hydraulic failure caused by xylem cavitation and embolism. Results demonstrated that the stem and root were characterized by low wood/root density and the presence of a few but quite large xylem vessels. Therefore, it is expected that stem and root tissue are likely to be more vulnerable to drought-induced embolism. Similarly Chapotin et al. (2006b,c) suggested that adult Malagasy baobab trees have developed such an embolism-avoidance strategy.

We assume the maintenance of a high  $\Psi_1$  in the baobab seedlings might also be supported by the large amounts of mucilage (i.e. a polysaccharide mixture) present in the leaves (epidermis and veins). Mucilages might act as hydraulic capacitors to buffer water status (Morse, 1990) and might also play a role in assimilate redistribution prior to defoliation (Clifford et al., 2002). They are widespread in succulent taxa (Ogbum and Edward, 2010) and several members of the Malvaceae (e.g. Woolfe et al., 1977; Wickens and Lowe, 2008; Ghanem et al., 2010).

### 4.2. Limited use of stored water despite the large amount of water stored

The use of stored water in trees should lead to fluctuations in stem diameter as (i) during daytime, leaf transpiration exceeds water uptake by the roots, thereby depleting stem water storage pools and (ii) during night time, stem water storage pools are fully replenished as water uptake by roots exceeds transpirational water losses. Indeed, we observed daily diameter fluctuations in baobab seedlings, which indicated stored water was used on a daily basis to buffer water deficits. Similar observations were made in other savanna tree species (cerrado trees growing in central Brazil) (Scholz et al., 2008). While Fenner (1980) also reported daily girth changes in adult African baobab trees (*A. digitata*), Chapotin et al. (2006a,b) did not find indications of daily use of stored stem water in adult Malagasy baobab trees (*A. za* and *A. robustipa*) (see further).

The use of stored water should also lead to time lags at the onset of sap flow between the base ( $F_b$ ) and top ( $F_t$ ) of a tree. This was not a pattern we observed in baobab seedlings.  $F_b$  and  $F_t$  were largely in phase, which indicated the daily amount of water withdrawn from storage was very small. It is often assumed that only in small trees no time lags exist between  $F_b$  and  $F_t$ , because it is expected that stem sap flow immediately responds to changes in leaf transpiration (Steppe and Lemeur, 2004). However, even in adult Malagasy baobab trees, Chapotin et al. (2006a) did not find time lags at the onset of sap flow between the base and crown, and therefore concluded that this baobab species did not use stored stem water to buffer daily water deficits. The fact that Chapotin et al. (2006a) did not find time lags with sap flow techniques on one of the most likely trees for significant stem water storage, raises some questions, whether time lags are a suitable metric for investigating stem water storage or why time lags are found in some trees and not in others (Burgess and Dawson, 2008). These latter



**Fig. 5.** Light microscopy images of the stem transverse section (A, B) and scanning electron microscope images of the bark surface (C, D) of baobab seedlings. Scale bars are represented. (A) Green layer in the inner bark (indicated by an arrow) and the abundant presence of parenchyma (p) and fibers (f). (B) Detail of a lenticels in the periderm. (C) Stem surface with cracks (indicated by arrows) and a stoma (s). (D) Detail of a non-functional stoma.

authors stated, based on their understanding of the cohesion–tension model, that absolute lags at the onset of sap flow between the base and crown should not occur.

The DWSC (i.e. the absolute amount of water withdrawn from storage) for baobab seedlings amounted  $\sim 8.5 \text{ g d}^{-1}$  (only  $\sim 6\%$  of total plant water) and contributed up to  $\sim 17.5\%$  of the total daily transpiration. Fenner (1980) reported that daily water losses in adult African baobab trees only represented 2% of total plant water. Although the DWSC was limited, the contribution to total daily water losses was comparable with values estimated for tropical trees of a seasonally dry forest (9–15%; Goldstein et al., 1998), including *Spondias mombin*, a deciduous tree with a low wood density and a high stem water storage (Borchert, 1994). It was also in the range of values estimated for cerrado savanna tree species (16–33%; Scholz et al., 2008).

The DWSC in baobab seedlings seems to be surprisingly small given the vast amounts of water stored in stem and root (see Section 4.3.), which indicates stored water is largely unavailable. The excessive withdrawal from stored water would lead to loss in turgor and conduit safety of both stem and root tissue (see Section 4.1.). Similarly Chapotin et al. (2006a,b,c) reported that the withdrawal of significant amounts of water from stem wood in adult Malagasy baobab trees would quickly result in turgor loss, as their turgor-loss point acts near actual field water potentials, and in a greater risk for hydraulic failure as their low-density wood is highly vulnerable to cavitation. Besides tissue water relations, these authors suggested that the use of stored stem water in adult Malagasy baobab trees might additionally be restricted by transport limitations (i.e. slow radial transport of stored water to the conductive pathway as a consequence of stem morphology and anatomy) and biomechanical considerations (i.e. low-density wood might lose mechanical stability).

#### 4.3. Data suggest root succulence rather than stem succulence

The stem of baobab seedlings contained a large amount of parenchymatic tissue ( $\sim 80\%$ ). Consequently,  $WC_s$  was high ( $\sim 75\%$ ) and WD

( $0.212\text{--}0.268 \text{ g cm}^{-3}$ ) low, which confirms the fourth hypothesis that stated that baobab seedlings survive drought by possessing a large amount of stored water in the stem. WD was comparable with *J. curcas* seedlings (which were found to have a WD of  $0.252\text{--}0.266 \text{ g cm}^{-3}$ ) (Maes et al., 2009) but higher than in adult Malagasy baobab trees (which had a WD of  $0.09\text{--}0.17 \text{ g cm}^{-3}$ ) (Chapotin et al., 2006c). However, the taproot of the baobab seedlings was found to be the most important plant part for water as it stored 68% of total plant water. These results agree with the observations of a previous study on African baobab seedlings (De Smedt et al., 2012). The thickened taproot was characterized by a very high  $WC_r$  ( $\sim 90\%$ ), a very low RD ( $0.095\text{--}0.242 \text{ g cm}^{-3}$ ) and the presence of abundant storage parenchyma ( $\sim 94\%$ ). Maes et al. (2009) reported lower  $WC_r$  in *J. curcas* seedlings ( $\sim 75\%$ ). No data was found of seedlings having  $WC_r$  and RD approaching the values measured in this study.

We might state that initially, baobab seedlings can be considered as mainly root succulent, as they have a swollen carrot-like taproot that functions as water storage organ. Stem-succulence is likely to become more pronounced with plant age as the lateral root system usually begins to develop during the second and third year (Wickens and Lowe, 2008).

#### 4.4. Use of stored water allows maintenance of a small number of leaves

Baobab trees, like all stem-succulent trees, are leafless during most of the dry season, suggesting a drought avoidance strategy (Borchert, 1994; Borchert and Rivera, 2001; Borchert and Pockman, 2005; Chapotin et al., 2006b). We, therefore, hypothesized that baobab seedlings also shed their leaves during a drought period (third hypothesis).

This was not strictly confirmed in our study, as at the end of the drought period, some baobab seedlings tended to retain part of their leaves ( $\sim 20\text{--}50\%$ ). Stem shrinkage (manifested in the decreasing trend in MDS) and a slight decrease in  $WC_s$  and  $WC_r$  ( $\sim 2\%$ ) of the drought-stressed seedlings, while at the same time sap flow was zero (Figs. 1

and 4), indicated no water was taken up by the roots nor or little water was lost by leaf transpiration. As such, these baobab seedlings seem to rely on stored stem and/or root water on a daily basis to maintain their upper leaves and to support some metabolic processes during the short-term drought period. We assume that the withdrawal of water from storage, together with the very low stomatal conductance (due to the tight stomatal control) and the presence of mucilage in the leaves (see Section 4.1.) reduces the decline in  $\Psi_1$  and extends leaf longevity (Nilson et al., 1990). Similarly Maes et al. (2009) found that stem water allowed the maintenance of a small number of leaves in *J. curcas* seedlings during the first weeks of a drought period.

The timescale during which baobab seedlings might use stored water and the degree to which it is relied upon, might depend on a combination of factors, such as genetic variation between seedlings, water storage (capacity) of stem and root (see Sections 4.2. and 4.3.), vulnerability to xylem cavitation (see Sections 4.1. and 4.2.) and soil water availability (see Section 4.5.). For example, the differences in use of stored water between seedling 1 and seedling 2 might be related to their genetic heterogeneity, which results from seed propagation (see Section 2.1.). The longer time needed to fully deplete the available water stored in stem and/or root (i.e. slower decrease in MDS) during the drought period evidenced the higher water storage capacity (i.e. the higher absolute amount of water that could be withdrawn from storage) in seedling 2 which therefore, kept part of its leaves, compared to seedling 1, which became leafless.

The maintenance of some upper leaves might help baobab seedlings to overcome short-term drought periods during the rainy season and to take advantage of scattered rainfalls after the onset of the dry season as the remaining leaves are on stand-by to photosynthesize (De Smedt et al., 2012).

By analogy, Chapotin et al. (2006b) ascertained in adult Malagasy baobab trees, that new leaves were flushed before the end of the dry season. Hereby, stem water content declined by up to 10–12%, while sap flow rate at the base of the trunk, branch sap flow rates and stomatal conductance remained very low. This indicated that adult baobab trees are almost entirely dependent on stored stem water to flush new leaves. The authors suggested that the advantage of leaf flushing before the end of the dry season (long-term drought period) allows adult Malagasy baobab trees to photosynthesize as soon as scattered rainfalls occur before the onset of the rainy season.

#### 4.5. Drought-enforced dormancy limits water use and growth

Through the tight stomatal control (see Section 4.1.) at the onset of the drought period, leaf transpiration and thus sap flow rate in the baobab seedlings rapidly declined at the expense of photosynthesis. Consequently DG reduced, less water was taken up by the roots and the overnight recharge of stored stem water to previous water content levels became incomplete as indicated by the substantial decline in the daily stem diameter fluctuations. Similarly, Fenner (1980) ascertained that during a drought spell, daily girth fluctuations in adult African baobab trees were minimal, which he attributed to very low transpirational water losses and little root water uptake. Stem shrinkage was expressed in a decreasing trend in MDS, which exhibited a decrease in the daily use of stored stem water. We suggested that this stored stem water was used to support the maintenance of a few upper leaves in some baobab seedlings during the short-term drought period (see Section 4.4.). Contrary to observations of Maes et al. (2009) and De Smedt et al. (2012), the seedlings in our study did not produce new leaves during this period, which might be related to differences in growth conditions, e.g. VPD, and the length of the drought period, between the different experiments.

When drought intensified, sap flow rates, DG and MDS of the baobab seedlings finally ceased to zero, which led to drought-enforced dormancy. These results confirm the second hypothesis which stated that baobab seedlings minimize water use and growth during drought, as they

become dormant. Although leaf photosynthetic activity seem to be inhibited during this phase, we suggest carbon assimilation might occur in the stem of the baobab seedlings. Indeed, their stem is characterized by a chlorenchyma layer underneath the periderm, similar to the stem of adult baobab trees (Baum, 1995). CO<sub>2</sub> diffusion in the baobab seedlings might take place through lenticels and longitudinal cracks, which we observed during peridermis development of the young stem. Stomata were only sporadically found on the stem, but considered as non-functional remains of primary stem growth, as described in Filippou et al. (2007). Several authors (Rouhi et al., 2007; Wittmann and Pfanz, 2008; Eyles et al., 2009; De Smedt et al., 2012) reported cuticular photosynthesis might be an important strategy of additional carbon assimilation without much water loss, especially during stress and leafless periods.

#### 4.6. Successful strategy for survival

Baobab seedlings should return easily to previous water content levels upon re-watering the soil as they only lost a small amount of stored water in response to drought stress. The replenishment of water storage pools in the seedlings requires water uptake by roots above that necessary to meet leaf transpiration and occurs therefore during night time (see Section 4.2.). Night-time sap flow might occur to support the replenishment of stored water but ceases when previous water content levels are reached. This was only the case for seedling 2 during the recovery period (Fig. 1) as stored water was previously used to maintain few upper leaves (see Section 4.4), while seedling 1 lost all its leaves during drought.

Stem and root anatomy (see Section 4.1.) seem to be optimized for the rapid transport of relatively large volumes of water in the stem. This was expressed in the increasing trends of MDS at points of water application. The rapidity with which water uptake followed re-watering indicates that fine roots were produced during the drought period and/or by the time leaves were flushed during the recovery period. Newly formed leaves were characterized by a higher  $g_s$  to maximize water uptake and photosynthesis to support rapid re-growth (DG). The gradual increase in sap flow indicated the increase in water use and final 100% recovery of the baobab seedlings.

## 5. Conclusions

Baobab seedlings traits confer drought avoidance and conservative water use to survive drought. Leaf transpiration, and thus sap flow, is minimized by a very tight control over stomatal closure, followed by a significant leaf loss when drought is experienced, thereby retaining a relative constant leaf water potential and high water content. The tap-root is the most important plant part for water storage, indicating root-succulence. A shift to stem-succulence is likely to become more pronounced with age. Although the use of stored stem/root water is small compared to daily water use and total plant water, its role is multifold: buffering daily water deficits, avoiding turgor loss and embolism during drought periods, and promoting rapid recovery. The amount of stored water which seems to be largely unavailable during short-term drought periods might become available during long-term drought periods, to maintain sufficiently high cell water content for plant metabolic processes and possible cuticular photosynthesis in the dormant phase. This study evidenced baobab seedlings can successfully survive short-term drought periods, which could be useful when managing baobab nurseries in times of water scarcity. The conservative water use of the species could be also advantageous when planted in parklands, as there will be less competition for water with other young trees and crops. Results recommend further research on long-term drought experiments and water requirements of baobab seedlings in the field.

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