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Functional responses of baobab (*Adansonia digitata* L.) seedlings to drought conditions: Differences between western and south-eastern Africa

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ABSTRACT

The baobab tree (*Adansonia digitata* L.) is an important multi-purpose tree species of dryland Africa, commonly found in the driest parts of the Savannah. Although seasonal drought is one of the major constraints for plant growth and survival in these regions, little has been reported about the mechanisms baobab seedlings use to deal with drought. Therefore, the aim of this study was to investigate the functional responses of baobab seedlings under a short-term soil drought stress. As genetic differences between baobab trees from western and south-eastern Africa have been reported, seeds were collected in both Mali and Malawi (western and south-eastern Africa, respectively).

Baobab seedlings were found to use a number of mechanisms to cope with drought. First, leaf area was reduced due to leaf shedding, though not all leaves were shed and even some leaves with altered morphology were formed. Second, under drought, relatively more biomass was allocated to the root system. Third, as baobab seedlings had a tight stomatal control, under drought conditions photosynthesis and transpiration were significantly reduced while leaf water potential barely changed. Non-stomatal limitations on photosynthesis became important as drought persevered. Part of the water stored in the taproot was being used for the salvation of part of the old leaves, for the formation of new ones, for the formation of new roots, and for the maintenance of metabolic processes. There was a significant correlation between water content of the taproot and stomatal closure. The former mechanisms, mainly related to water conservation, allow baobab seedlings to keep a high water status during drought events which helps to prevent xylem cavitation and allow them to survive dry periods.

Differences between the drought responses of baobab seedlings from different origins were observed: Malawian seedlings were able to retain more leaves and to form more new ones, while Malian seedlings tended to allocate more biomass to their root system. Therefore, baobab seedlings from western Africa showed more drought-avoidance characteristics. These different strategies between Malawi and Mali agree with genetic differences between baobab clades, and suggest that superior planting material in terms of drought tolerance can be selected.

1. Introduction

The African baobab, *Adansonia digitata* L. (Family Malvaceae), is a deciduous stem-succulent tree native to the dry regions of tropical Africa (Sidibé and Williams, 2002; Wickens and Lowe, 2008). In total, more than 300 uses have been reported for this species, with the most important ones being related to food, medicine and income generation (Buchmann et al., 2010; De Caluwé et al., 2009; Wickens and Lowe, 2008). The highly nutritious fruits, seeds and leaves (Nordeide et al., 1996) are frequently consumed by rural people in both western and south-eastern Africa, thereby supplementing the local diet and improving food security (Buchmann et al., 2010; Wickens and Lowe, 2008). Recently, baobab fruit pulp has entered the European and the US food market (European Commission, 2008; FDA, 2009), raising the potential international commercial value of this species to an estimated one billion US dollar (Akinnifesi et al., 2007). Despite its known importance, lack of recruitment seems to threaten the species, as little natural regeneration has been reported in different parts of Africa (e.g., Assogbadjo et al., 2005; Edkins et al., 2008; Schumann et al., 2010). Low natural regeneration of the baobab tree has been partially related to increasing drought events (Wickens and Lowe, 2008), which are predicted to become more common in dryland Africa according to future climate projections (IPCC, 2007). To our knowledge, the physiological mechanisms behind the drought withstanding capability of the baobab tree, which are very important for its survival in its natural environment, have never been accurately characterised. An understanding of these mechanisms is also of importance for

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the selection of drought-adapted plant material for reforestation purposes.

Other deciduous stem-succulent trees, often occupying the driest parts of tropical dry forests, maintain high water stem potentials throughout the dry season (>−0.5 MPa) by shedding their leaves rapidly at the onset of the drought (Borchert, 1994; Borchert and Rivera, 2001). Also adult baobab trees are known to shed their leaves at the onset of a dry period in order to prevent water losses (Fenner, 1980; Wickens and Lowe, 2008). However, no information is available about the drought tolerance mechanisms at seedlings stage, a critical stage for this species' survival (Wickens and Lowe, 2008).

A recent study on chloroplast DNA has shown that there are genetic differences between baobab populations from western and south-eastern Africa (Pock Tsy et al., 2009). Due to the presence of equatorial rain forest and the Mega-Chad Lake in the Quaternary, these populations have been isolated from one another for a long period of time (Pock Tsy et al., 2009; Wickens and Lowe, 2008). This might indicate that both genetic clades have developed different mechanisms to cope with drought. Genotypic effects in the strategies seedlings use to deal with drought have been shown to be important in *Pinus sylvestris* L., *Fagus sylvatica* L. and *Quercus ilex* L., and in the tropical tree species *Parkia biglobosa* (Jacq.) Benth. which is commonly found in similar environments as the baobab tree (Gratani et al., 2003; Palmroth et al., 1999; Peuke et al., 2002; Teklehaimanot et al., 1998).

This study aims to: (i) investigate the morphological and ecophysiological mechanisms baobab seedlings use to cope with soil drought, and (ii) determine if these mechanisms differ between seedlings originating from western and south-eastern Africa. This information could be used for the selection of superior, drought-adapted plant material, which can be used for reforestation programs in dryland Africa.

2. Materials and methods

2.1. Plant material and experimental set-up

Given the existence of genetic differences between baobab trees from western and south-eastern Africa (Pock Tsy et al., 2009), seeds were collected in two countries representative of these two clades: Mali in western Africa and Malawi in south-eastern Africa. In each country, seeds were collected from five study sites (supplementary Fig. 1). Study sites were selected in different climatic zones and on the basis of the presence of a well-established fruit-bearing baobab population. Climatic details of the different study sites are given in Cuní Sanchez et al. (2010) and De Smedt et al. (2011). Seeds from each study site were pre-treated, germinated and grown in the greenhouse of the University of Antwerp (Antwerp, Belgium), as described by Cuní Sanchez et al. (2011).

From each study site, 20 healthy-looking seedlings were selected and grown for 14 weeks following a randomised block design with twenty blocks ($n = 200$). Pots were moved around once a week in order to standardize environmental conditions. They were irrigated twice a week with standard Hoagland solution and once a week with tap water. Water or nutrient solution when applied was added until an excess drained from the bottom of the pot. After 14 weeks, the plants were randomly divided into two groups: half of the seedlings continued to be grown in the described conditions (control treatment) while the other half were imposed a drought stress by withholding irrigation completely (drought treatment). Four weeks later (18 weeks after sowing), all plants were harvested. Between the 14th and 18th week, the number of leaves on each plant was counted on a regular basis (in total six times)

2.2. Biomass, biomass allocation and water content

After harvesting, the seedlings' roots were rinsed with water in order to remove the sand, and dried with paper towel. Seedlings were divided into taproot, other (fine) roots, stem and leaves. Fresh weights of each plant part (in g) were determined using a precision balance (named FW_t, FW_r, FW_s and FW_l, respectively). The leaves of each seedling were scanned with a flatbed scanner and total leaf area per plant (LA, dm^2) was determined with ImageJ software (Abramoff et al., 2004). All seedling parts were dried in an oven at 70 °C for 48 h (for taproots 72 h). Dry weights of the total plant and of the different plant parts (in g) were determined using a precision balance (named DW_{tot}, DW_t, DW_r, DW_s and DW_l). The water content (in %) of each plant part ($WC_{plant\ part}$) was calculated as $100 \times (1 - DW_{plant\ part}/FW_{plant\ part})$. Since the response on drought of the root/shoot ratio, calculated by dividing total belowground by total aboveground biomass, might be blurred because of leaf shedding, an adjusted root/shoot ratio (R/S ratio, $g\,g^{-1}$) was calculated as $(DW_t + DW_r)/FW_s$. Leaf weight ratio (LWR, $g\,g^{-1}$) and leaf area ratio (LAR, $dm^2\,g^{-1}$) were calculated by dividing DW_l and LA by DW_{tot}, respectively.

2.3. Leaf characteristics

After harvesting, the first fully developed leaf (counted from the top of the plant) was punched three times with a cork borer of known diameter; the discs were dried in an oven at 70 °C and weighed with a precision balance after 48 h. Specific leaf area (SLA, $dm^2\,g^{-1}$) was calculated by dividing the punched area by the dry weights of the discs.

Nail polish impressions were made of the abaxial surface of the first fully developed leaf (counted from the top of the plant) for all seedlings, avoiding the midrib and the leaf margin (Kardel et al., 2010). For a limited number of seedlings, nail polish impressions were also taken of the stem surface. The stomatal imprints were analysed at a magnification of 10 × 40 using a bright field microscope (Olympus CX41, Olympus Corporation, Tokyo, Japan). For each sample, six microscopic fields were randomly selected and stomata were counted using Cell^D software (Olympus Corporation, Tokyo, Japan). For each plant, stomatal density (SD, in number of stomata mm^{-2}) was then calculated as the mean of these counts.

Predawn water potential (Ψ_l, MPa) of the first fully developed leaf was measured immediately after harvesting with a scholander pressure chamber (Model 1000, PMS Instrument Company, Albany, Orgeon, USA).

2.4. Gas-exchange and water-use efficiency measurements

Net photosynthesis (A, $\mu mol\,CO_2\,m^{-2}\,s^{-1}$), stomatal conductance to water vapour diffusion (g_s, $mol\,H_2O\,m^{-2}\,s^{-1}$), transpiration rate ($E$, $mmol\,H_2O\,m^{-2}\,s^{-1}$) and internal CO_2 concentration (C_i, ppm) were measured using a portable infrared gas analyser (LI-6400, LiCor Environmental Sciences, Lincoln, Nebraska, USA).

Plants were first acclimated to light having a photon flux density of about 700 $\mu mol\,m^{-2}\,s^{-1}$ (supplied by 600 W metal-halide light bulbs) for at least 30 min before clamping the leaves with the leaf chamber. The CO_2 concentration of the reference air entering the leaf chamber (C_a) was adjusted to 500 ppm with a CO_2 mixer control unit. The leaf temperature was kept at 30 °C while the relative humidity of the reference air was kept close to ambient (60–70%). The air flow rate was 500 $mL\,min^{-1}$. In order to avoid the midday depression of the photosynthesis, gas exchange measurements were made between 8 am and noon. The instantaneous water use efficiency (WUE) was calculated as the ratio between A and E ($\mu mol\,CO_2$ assimilated per $mmol\,H_2O$ transpired, Palmroth et al., 1999)

All mentioned gas-exchange parameters (A, g_s, E, C_i) and WUE were estimated after 14 days of drought stress on the 4th completely expanded leaf (counted from the top of the plant) of each seedling

2.5. Statistical analyses

Give the grouped structure of the data (several seedlings were taken from one study site, and seedlings were thus nested in sites) mixed effects models were used to test for treatment effects on all measured morphological and physiological seedling traits (Pinheiro and Bates, 2002). Water regime (drought/control), country of origin (Mali/Malawi) and their interaction were included in the models as fixed effects. Study site was included as a random effect. Net photosynthesis, stomatal conductance, transpiration rate and instantaneous water use efficiency were log-transformed for normalization purposes. Correlations between seedlings traits were tested using Spearman's rank order coefficients. Following Brodribb (1996) and Rouhi et al. (2007), a function was fitted that describes the relationship between g_s and C_i/C_a under conditions where assimilation is not limited by non-stomatal factors. R statistical software was used for statistical analyses (R Development Core Team, 2008) and mixed models were constructed using the nlme-package (Pinheiro et al., 2008).

3. Results

3.1. Biomass and biomass allocation

Water regime had a significant effect on total plant biomass. Droughted seedlings had significantly lower total plant biomass than seedlings under control treatment (Table 1). Country of origin had no significant effect on total plant biomass, since both under control and drought treatment Malian and Malawian seedlings had similar plant weights (Table 1).

Water regime also had a significant effect on biomass allocation. While seedlings under drought had lower leaf weight ratios than seedlings under well-watered conditions, seedlings under drought also allocated more biomass to their root system (see LWR and R/S ratio in Table 1). Under drought conditions, country of origin also had a significant effect on seedlings' biomass allocation. Both the increment in R/S ratio under drought and the reduction in LWR were significantly larger in Malian seedlings compared with Malawian ones (Table 1).

Leaf shedding started the 7th day after water withholding (Fig. 1). From that point onwards, a great percentage (up to 40% for individual plants) of the leaves of droughted plants was shed in only 3–4 days. Then, leaf shedding gradually declined and stopped after approx. 25 days of water withholding while most plants still had some leaves. Leaf shedding started at the bottom of the stem and progressed upwards. Due to leaf shedding, seedlings under drought had significantly lower total leaf area and leaf area ratio than seedlings under control conditions (Table 1).

Compared with the number of leaves at the onset of the experiment, Malawian seedlings produced more leaves (60%) than Malian ones (30%) under control conditions (Fig. 1). Under drought conditions, Malawian seedlings shed relatively fewer leaves (20% of initial leaves) compared with Malian ones (60% of initial leaves, see Fig. 1), which resulted in a higher LAR at the end of the experiment (Table 1).

3.2. Leaf characteristics

Water regime, country of origin and their interaction had a significant effect on SLA (Table 1). Under control conditions, Malian seedlings had significantly lower SLA than Malawian ones. However, under drought conditions, Malawian seedlings significantly reduced their SLA, while Malian seedlings did not.

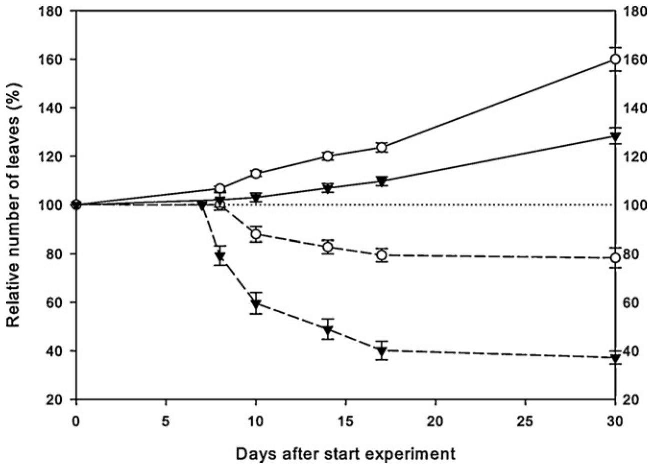

Fig. 1. The mean relative number of leaves of the baobab seedlings compared with the number of leaves at the beginning of the experiment. Closed symbols represent seedlings from Mali, open ones from Malawi. Continuous lines represent control seedlings, dashed lines droughted seedlings. Bars indicate standard errors ($n = 50$).

Although there were no significant differences in stomatal density between drought and control conditions (Table 1), there was a trend, with seedlings under drought producing leaves with higher stomatal density than seedlings under control conditions. Country of origin was not found to have a significant effect on stomatal density under any water regime.

Apart from modifying their leaves, seedlings under drought also reduced their predawn leaf water potential (Ψ_l) (Table 1). Malian seedlings reduced their Ψ_l much more than Malawian seedlings.

3.3. Water content of the different plant parts

Under both water regimes, the taproot was found to be the plant part which accumulated most plant water (on average 60% of total plant water). Also the stem stored an important percentage of total plant water (about 20%).

Water regime, country of origin and their interaction had a significant effect on water content of the different plant parts (Table 1). Seedlings under drought had lower water content of the different plant parts compared with seedlings under control conditions. Under control conditions, Malian seedlings had higher water content of the stem (WC_s) than Malawian seedlings. However, this was not the case for WC of the other plant parts. It should be noted that although seedlings from both countries had similar WC_r under control conditions, under drought conditions Malian seedlings reduced their WC_r more than Malawian ones.

3.4. Gas exchange measurements

Water regime also had a significant effect on all the gas exchange variables measured (Table 1). Under drought, seedlings reduced photosynthesis (A), transpiration (E), stomatal conductance (g_s) and internal CO_2-concentration (C_i). No differences between countries of origin could be detected on any gas exchange variables measured. With regard to WUE, while treatment had a significant effect on WUE (with droughted seedlings increasing their WUE, see Table 1), country of origin had no significant effect on WUE. The natural logarithm of g_s and WC_t were found to be significantly positively correlated (Fig. 2). Fig. 3 shows the response of C_i/C_a to decreasing g_s. The curve shown in Fig. 3 represents the expected response if assimilation was limited only by g_s, i.e. when

Table 1

Effects of water regime, country of origin and their interaction on the measured morphological and physiological traits. *P*-values in bold are statistically significant ($P < 0.05$). Mean values with their standard deviation are also given. Different characters in the right column indicate significant differences ($P < 0.05$) between treatments (drought/control and Mali/Malawi).

Plant trait	Abbreviation	Unit	Water regime	Country	Water regime × country	Control		Drought	
						Mali	Malawi	Mali	Malawi
Biomass and biomass allocation									
Total biomass	DW_{tot}	g	<**0.001**	0.80	0.89	14.72 ± 5.32 a	15.07 ± 4.58 a	11.5 ± 2.87 b	12.1 ± 3.03 b
Root/shoot ratio	R/S ratio	$g\,g^{-1}$	**0.013**	0.88	**0.036**	1.29 ± 0.42 a	1.26 ± 0.48 a	1.77 ± 0.52 c	1.47 ± 0.45 b
Leaf weight ratio	LWR	$g\,g^{-1}$	<**0.001**	0.17	<**0.001**	0.26 ± 0.04 a	0.25 ± 0.05 a	0.10 ± 0.04 b	0.16 ± 0.04 c
Leaf area ratio	LAR	$dm^2\,g^{-1}$	<**0.001**	0.49	<**0.001**	0.51 ± 0.10 a	0.54 ± 0.13 a	0.15 ± 0.10 c	0.29 ± 0.09 b
Total leaf area	LA	dm^2	<**0.001**	0.48	0.12	7.63 ± 3.43 a	8.27 ± 3.61 a	1.72 ± 1.20 b	3.58 ± 1.55 b
Leaf characteristics									
Specific leaf area	SLA	$dm^2\,g^{-1}$	<**0.001**	**0.033**	<**0.001**	2.75 ± 0.39 a	2.99 ± 0.42 b	2.75 ± 0.34 a	2.44 ± 0.31 c
Stomatal density	SD	mm^{-2}	0.07	0.12	0.99	208 ± 33 a	176 ± 38 a	221 ± 46 a	190 ± 47 a
Predawn leaf water potential	ψ_l	MPa	<**0.001**	0.66	<**0.001**	−0.46 ± 0.09 a	−0.43 ± 0.07 a	−1.10 ± 0.21 c	−0.8 ± 0.14 b
Water content of different plant parts									
Taproot water content	WC_t	%	<**0.001**	0.66	0.49	92.6 ± 1.4 a	92.4 ± 1.4 a	90.0 ± 1.4	89.5 ± 1.6 b
Fine roots water content	WC_r	%	<**0.001**	0.65	**0.004**	88.2 ± 1.9 a	88.8 ± 2.1 a	67.6 ± 7.2 b	72.5 ± 5.9 b
Stem water content	WC_s	%	<**0.001**	**0.001**	0.88	82.3 ± 1.5 a	79.4 ± 1.5 b	79.3 ± 1.4 c	76.4 ± 1.6 d
Leaf water content	WC_l	%	<**0.001**	0.22	0.19	84.9 ± 1.0 a	84.3 ± 1.1 a	83.5 ± 1.4 b	82.5 ± 1.6 b
Gas exchange									
Photosynthesis	A	$\mu mol\,CO_2\,m^{-2}\,s^{-1}$	<**0.001**	0.68	0.80	10.49 ± 2.43 a	11.52 ± 1.85 a	2.11 ± 2.12 b	2.42 ± 2.30 b
Transpiration	E	$mmol\,H_2O\,m^{-2}\,s^{-1}$	<**0.001**	0.69	0.80	3.12 ± 0.98 a	3.45 ± 1.06 a	0.28 ± 0.4 b	0.33 ± 0.3 b
Conductance to water vapour	g_s	$mol\,H_2O\,m^{-2}\,s^{-1}$	<**0.001**	0.52	0.89	0.20 ± 0.08 a	0.24 ± 0.10	0.02 ± 0.02 b	0.02 ± 0.02 b
Internal CO_2-concentration	C_i	$\mu mol\,CO_2\,mol^{-1}$	**0.003**	0.98	0.76	376 ± 38 a	375 ± 40 a	316 ± 100 b	324 ± 104 b
Water-use efficiency	WUE	$\mu mol\,CO_2\,mmol\,H_2O^{-1}$	<**0.001**	0.59	0.93	3.53 ± 0.51 a	3.62 ± 0.15 a	7.12 ± 0.51 b	6.79 ± 0.37 b

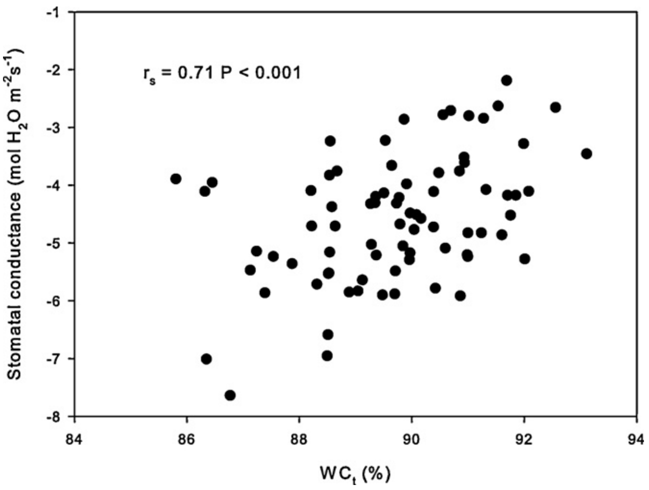

Fig. 2. Correlation between WC_t and stomatal conductance. *Y*-axis is plotted on a natural logarithmic scale.

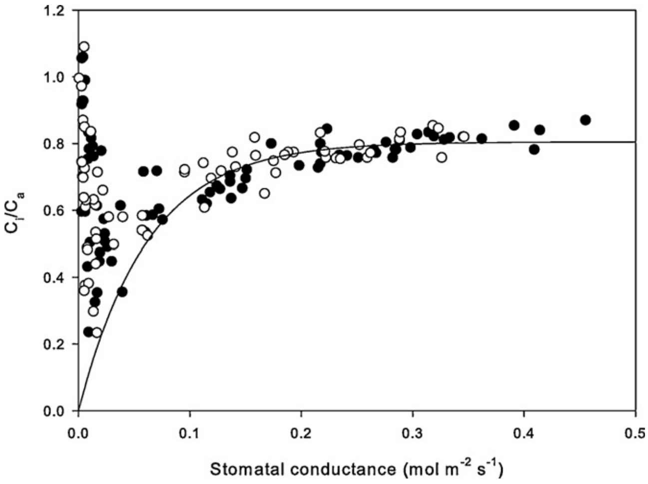

Fig. 3. Changing ratios of C_i/C_a as a function of stomatal conductance (g_s). The curve represents the relationship between g_s and C_i/C_a under conditions in which non-stomatal limitations of photosynthesis are absent (following Brodribb, 1996 and Rouhi et al., 2007). Open circles represent seedlings from Mali, closed ones from Malawi.

non-stomatal limitations were absent. Points falling at increasing distances above this curve reflect an increasing component of non-stomatal limitation. The lowest values of C_i/C_a occurred at a g_s of about 0.01 mol m^{-2} s^{-1}. Values for C_i/C_a rapidly increased when g_s dropped below this value.

4. Discussion

4.1. Drought coping mechanisms

Results from this study indicate that baobab seedlings use a number of morphological and physiological mechanisms to withstand drought. At the end of the experiment, total leaf area of the drought stressed seedlings was reduced sharply due to leaf shedding. Deciduousness in response to drought is a common trend across a large number of tree species from semi-arid environments (Borchert, 1994; Osonubi and Fasehun, 1987; Poorter and Markesteijn, 2008). In most of these species, apart from adult trees, seedlings also shed their leaves in order to survive the dry season (Poorter and Markesteijn, 2008). While most adult stem-succulent trees are completely deciduous under drought conditions (Borchert, 1994), baobab seedlings' leaf shedding stopped after some time, and, as a result, most plants kept some top leaves. Maes et al. (2009) found that the leaves of *Jatropha curcas* L. seedlings (another stem-succulent tree) were not abruptly but rather gradually shed in response to a drought event, and mostly, some leaves were kept. It has been suggested that some seedlings might not be able to lose all their leaves under adverse conditions because they do not possess sufficient carbohydrate reserves for leaf replacement (Poorter and Markesteijn, 2008). This might be the case for both baobab and *J. curcas* seedlings. Chapotin et al. (2006a), who studied two species of Malagasy baobab trees, suggested that the presence of leaves at the end of the drought season allows adult baobab trees to take advantage of scattered rainfall events occurring before the real onset of the rainy season. Similarly, the advantage of keeping some leaves might help baobab seedlings to overcome short droughts and to take advantage of scattered rainfall after the start of the dry season

Apart from shedding some leaves, baobab seedlings under drought stress also produced new leaves with lower SLA and higher stomatal density compared with the older leaves, characteristics often related to drought adaptation (Abrams et al., 1990). Other stem-succulent species have also been reported to produce new leaves with altered morphology under drought conditions. For example, Maes et al. (2009) reported that *J. curcas* seedlings under drought stress produced new leaves with higher stomatal density. The production of drought-adapted leaves might enable baobab seedlings to keep some leaves physiologically active during the beginning of the drought period in order to benefit from scattered rainfall events.

While under drought conditions leaf biomass was significantly reduced (due to leaf shedding), biomass allocation to the root system was significantly increased (higher R/S ratio). A relative increase in biomass allocation to the root system under drought conditions has also been reported for a number of other semi-arid tree species such as *J. curcas* and *P. biglobosa* (Achten et al., 2010; Osonubi and Fasehun, 1987). Greater investment in the root system (e.g., root elongation or production of thinner roots) is a common strategy seedlings might use to overcome drought (Fitter and Hay, 2002). The observed values of R/S ratios (without leaves) for baobab seedlings under drought (1.47–1.77) are much higher than the values observed for droughted *J. curcas* seedlings (~0.5) (Achten et al., 2010), illustrating the relative importance of the taproot in droughted baobab seedlings.

In this study the taproot was found to be the plant part which accumulated most plant water, which agrees with the suggestion that the taproot is the key organ for baobab seedling survival under drought conditions (Wickens and Lowe, 2008). The presence of a thickened taproot which accumulates water is one of the most common traits of seedlings of semi-arid tree species (Poorter and Markesteijn, 2008). However, as the stem was also found to accumulate an important part of total plant water, it should be noted that even at seedling stage, the stem plays an important role as water storing organ. As the water storage role of the taproot is overtaken by the stem in the adult stage for the baobab tree (Wickens and Lowe, 2008), it can be argued that with increasing plant age the role of the stem becomes more important for baobab seedlings. For *J. curcas* seedlings, it has also been reported that the stem plays an important role as water storing organ (Maes et al., 2009). In adult Malagasy baobab trees, the water stored in the parenchymous stem tissue is conserved under drought conditions because it will be used for the formation of new leaves in the following growing season (Chapotin et al., 2006a). For *A. digitata* seedlings, the water stored both in the taproot and the stem might also be used for this purpose.

As water content of the taproot of drought stressed seedlings was much lower than those under control conditions, it seems that the water stored in the taproot is being used for (i) maintaining part of the old, physiologically active, leaves, (ii) for the formation of new leaves with altered morphology, (iii) for the formation of new roots and (iv) for the maintenance of metabolic processes of the remaining tissues. Baobab shoots have a green photosynthesizing bark layer (Baum, 1995) and imprints showed the presence of stomata on the stems of the studied baobab seedlings. Therefore, the observed taproot water losses might, at least partly, be related to stem surface transpiration associated with bark photosynthesis. Non-foliar photosynthesis is often an important strategy of additional carbon acquisition, especially during stress and leafless periods (Rouhi et al., 2007).

Compared with other tree species found in the same environment, baobab seedlings substantially reduced stomatal conductance (g_s) (and concomitantly photosynthesis and transpiration) under drought stress. For example, it has been reported that *P. biglobosa* seedlings reduced g_s about 50% after two weeks of water withholding (Osonubi and Fasehun, 1987; Teklehaimanot et al., 1998) while in our experiment baobab seedlings reduced their g_s about 80% after two weeks of drought (see Table 1). Also compared with *Ziziphus mauritania* seedlings (Kulkarni et al., 2010), baobab was found to be more conservative in terms of water usage: while *Z. mauritania* seedlings reduced their transpiration (E) by only ~25% after two weeks of drought, baobab seedlings reduced E by >90% after the same drought period (Table 1). The tight control of baobab seedlings over stomatal water losses enables the plants of keeping a high water status during drought events, which helps to prevent xylem cavitation (see further). Chapotin et al. (2006b) found that transpirational water losses were also under strong stomatal control in adult individuals of two Malagasy baobab species, even during short drought events taking place in the rainy season. While baobab trees are stem-succulent species (a functional group that is known to have a tight stomatal control over transpirational water losses), *P. biglobosa* and *Z. mauritania* are deciduous hardwood species, which are much less capable of lowering g_s (Borchert, 1994).

In this experiment WC_t was found to be strongly positively correlated with g_s (see Fig. 2). It is known that stomata can respond directly to the plant water status due to loss of leaf turgor (Fitter and Hay, 2002). However, we did not detect a loss of leaf turgor of the seedlings subjected to drought (visual obs.); thus, it can be hypothesized that stomatal closure (aimed at preventing further water loses) is mainly governed by the activation of ABA synthesis in the taproot (Pei et al., 2000).

Under control conditions, when g_s was relatively high, photosynthesis was mainly controlled by stomatal opening (Fig. 3). However, as there was a divergence between the expected C_i/C_a and the observed one with decreasing photosynthesis, it can be concluded that as drought persevered, non-stomatal limitations became important (see Brodribb, 1996). Non-stomatal limitations at low conductances have also been shown to be important in a number of almond (*Prunus* spp.) species (Rouhi et al., 2007).

The sharp reduction in g_s of baobab seedlings under drought stress took place when leaves still had a relatively high Ψ_l, which is a typical characteristic of isohydric species like stem-succulents (Borchert, 1994; McDowell et al., 2008). In general, isohydric species reduce stomatal conductance as soil water potential decreases and vapour pressure deficit of the air drops, thereby maintaining a relatively constant Ψ_l (in contrast to anisohydric species, which maintain a higher g_s but allow Ψ_l to decline as soil water potential drops, McDowell et al., 2008). Chapotin et al. (2006b) mentioned that, in conjunction with a sharp drop in g_s, Ψ_l rarely dropped below −1.2 MPa. The results obtained in this study for *A. digitata* seedlings agree with the findings of Chapotin et al. (2006b). Low leaf water potentials increase the risk of hydraulic failure by xylem cavitation, particularly in plants with wide vessels, which is typical for trees with a low wood-density (Hacke et al., 2001), such as the baobab tree (Wickens and Lowe, 2008). Thus, it seems that in baobab seedlings, xylem cavitation is avoided by the development of a tight control over g_s, thereby alleviating the need for Ψ_l to drop substantially.

4.2. Differences in drought response between country of origin

The described mechanisms used by baobab seedlings to deal with drought were, in general, used by all seedlings regardless of the country. However, there were some remarkable differences between both countries in the extent to which some of the described mechanisms were used.

Under drought conditions, seedlings from Mali shed a higher proportion of their leaves and produced a lower number of new leaves with altered morphology than Malawian seedlings. They tended also to allocate relatively more biomass to their root system. Since leaf shedding and an increase of the R/S ratio are typical drought avoidance mechanisms (Fitter and Hay, 2002; Poorter and Markesteijn, 2008), it seems that baobab seedlings from western Africa are more water conservative than seedlings from south-eastern Africa.

Under drought conditions, Malawian seedlings showed a lower reduction of their leaf water potential (Ψ_l) compared with Malian ones. However, both maintained relatively constant levels of leaf water potential, which is known to be a typical drought-avoidance strategy (Borchert, 1994; Rouhi et al., 2007).

The observed differences in drought responses between seedlings from Mali and Malawi follow the genetic differences between western and south-eastern baobab populations reported by Pock Tsy et al. (2009). In a recent study, phenotypic differences in fruit characteristics between baobab trees from western and south-eastern Africa have also been reported, as well as differences in seedling growth patterns (Cuní Sanchez et al., 2010, 2011). The differences in mechanisms used by baobab seedlings to cope with drought conditions might have been evolved in response to differences in the environment where these baobab tree populations are found. It has been suggested that baobab trees from western and south-eastern Africa have different ecological requirements (Cuní Sanchez et al., 2011). For example, probability of erratic rains during the onset of the dry season, extremely low air humidity during the Harmattan season (dry wind from the Sahara desert blowing southwards in the Sahel) or soil type might explain why baobab seedlings from western Africa show more drought-avoidance characteristics compared with baobab seedlings from south-eastern Africa.

Considering that there are important differences in the way baobab seedlings cope with drought, there is room for selecting planting material with greater drought tolerance. Our results indicate that plant material from western Africa can be used for reforestation and cultivation purposes in dry regions of south-eastern Africa. However, longer (more than 4 weeks) drought stress experiments with more accessions and *in situ* seedlings experiments are needed to provide further information on baobab seedling responses to drought and on differences between accessions.

5. Conclusions

Results indicate that baobab seedlings use a number of mechanisms to cope with drought conditions, most of them being drought avoidance ones. Part of the leaves is rapidly shed after the onset of drought, and some new leaves with altered morphology are formed. In order to prevent water losses, baobab seedlings have a very tight control over stomatal closure, thereby maintaining relatively constant leaf water potentials. The drought tolerance of baobab seedlings seems thus to be due to their conservative water use. However, some water is still being used for keeping some leaves physiologically active, for the formation of new leaves and roots, and for the maintenance of metabolic processes. While photosynthesis is mainly controlled by stomatal opening at the onset of drought, non-stomatal limitations become important as drought perseveres. Differences in the way baobab seedlings from western and south-eastern African cope with drought could be observed, with seedlings from Mali using more drought avoidance mechanisms compared with seedlings from Malawi. As seedlings from western Africa seem to be better adapted to extreme drought events, they could be used for reforestation and cultivation purposes in dry areas in both western and eastern Africa. However, we recommend future research on longer and *in situ* drought experiments in order to confirm this hypothesis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envexpbot.2011.09.011.

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