Baobab (*Adansonia digitata* L.) seedlings under drought: differences between genetic clades

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Introduction

The African baobab, *Adansonia digitata* L., is a stem-succulent tree native to the dry regions of tropical Africa (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). Lack of recruitment, partially related to increasing drought events, threatens the species (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 (WICKENS AND LOWE, 2008), which might indicate that both genetic clades have developed different mechanisms to cope with drought. This study aims to investigate the morphological and eco-physiological mechanisms baobab seedlings use to cope with soil drought, and to determine if these mechanisms differ between seedlings originating from western and south-eastern Africa.

Material and Methods

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. Climatic details of the study sites are given in CUNÍ SANCHEZ ET AL. (2011A) and DE SMEDT ET AL. (2011). From each study site, 20 seedlings were grown for 14 weeks under well-watered conditions. After 14 weeks, half of the seedlings continued to be grown in the described conditions (control treatment) while from the other half irrigation was withheld (drought treatment). Four weeks later, all plants were harvested. During these four weeks, number of leaves on each plant was counted on a regular basis.

After harvesting, seedlings were divided into taproot, other (fine) roots, stem and leaves. Fresh weights of each plant part (in g) were determined (named FW_t, FW_r, FW_s and FW_l, respectively). The leaves of each seedling were scanned and total leaf area per plant (LA, dm^2) was determined. All seedling parts were dried and dry weights of the total plant and of the different plant parts (in g) were determined (named DW_{tot}, DW_t, DW_r, DW_s and DW_l). The water content of each plant

part ($WC_{plant\ part}$) could then be calculated. 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. 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. Specific leaf area (SLA, $dm^2\ g^{-1}$) was calculated by dividing the punched area by the dry weights of the discs. Stomatal density (SD, in number of stomata mm^{-2}) was assessed as in DE SMEDT ET AL. (2012). Predawn water potential (Ψ_l, MPa) of the first fully developed leaf was measured immediately after harvesting with a scholander pressure chamber. Net photosynthesis (A, $\mu mol\ CO_2\ m^{-2}\ s^{-1}$), stomatal conductance to water vapour diffusion (g_s, $mol\ H_2O\ m^{-2}\ s^{-1}$), and transpiration rate (E, $mmol\ H_2O\ m^{-2}\ s^{-1}$) were measured using a infrared gas analyser. Details on gas exchange measurements can be found in DE SMEDT ET AL. (2012). All gas-exchange parameters were estimated after 14 days of drought stress on the 4th completely expanded leaf (counted from the top of the plant).

Mixed effects models were used to test for treatment effects on all measured morphological and physiological seedling traits. 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. Correlations between traits were tested using Spearman's rank order coefficients.

Results and Discussion

Results are summarized in Table 1. Droughted seedlings had significantly lower total plant biomass than seedlings under control treatment. While seedlings under drought had lower LWR than seedlings under well-watered conditions, seedlings under drought also allocated more biomass to their root system (higher R/S ratio), which is a common strategy to overcome drought periods. Both the increment in R/S ratio under drought and the reduction in LWR were larger in Malian seedlings compared with Malawian ones.

Leaf shedding started the 7th day after water withholding. 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 top leaves. This is in contrast with most other stem-succulent trees, which are completely deciduous under drought conditions (BORCHERT, 1994). Keeping a few leaves during the first drought events at the end of the rainy season might help baobab seedlings to take advantage of scattered rainfall after the start of the dry season. Due to leaf shedding, seedlings under drought had significantly lower leaf area ratio (LAR) than seedlings under control. Malawian seedlings shed relatively fewer leaves (20% of initial leaves) compared with Malian ones, which resulted in a higher LAR at the end of the experiment.

Water regime, country of origin and their interaction had a significant effect on SLA. 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. Although there were no significant differences in stomatal density between drought and control conditions, there was a trend, with seedlings under drought producing leaves with higher stomatal density than seedlings under control conditions. The production of leaves with a lower SLA and a higher SD is often related to drought adaptation (ABRAMS ET AL., 1990). 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. Apart from modifying their leaves, seedlings under drought also reduced their predawn leaf water potential (Ψ_l). Malian seedlings reduced their Ψ_l much more than Malawian seedlings.

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).

Trait	Unit	Water regime	Country	Water regime x country	Control Mali				Control Malawi				Drought Mali				Drought Malawi			
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
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
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
LAR	dm² 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
SLA	dm² 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
SD	mm^{-2}	0.07	0.12	0.99	208	±	33	a	176	±	38	a	221	±	46	a	190	±	47	a
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
WC_t	%	**< 0.001**	0.66	0.49	92.6	±	1.4	a	92.4	±	1.4	a	90.0	±	1.4	b	89.5	±	1.6	b
WC_r	%	**< 0.001**	0.65	**0.004**	88.2	±	1.9	a	88.8	±	2.1	a	67.6	±	7.2	c	72.5	±	5.9	b
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
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
A	µmol 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
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
g_s	mol H_2O m^{-2}s^{-1}	**< 0.001**	0.52	0.89	0.20	±	0.08	a	0.24	±	0.10	a	0.02	±	0.02	b	0.02	±	0.02	b

The taproot was found to be the plant part which accumulated most plant water (on average 60 % of total 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). 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.

Since stomatal conductance (g_s) (and concomitantly photosynthesis and transpiration) was considerably reduced under drought stress, baobab seedlings seem to be conservative in terms of water usage. The natural logarithm of g_s and WC_t were found to be significantly positively correlated (r_s = 0.71, P < 0.001), which might indicate that stomatal closure (aimed at preventing further water losses) is mainly governed by the activation of ABA synthesis in the taproot. 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). 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, 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.

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, it seems that baobab seedlings from western Africa are more water conservative than seedlings from south-eastern Africa. These 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) and the morphological differences observed by CUNÍ SANCHEZ ET AL. (2011A, 2011B). 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. 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.

Conclusions and Outlook

Results indicate that baobab seedlings use a number of mechanisms to cope with drought conditions, most of them being drought avoidance ones. The drought tolerance of baobab seedlings seems thus to be due to their conservative water use. Some water is however being used for keeping some leaves physiologically active, for the formation of new leaves and roots, and for the maintenance of metabolic processes. 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.

References

ABRAMS, M.D., KUBISKE, M.E. AND STEINER, K.C. (1990). Drought adaptations and responses in 5 genotypes of *Fraxinus pennsylvanica* Marsh - Photosynthesis, water relations and leaf morphology. Tree Physiology 6: 305-315.

BORCHERT, R. (1994). Soil and stem water storage determine phenology and distribution of tropical dry forest trees. Ecology 75: 1437-1449.

BUCHMANN, C., PREHSLER, S., HARTL, A. AND VOGL, C.R. (2010). The importance of baobab (*Adansonia digitata* L.) in rural West African subsistence - Suggestion of a cautionary approach to international market export of baobab fruits. Ecology of Food and Nutrition 49: 145-172.

CUNÍ SANCHEZ, A., DE SMEDT, S., HAQ, N. AND SAMSON R. (2011A). Comparative study on baobab fruit morphological variation between western and south-eastern Africa: opportunities for domestication. Accepted for publication in Scientia Horticulturae.

CUNÍ SANCHEZ, A., DE SMEDT, S., HAQ, N. AND SAMSON, R. (2011B). Variation in baobab seedling morphology and its implications for selecting superior planting material. Scientia Horticulturae 130: 109-117.

DE SMEDT, S., ALAERTS, K., KOUYATÉ, A.M., VAN DAMME, P., POTTERS, G. AND SAMSON, R. (2011). Phenotypic variation of baobab (*Adansonia digitata* L.) fruit traits in Mali. Agroforestry Systems 82: 87-97.

DE SMEDT, S., CUNÍ SANCHEZ, A., VAN DEN BILCKE, N., SIMBO, D., POTTERS, G. AND SAMSON, R. (2012). Functional responses of baobab (*Adansonia digitata* L.) seedlings to drought conditions: Differences between western and south-eastern Africa. Environmental and Experimental Botany 75: 181-187.

POCK TSY, J.M.L., LUMARET, R., MAYNE, D., VALL, A.O.M., ABUTABA, Y.I.M., SAGNA, M., RAOSETA, S.O.R. AND DANTHU, P. (2009). Chloroplast DNA phylogeography suggests a West African centre of origin for the baobab, *Adansonia digitata* L. (Bombacoideae, Malvaceae). Molecular Ecology 18: 1707-1715.

WICKENS, G.E. AND LOWE, P. (2008). The baobabs: pachycauls of Africa, Madagascar and Australia. Springer, London, UK.