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14 **Title**

15 Plant-water relationships and growth strategies of *Jatropha curcas* L. seedlings under
16 different levels of drought stress

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34 **Abstract**

35 Although *Jatropha curcas*, an important tropical biofuel crop, is reputed for its drought
36 resistance, its ability to perform under dry conditions has hardly been investigated. In a
37 greenhouse experiment we investigated the plant-water relationships of *Jatropha* seedlings
38 from different accessions under different levels of drought stress.

39 There was little difference in plant water relations between accessions.

40 Drought significantly reduced leaf area, biomass and relative growth rate, but had no effect
41 on specific leaf area, daily range in leaf water potential, leaf water content, transpiration
42 efficiency or aboveground biomass water productivity, corrected for atmospheric
43 conditions. Stem wood density was equally low ($0.26 \text{ g}\cdot\text{cm}^{-3}$) for all treatments. Stem water
44 content was lowest for dry treatment seedlings.

45 Based on these results, *Jatropha* could be characterized as a stem succulent tree. In contrast
46 to other stem-succulent deciduous trees, leaves were not shed immediately after the
47 seedlings were confronted with drought. Instead, at the onset of drought, leaves with a
48 higher adaxial stomatal density were formed, after which leaves were only gradually shed.
49 The role of the succulent stem in the water economy of *Jatropha* was confined to balancing
50 the small water losses of the leaves during drought.

51

52 **Keywords**

53 Biodiesel, leaf water potential, Physic nut, stem succulent, stomatal density, stomatal
54 conductance

55 **1. Introduction**

56 Investments in fossil fuel substitution by biofuels should focus on biofuels extracted from
57 perennials grown on abandoned agricultural or degraded lands, as these do not cause a
58 carbon debt at land use change (Fargione et al., 2008; Searchinger et al., 2008). In this
59 context, *Jatropha curcas* L. is promising as a sustainable biofuel option. With its seeds
60 containing up to 35% oil easily convertible into biodiesel, its potential to reclaim
61 wastelands, with positive effects on ecology and socio-economic development (Francis et
62 al., 2005), and with its reputation of being a drought-resistant and easily establishing
63 species, this small tree originating from Central- and South-America is now planted
64 worldwide on wastelands in the (semi-arid) tropics (Fairless, 2007; Achten et al., 2008).
65 Yet, there are two major concerns that might abate the expectations. First, there is a
66 surprising lack of scientific knowledge about basic agronomic properties (Fairless, 2007).
67 To our knowledge, the physiological mechanism behind the high drought resistance of *J.*
68 *curcas* has never been described. Second, *J. curcas* still has to be considered a wild plant
69 (Achten et al., 2007; Fairless et al., 2007) with little information available about the
70 performance of seed sources and accessions.

71 We investigated the plant-water relations of *J. curcas* from different accessions and aimed
72 to quantify its physiological adaptations to drought stress.

73 Three known characteristics of *J. curcas* allow formulating hypotheses about its plant-water
74 relations. The plant (i) originates from the seasonal tropical forest (Achten et al., 2007) (ii)
75 is deciduous, and (iii) has a succulent stem (Foidl et al., 1996) with soft wood (Henning,
76 2006). This categorizes *J. curcas* as a tree species of the D_{light} type (sensu Borchert, 1994),
77 i.e. deciduous lightwood trees with large stem water storage, or deciduous stem-succulent
78 trees. Trees of this type have several plant-water relations in common.

79 The most characteristic property D_{light} -species share is the stem succulence, manifested
80 through a high stem water content and a low wood density (Borchert, 1994; Holbrook et al.,
81 1995). The stem water seems to play a role in regulating the leaf water potential (ψ_{leaf}),
82 serving as a buffer against low soil water potential (\bullet_{soil}): predawn leaf water potential (ψ_{pd})
83 is high and uncorrelated with \bullet_{soil} and the diurnal range in ψ_{leaf} ($\bullet\bullet$) is low (Nilsen et al.,

1990; Borchert, 1994; Chapotin et al., 2006a). The leaves, which have a high water content, low specific leaf area (SLA) and few drought adaptations (Nilsen et al., 1990; Borchert, 1994), are typically shed at very low $\Delta\psi$ -values (Borchert, 1994), early after or even before the start of the dry season (Lobo et al., 2003; Chapotin et al., 2006b). The stem water does not play a role in maximizing stomatal conductance, which is generally lower than that of co-occurring species and which decreases during the day (Chapotin et al., 2006a, b).

Based on the general postulate that *J. curcas* is a deciduous stem-succulent tree we investigated the following specific hypotheses:

- 1) *J. curcas* has a low wood density and a high stem water content.
- 2) *J. curcas* has leaves with a high water content and small SLA, with high ψ_{pd} and small $\Delta\psi$. Stomatal conductance will be closely regulated, resulting in decreased values during the day.
- 3) Leaf and stem growth stops and leaves are shed shortly after *J. curcas* is faced with drought stress.

Furthermore, we calculated the transpiration efficiency and biomass accumulation, important characteristics for a biofuel crop, and investigated if all above characteristics differed between plants of different accessions.

2. Materials and Methods

2.1. Experimental Set-up

J. curcas seeds were sown in the center of 6.5L pots (205mm height, river sand:peat, 400 mm spacing) on July 3, 2007 (Day 0), in a tropical glasshouse at K.U.Leuven, Belgium. A randomized Latin Square design of 3 accessions (Ethiopian, Indian and Thai seeds) \times 3 Drought treatments \times 9 replicates was used. An additional 14 pots were sown with the Ethiopian accession. Pots were kept at field capacity until Day 62 (juvenile phase, JP) after which irrigation treatments of ‘dry’ (unwatered), ‘medium’ (40% plant available water, PAW) and ‘wet’ (slightly below field capacity) were applied until Day 114 (treatment phase, TP). During TP, all plants were manually watered three times a week to a target

112 weight. Pot mass before and after watering were recorded. The irrigation water contained a
113 balanced nutrient mixture. Aluminium foil covered the soil surface and bottom of the pots
114 prevented water evaporation.

115 Target weights were based on the established soil pF curve and the volumetric soil water
116 content θ_v , measured with a TRIME TDR sensor (Imko, Ettlingen) on days 2, 62, 76 and
117 90, and was regularly corrected for plant weight.

118 Air temperature (T_a , [°C]) was 17-27°C during the entire experiment, relative air humidity
119 (RH, [%]) was kept at 70% during JP and lowered to 30-40% during TP. T_a , RH, and PAR
120 were measured every half hour. Mean PAR and VPD during daytime of TP are given in
121 table 1. Atmospheric CO₂ concentration was 500-600 ppm.

122 2.2. Non-destructive Measurements

123 On Days 62, 76, 90, 104 and 114 the number of leaves (nL) and branches (nB), stem length
124 (L ; [cm]) and diameter at base (D_b ; [mm]) and at several intervals were measured and stem
125 volume (including the branches) (Vol; [cm³]) was derived using Smalian's sectional
126 volume formula (West 2004).

127 Leaf stomatal conductance (g_s ; [mmol·m⁻²·s⁻¹]) was measured with a steady-state porometer
128 (Model SC-1, Decagon Devices, Washington) on Days 72 (two top leaves of all seedlings,
129 measured in two rounds between 10:00 and 17:00 local time), 79 (three top leaves of 27
130 plants of the central three rows, measured 7 times (7 rounds) between 08:00 and 18:30 lt)
131 and 107 (idem, but measured 4 times (4 rounds) between 11:00 and 17:00 lt) .

132 The daily mean ($g_{s,m}$) was calculated per seedling per day. On day 79, the mean g_s per
133 round ($g_{s,pr}$) was additionally calculated, as well as mean VPD and mean PAR per round.
134 Climatic conditions on days 72, 79 and 107 are given in table 1.

135 Midday leaf water potential (\bullet_{md}) was measured on Day 104, \bullet_{pd} on Day 105, using a
136 Scholander pressure chamber (3000 Plant Water Status Console; Soil moisture equipment)
137 on one randomly selected top leaf of 54 seedlings (no measurements on plants of the central
138 three rows). Per seedling $\Delta\bullet$ was calculated as $\psi_{pd}-\psi_{md}$. Climatic conditions of the morning
139 (up to 14:00 local time) of Day 105 are given in table 1.

140 Stomatal density impressions of the adaxial and abaxial side were taken from one full-
141 grown top leaf, formed during TP, per seedling (Paoletti et al., 2007) of the three central
142 rows at Day 114. Impressions were made using colorless nail polish and adhesive
143 cellophane tape (Ceulemans et al., 1995; Herrick et al., 2004), were examined with a light
144 microscope connected to a computer and analyzed with Cell^B v. 2.6 software (Olympus
145 Soft Imaging Solutions, Hamburg).

146 The abaxial stomatal density (SD_{ab}) per seedling was estimated as the mean of the number
147 of stomata in three randomly selected areas of $500 \times 500 \mu\text{m}^2$. The stomatal index (SI_{ab}) was
148 calculated as $SI_{ab} = 100 \cdot \frac{S}{(S + E)}$ (Radoglou and Jarvis 1990; Ceulemans et al., 1995) with

149 S and E the total number of stomata and epidermal cells present in three randomly chosen
150 $250 \times 250 \mu\text{m}^2$ areas, respectively. From these stomata, the length ($L_{stom,ab}$) was measured.
151 A preliminary analysis on the adaxial side of the leaf revealed that the stomata were not
152 randomly distributed but concentrated near the veins. A grid of $250 \times 500 \mu\text{m}^2$ was laid over
153 three randomly selected areas with the long side parallel with the veins. The number of
154 stomata was counted in three adjacent subareas. The mean SD_{ad} per subarea was calculated
155 per leaf.

156

157 (Insert table 1)

158

159 2.3. Destructive Measurements

160 On Day 114, 58 of the 81 seedlings were harvested (15 dry, 21 medium, 22 wet; all
161 seedlings of the three central rows). Two seedlings were removed from the experiment after
162 infection by Spider mite (*Tetranychus sp.*), the remaining were used in an additional
163 experiment. Fresh weight (FW) and dry weight of leaves (W_l), stem (including branches,
164 W_s), roots (W_r) and of the whole plant ($W_{t,114}$) were measured. The water content was
165 calculated as $100 \cdot (1 - W \cdot FW^{-1})$ for leaves (WC_l ; [%]), stems including branches (WC_s ; [%])
166 and roots (WC_r ; [%]). Leaf weight ratio (LWR; [$\text{g} \cdot \text{g}^{-1}$]) was calculated as $LWR = W_l \cdot W_{t,114}^{-1}$
167 ¹.

168 Of the 27 seedlings in the central three rows, Vol, FW and W of the woody part of the stem
169 were separately measured and water content of the woody part (WC_w ; [%]) and wood
170 density (WD ; [$g \cdot cm^{-3}$]) calculated.

171 The total leaf area (LA ; [cm^2]) of these 27 plants was recorded with a LI-COR 3050C leaf
172 area meter (LI-COR, Nebraska) and average leaf size (A_i ; [cm^2]), specific leaf area ($SLA =$
173 $LA \cdot W_i^{-1}$; [$cm^2 \cdot g^{-1}$]) and leaf area ratio ($LAR = LA \cdot W_{t,114}^{-1}$, [$cm^2 \cdot g^{-1}$]) were calculated.

174 The 14 seedlings of the parallel experiment were harvested between 8 and 41 days after
175 germination, and their dry and fresh root, stem and leaf and total biomass were obtained.
176 One seedling had reduced root growth and was omitted from further analyses.

177

178 2.5. Additional calculations

179 A stepwise approach was followed to fit a polynomial function through $\ln(W_i)$ of the
180 parallel experiment plotted against day after germination (Hunt and Parsons, 1974). The
181 best fitting function was a second order polynomial ($R^2 = 0.97$), whose linear term was used
182 as an estimate of maximum relative growth rate (RGR_{max} ; [$g \cdot g^{-1} \cdot day^{-1}$]) (Fernández et al.,
183 2002; Poorter and Garnier 2007).

184 The transpiration rate at day i (TR_i ; [$g \cdot day^{-1}$]) was calculated based on the weights before
185 and after watering and corrected for leaf loss.

186 Two forward linear regression models, estimating $W_{t,i}$ and aboveground biomass ($W_{AG,i}$) on
187 Day i as a function of several plant characteristics were established (both models: $R^2_a =$
188 0.90 , and $\log(Vol)$, L and nL as input variables) and $W_{t,62}$, $W_{AG,62}$, $W_{AG,76}$, $W_{AG,90}$, $W_{AG,104}$
189 and $W_{AG,114}$ were calculated of each seedling. In case of leaf loss by Day i , nL_{max} of the
190 seedling was used for nL_i to calculate $W_{AG,i}$

191 The relative growth rate during TP (RGR_{TP} ; [$g \cdot g^{-1} \cdot day^{-1}$]) was calculated as

192 $RGR_{TP} = (\ln(W_{t,114}) - \ln(W_{t,62})) / 52$ (Evans, 1972). Transpiration efficiency (TE ; [$mg \cdot g^{-1}$])

193 was calculated as $TE = (W_{t,114}^{corr} - W_{t,62}) / \sum_{i=62}^{114} TR_i$, where $W_{t,114}^{corr}$ is $W_{t,114}$ added with the

194 estimated W_1 of the lost leaves.

195 The cumulative transpiration on day i (CT_i ; [$\text{l}\cdot\text{kPa}^{-1}$]), normalized for VPD was calculated
 196 as $CT_i = \sum_{j=62}^i TR_j / VPD_j$ with VPD_j the mean vapor pressure deficit on day j (Steduto et al.,
 197 2007). Biomass water productivity, normalized for atmospheric conditions (WP ; [$\text{g}\cdot\text{kPa}\cdot\text{l}^{-1}$])
 198 was calculated as the regression coefficient of the linear regression with CT_i , Accession
 199 and Drought treatment as input, and $W_{AG,i}$ as dependent variable. This value was corrected
 200 for CO_2 -concentration as (Steduto et al., 2007):

$$201 \quad WP_{ref} = WP \cdot \frac{\sum_{i=1}^n (c_{a,0})_i}{\sum_{i=1}^n (c_a)_i} \cdot \frac{\sum_{i=1}^n (\Delta w)_i}{\sum_{i=1}^n (\Delta w_0)_i} \quad (eq. 1)$$

202 With $(c_a)_i$ the mean CO_2 -concentration at day i , $(c_{a,0})_i$ the reference CO_2 -concentration (360
 203 ppm), and $\sum_{i=1}^n (\Delta w)_i / \sum_{i=1}^n (\Delta w_0)_i$ a factor correcting for differences in water vapor
 204 concentration between the leaf intercellular air space and the atmosphere, for which a value
 205 of 1 was taken (Steduto et al., 2007).

206 As both growth and water use of the dry treatment seedlings were limited, TE, RGR_{TP} and
 207 WP were only estimated for seedlings of the wet and medium drought treatment.

208 2.6. Statistical Analysis

209 A two-factor type III ANOVA with factors Drought treatment (three levels, but two levels
 210 for TE) and Accession (three levels) was performed separately on $\log_{10}(g_{s,m}(72))$,
 211 $\log_{10}(g_{s,m}(79))$, $\log_{10}(g_{s,m}(107)+1)$, W_t , LA, RGR_{TP} , TE, SLA, ψ_{pd} , ψ_{md} , $\Delta\psi$, SD_{ab} , SI_{ab} ,
 212 $L_{stom,ab}$, WC_l , WC_s , WC_r , WC_w and WD. Tukey post-hoc tests were calculated.
 213 Repeated Measures ANOVA with between-subject factors Drought and Accession was
 214 applied on nL (within-subject factor = Day, 5 levels), $TR_i^{1/2}$ (within-subject factor = Day,
 215 20 levels=irrigation events), $\log(g_{s,pr})$ (of Day 79, within-subject factor = Round, seven
 216 levels), $(SD_{ad})^{1/2}$ (within-subject factor = Distance to vein, 3 levels: near the vein, in the
 217 middle, and further away). Pillai's Trace statistic was used for multivariate tests; if
 218 Mauchly's Test was significant, the Huyn-Feldt statistic was used for within-subject tests.
 219 Bonferroni was applied for post-hoc testing and confidence interval estimation.

220 A paired samples t-test was used to verify if the difference between ψ_{pd} and ψ_{md} were
221 different from zero (Gebrehiwot et al., 2005).

222 All statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, IL).

223 3. Results

224 3.1. Water use and growth

225 ANOVA output and posthoc results for Drought treatment of this section are given in table
226 2a.

227 3.1.1 Transpiration rate

228 Transpiration rate was only significantly influenced by Drought and Time×Drought
229 ($P<0.001$) (see Figure 1a). After a strong decline between Days 62-76, TR of the dry
230 treatment seedlings was very small, declining steadily from Day 76 onwards, when θ_v had
231 dropped to $4.4 \pm 0.3 \%$, close to wilting point (3.5%).

232 After an initial decline, the TR of the medium treatment stabilized during the first weeks of
233 the experiment and was closely correlated with atmospheric demand. From Day 80
234 onwards the TR decreased slightly and was less correlated with atmospheric demand. The
235 TR of the wet treatment seedlings increased sharply in the first weeks of the experiment
236 and decreased slightly afterwards, remaining closely correlated with the atmospheric
237 demand.

238

239 (Insert Figure 1)

240

241 3.1.2 Stomatal conductance

242 On the three measuring days $g_{s,m}$ differed between Drought treatments in the order
243 $g_{s,m}(\text{wet}) > g_{s,m}(\text{med}) > g_{s,m}(\text{dry})$, although on Day 107 the difference between $g_{s,m}(\text{wet})$ and
244 $g_{s,m}(\text{med})$ was not significant. The other factors were not significant.

245 On Day 79, the Drought-Round interaction was significant ($P < 0.001$), see Figure 2. The
246 $g_{s,pr}$ of the wet treatments had a sharp rise during the morning but started declining before
247 PAR and VPD had reached their maximal values in the late afternoon.

248

249 (Insert Table 2)

250 (Insert Figure 2)

251

252 3.1.3 Biomass growth, growth rate and efficiency

253 The number of leaves changed through time and was different between the drought
254 treatments (Figure 3). At the time of harvest (Day 114), nL was not maximal for any of the
255 three drought treatments. Leaf loss occurred first for the dry treatment-seedlings and last for
256 the wet treatment seedlings. Total biomass, LA, LAR and LWR differed significantly
257 between the Drought treatments in the order wet>medium>dry, although the difference
258 between the medium and wet treatment was not significant for LAR (see Table 3). RGR_{TP}
259 differed significantly between medium and wet treatment, RGR_{max} was $0.30 \pm 0.06 \text{ g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$.

261 Although higher for the medium treatment, TE was not significantly influenced by
262 Drought, and mean TE was $5.81 \pm 0.19 \text{ mg}\cdot\text{g}^{-1}$.

263 The aboveground biomass growth is plotted against CT_i in Figure 4. The linear regression
264 model was highly significant ($P < 0.001$; $R^2 = 0.88$). Drought treatment was not significant
265 ($P = 0.768$). WP was $11.1 \pm 0.2 \text{ g}\cdot\text{kPa}\cdot\text{l}^{-1}$, WP_{ref} was $5.8 \pm 0.1 \text{ g}\cdot\text{kPa}\cdot\text{l}^{-1}$.

266 Accession did not have any significant effect on the above mentioned growth variables.

267

268 (Insert Figure 3)

269

270 3.2. Leaf traits

271 ANOVA output and post-hoc results for Drought treatment are given in table 2b.

272 Only drought treatment influenced leaf size, with $A_l(\text{wet}) > A_l(\text{med}) > A_l(\text{dry})$. There were no
273 significant differences in WC_1 , except for the leaves of the dry treatment of the Thai
274 accession, which had a lower WC_1 ($81.82 \pm 0.48\%$) than the two other treatments ($84.11 \pm$
275 0.48%). No factor significantly influenced SLA, ψ_{md} or $\Delta\psi$. The paired samples t-test
276 revealed significant differences between ψ_{pd} and ψ_{md} for all Drought treatments ($P < 0.001$).
277 Drought treatment and Accession significantly influenced ψ_{pd} . Dry treatment-leaves had
278 less negative ψ_{pd} than those of the other treatments. Indian accession leaves had a less
279 negative ψ_{pd} ($\psi_{\text{pd}} = -0.28 \pm 0.03$ MPa) than those of the Thai accession ($\psi_{\text{pd}} = -0.37 \pm 0.03$
280 MPa), leaves of the Ethiopian accession had intermediate ψ_{pd} ($\psi_{\text{pd}} = -0.31 \pm 0.03$ MPa).
281 SD_{ab} and $L_{\text{stom,ab}}$ were only significantly influenced by Drought treatment, in the orders and
282 $SD_{\text{ab}}(\text{dry}) > SD_{\text{ab}}(\text{medium}) = SD_{\text{ab}}(\text{wet})$ and $L_{\text{stom,ab}}(\text{wet}) > L_{\text{stom,ab}}(\text{medium}) > L_{\text{stom,ab}}(\text{dry})$. No
283 factor influenced SI_{ab} .
284 The distance to the vein significantly influenced the SD_{ad} ($P < 0.001$) with decreasing
285 stomatal density from the vein towards the lamina. The drought treatment influenced SD_{ad}
286 ($P < 0.001$) in the order $SD_{\text{ad}}(\text{dry}) < SD_{\text{ad}}(\text{wet}) = SD_{\text{ad}}(\text{medium})$ (see Figure 4). No other
287 factors or interactions were significant.

288

289 (Insert Figure 4)

290

291 3.3. Stem and root water content and density

292 ANOVA output and posthoc results for Drought treatment are given in table 2c. The
293 $WC_r(\text{dry})$ was lower than that of the two other treatments, who had equal WC_r . The same
294 applied for WC_s and WC_w but, additionally, Accession was significant with the Ethiopian
295 accession having lower WC_s ($76.51 \pm 0.43\%$) and WC_w ($72.72 \pm 0.38\%$) than the Indian
296 one ($WC_s = 81.55 \pm 0.43\%$, $WC_w = 78.67 \pm 0.38\%$), with the Thai accession having
297 intermediate WC_s ($81.09 \pm 0.43\%$) and higher WC_w ($78.74 \pm 0.38\%$). Wood density was not
298 influenced by any factor.

299 **4. Discussion**

300 *Limited differences between accessions*

301 The growing scientific interest in the genetic diversity of *J. curcas* has so far mainly
302 focused on Indian accessions (Ram et al., 2008; Ranade et al., 2008). Genetic differences
303 between accessions were related with significant differences in seed morphology (e.g.
304 Kaushik et al., 2007; Mukta and Murthy, 2008) and in seedling (Ginwal et al., 2005) and
305 plant height (Rao et al., 2008). These differences in growth were not confirmed in this
306 study. The accessions differed in only a few properties and not in growth. The Ethiopian
307 accession had a lower root, wood and stem water content, while the dry treatment-leaves of
308 the Thai accession had lower WC_1 . This did not influence other leaf traits or growth
309 characteristics. Given the great distance between and the different growth conditions in the
310 regions of origin of the accessions, the uniformity is remarkable and a likely consequence
311 of the undomesticated nature of the plant (Rao et al., 2008).

312

313 *Growth rate and transpiration efficiency*

314 RGR_{max} was high for a woody species (Poorter and Garnier, 2007). Compared with 10
315 deciduous woody species of the same habitat and area of origin in Central America, studied
316 by Huante and Rincón (1998), W_i was high. While in their study the plants with highest W_i
317 had relatively low LAR, *J. curcas* had in comparison one of the highest values for LAR,
318 despite leaf loss. Consequently, LA of *J. curcas* was much higher than those of the species
319 studied by Huante and Rincón (1998). However, due to a low SLA, the LWR of *J. curcas*,
320 (hence, the relative cost for making leaves), was comparable to that of the other species
321 (Huante and Rincón, 1998).

322 With normal TE values generally ranging from 1.5 to 6 $mg \cdot g^{-1}$ (Mengel and Kirkby, 2004),
323 *J. curcas* has a high TE, probably even when CO_2 concentration would be at ambient
324 levels.

325 The observed linear relation between CT_i and biomass formation and the fact that drought
326 treatment did not influence WP confirm the hypothesis that WP is approximately constant
327 for a given crop species, regardless of the growth conditions (Steduto et al. 2007). WP of *J.*
328 *curcas* will probably decrease in the reproductive phase, due to the high oil content in the

329 seeds (Azam-Ali and Squire, 2002; Steduto et al., 2007). Contrary to the expected
330 uniformity among all C₃-species (Steduto et al., 2007), WP_{ref} (5.98 g·m⁻²·mm⁻¹·kPa) was
331 50% higher than that of other C₃-species (3.8 g·m⁻²·mm⁻¹·kPa (Steduto and Albrizio,
332 2005)). However, those data were based on evapotranspiration, not transpiration.
333 Furthermore, possibly *J. curcas* does not have a pure C₃-metabolism, but rather a CAM-
334 metabolism in the succulent stem with leaves shifting from C₃- to CAM-metabolism under
335 drought (Ting et al., 1983; Lüttge, 2008), as do other stem succulent species with green
336 stems (e.g. *Frerea indica* -Lange and Zuber, 1977). The metabolism of *J. curcas* deserves
337 further attention.

338 The CO₂-concentration in the greenhouse was 500-600 ppm, higher than ambient values.
339 This may have affected the water use (lower g_s and TR (Paoletti and Grulke, 2005;
340 Ainsworth and Rogers, 2007), biomass growth (higher W_t (Norby et al., 1999), TE (Korner,
341 2006)) and leaf traits (e.g. lower SD (Woodward, 1987; Ceulemans and Mousseau 1994)),
342 therefore these results should be interpreted with caution.

343

344 *J. curcas* has a succulent stem and leaf traits similar to other stem-succulent trees

345 The low WD and high WC_w confirm the first hypothesis. Indeed, WD is among the lowest
346 and WC_w among the highest of reported values for deciduous stem-succulent species by
347 Borchert (1994).

348 The high WC_l, small SLA, high ψ_{pd} and small $\Delta\psi$ confirm the second hypothesis. The fact
349 that g_s drops when $\Delta\psi$ is still low indicates that *J. curcas* has an embolism avoidance
350 strategy (Sperry et al., 2003). The conservative water use is reflected in the low stomatal
351 conductance measured on day 107, when $g_{s,m}$ was much lower than on earlier days,
352 although the conditions were comparable. Indeed, the transpiration rate did not follow the
353 increase in LA during the experiment (see figures 1 and 3).

354

355 *Onset of drought: limited stem growth and investment in leaves with altered anatomy.*

356 The third hypothesis stated that leaf and stem growth stops when *J. curcas* is faced with
357 drought stress and that leaves are shed very soon afterwards, as do other stem-succulent
358 deciduous trees. This was not confirmed in this study.

359 Medium treatment seedlings were able to maintain the water contents in stems and roots at
360 the same level as the wet treatment, and kept growing considerably during the rest of the
361 drought experiment.

362 Furthermore, during the first two weeks of TP, dry treatment seedlings kept investing in
363 shoot growth and leaf formation even though θ_v and ψ_{soil} decreased rapidly. In this period,
364 they formed leaves that had the same SLA and WC_l as leaves of the other treatments, but
365 they differed from them in leaf anatomy. The absence of significant differences in SI_{ab}
366 indicate that the higher $SD_{\text{ab}}(\text{dry})$ is probably due to smaller A_l (Peñuelas and Matamala,
367 1990; Wang et al., 2007) and does not necessarily reflect an adaptive mechanism to drought
368 stress (Banon et al., 2004). However, the much higher $SD_{\text{ad}}(\text{dry})$ indicates that drought
369 stress induces formation of leaves with an altered leaf anatomy.

370

371 *First weeks of drought period: stem water allows maintaining small number of leaves*

372 After two weeks of drought, TR was very low and seedlings started shedding leaves, yet, at
373 the end of the experiment, all dry treatment seedlings still had some leaves left.

374 Parahelionasty was observed in seedlings of the dry and medium treatment. This is a
375 common stress response to prevent overheating (Begg, 1976) and reflects an active plant
376 response to protect – hence, keep- its leaves under dry conditions.

377 The investment in new leaves with an altered leaf anatomy at the onset of drought and the
378 maintenance of a part of its leaves for several weeks during drought, are in contrast with
379 observations on other stem succulent trees (Holbrook et al., 1995; Borchert and Rivera,
380 2001).

381 Baobab trees use their stem water reserves for flushing new leaves before the end of the dry
382 season (Chapotin et al., 2006b). Other deciduous stem-succulent species use this water for
383 flowering during the dry season (Borchert, 1994). In contrast, *J. curcas* has a strictly
384 opportunistic leaf flushing behavior, starting immediately after the beginning of the rainy
385 season, and flowering of *J. curcas* occurs in the wet season (Achten et al., 2008; Kumar and
386 Sharma, 2008).

387 Despite very low g_s and TR of the dry treatment seedlings, some water inevitably got lost
388 through the leaves. As ψ_{soil} was below wilting point and soil water was not extractable,

389 replenishment had to come from the roots and from the stem, which indeed had lower WC
390 than those of the other treatments. Stem shrinkage derived from the volume estimates was
391 $6.4 \pm 3.4 \text{ cm}^3$ or $5.3 \pm 2.0 \%$ of stem volume. As such *J. curcas* plants seem to use the stem-
392 stored water to keep their leaves for several weeks after the start of the dry season. The fact
393 that stems are replenishing leaves only in dry conditions probably explains the significantly
394 higher ψ_{pd} of the dry treatment in comparison with the other treatments, whose leaves were
395 still in equilibrium with soil water.

396 Chapotin et al. (2006b) suggested that the physiological advantage of the leaf-flushing
397 before the end of the dry season allows Baobab trees to take advantage of scattered rainfall
398 events occurring before the start of the rainy season. By analogy, the physiological
399 advantage of keeping the leaves after the start of the dry season might be that the species
400 can overcome short drought periods without significant leaf loss or that it can take
401 advantage of scattered rainfall after the start of the dry season.

402

403 *Are plant-water relations of seedlings valid for adult trees?*

404 The higher drought sensitivity at the seedling stage can alter plant water relations with age
405 directly (lower ψ_{pd} and g_s at seedling stage due to lower access to soil water) (Epron and
406 Dreyer, 1993; Cavender-Bares & Bazzaz, 2000; He et al., 2005) and indirectly (higher g_s
407 and R:S ratio but lower TE at seedling stage in order to have a more rapid access to deeper
408 soil water) (Donovan and Ehleringer, 1991). Hence, generalizing plant water relations
409 measured on seedling to mature plants has to be done under precaution (Cavender-Bares &
410 Bazzaz, 2000). Yet, it is likely that plant water relations of stem succulents do not change
411 with age, as stem succulent species do not avoid drought stress through a better access to
412 soil water (they generally have shallow rooting systems), but through their stem water
413 reserves (Borchert, 1994). The results from our experiment indicate this strategy is already
414 applied at seedling stage, and the plant water relations of *J. curcas* seedlings were very
415 much in line with those of mature trees of other stem succulent species. Hence, we consider
416 it likely that plant water relations of the species are not age-dependent, although the plant
417 water relations of mature *J. curcas* trees deserves further research.

418

419 **5. Conclusions**

420 The two first research hypotheses, that *J. curcas* has a low wood density and several leaf
421 traits in common with other stem-succulent deciduous trees, were confirmed. However, the
422 third hypothesis, that leaves are not shed immediately when the seedlings are confronted
423 with drought, was not. Instead, at the onset of drought, leaves with a different leaf anatomy
424 are formed, after which leaves are only gradually shed. The role of the succulent stem in
425 the water economy of *Jatropha* seems confined to balance the small water losses of the
426 leaves during drought.

427 The conservative transpiration rate, high growth rate, transpiration efficiency and water
428 productivity are promising characteristics for a quick establishment of the species on
429 degraded or unvegetated sites and for high production on suitable sites, whilst not
430 consuming too much water. Yet, future research should focus on the water requirements in
431 field conditions and the possible hydrological impact of plantations on watersheds.

432 In contrast with other studies focusing on differences between (mainly Indian) accessions,
433 no influence of accession on growth rate, plant-water relationships or drought resistance
434 was observed. The genetic diversity of the accessions worldwide and its consequences for
435 growth and drought resistance deserve further attention, particularly of accessions in its
436 natural distribution area.

437

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581

582 **Figure Captions**

583 Figure 1. Transpiration rate of *J. curcas* seedlings under dry, medium and wet treatments as
584 a function of days after sowing and during the drought treatment. Root square
585 transformation is used to represent the transpiration rate and PAR data. Bars represent
586 standard errors. The PAR and VPD data represent the mean values during the day.

587

588 Figure 2. Daily pattern of stomatal conductance of leaves of *J. curcas* seedlings under dry,
589 medium and wet treatment and their relation with the mean PAR and VPD as measured on
590 day 79 after sowing (20/09/07). Log scale is used to represent the stomatal conductance
591 data. The bars represent standard errors.

592

593 Figure 3. The number of leaves of *J. curcas* seedlings under dry, medium and wet
594 treatments as a function of days after sowing and during the drought treatment. Bars
595 represent standard errors.

596

597 Figure 4. Biomass growth of the medium and wet treatment seedlings as a function of
598 cumulative transpiration, normalized for vapor pressure deficit ($CT_i = \sum_{i=62}^n TR_i / VPD_i$, see
599 text).

600

601 Figure 5. Adaxial stomatal density as a function of distance to veins of leaves of *J. curcas*
602 seedlings under dry, medium and wet treatment. The root squares of stomatal densities are
603 used to represent the stomatal density. The bars represent standard errors.

604

605

606

607 **Tables**

608

609 Table 1. Overview of the mean climatic conditions during the drought treatment period and
 610 of the climatic conditions on the measuring days of leaf stomatal conductance (g_s) and leaf
 611 water potential. Data for PAR are in $W \cdot m^{-2}$, for VPD in kPa.

| Measuring period | Variable | PAR | VPD |
|-------------------------|------------------------------------|------------|------------|
| TP (Entire day) | | 59.0 | 1.07 |
| TP (Morning) | | 59.0 | 0.97 |
| Day 72 | g_s | 107.3 | 1.33 |
| Day 79 | g_s | 61.0 | 1.44 |
| Day 107 | g_s | 57.0 | 1.42 |
| Day 104 | $\Psi_{pd}, \Psi_{md}, \Delta\Psi$ | 92.0 | 1.57 |

612

613 Table 2. Results of the two-way ANOVA and estimated mean values with their standard error for the three drought treatments
 614 for a) the water use and growth data, b) the leaf traits and c) stem and root water content and density.

| | Variable | Unit | Corr. Model | Dr | Acc | AccxD r | Dry | Medium | Wet | | |
|--------------------------------|-----------------------------|--|-------------|--------|--------------|--------------|-------------------------|----------|-------------------------|-----------------------|--------------------------|
| a) Water use and growth | | | | | | | | | | | |
| Stomatal conductance | | | | | | | | | | | |
| | log(g _{s,m} (76)) | Log(mmol·m ⁻² ·s ⁻¹) mmol·m ⁻² ·s ⁻¹ | <0.001 | <0.001 | 0.79 | 0.53 | 1.297 ± 0.040 (19.8) | <i>a</i> | 1.845 ± 0.039 (69.9) | <i>b</i> | 2.354 ± 0.041 (225.9) |
| | log(g _{s,m} (83)) | Log(mmol·m ⁻² ·s ⁻¹) mmol·m ⁻² ·s ⁻¹ | <0.001 | <0.001 | 0.79 | 0.33 | 1.090 ± 0.049 (12.3) | <i>a</i> | 1.833 ± 0.049 (68.1) | <i>b</i> | 2.307 ± 0.049 (202.9) |
| | log(g _{s,m} (107)) | Log(mmol·m ⁻² ·s ⁻¹) mmol·m ⁻² ·s ⁻¹ | <0.001 | <0.001 | 0.89 | 0.97 | 0.733 ± 0.084 (4.4) | <i>a</i> | 1.722 ± 0.084 (51.7) | <i>b</i> | 2.044 ± 0.084 (109.6) |
| Total biomass | W _t | g | <0.001 | <0.001 | <u>0.036</u> | 0.93 | 32.8 ± 2.2 | <i>a</i> | 65.4 ± 2.6 | <i>b</i> | 109.2 ± 4.0 |
| Leaf area | LA | m ² | <0.001 | <0.001 | 0.42 | 0.48 | 0.10 ± 0.04 | <i>a</i> | 0.33 ± 0.04 | <i>b</i> | 0.71 ± 0.04 |
| Leaf-area ratio | LAR | cm ² ·g ⁻¹ | 0.005 | <0.001 | 0.55 | 0.37 | 34.0 ± 4.1 | <i>a</i> | 51.3 ± 4.5 | <i>b</i> | 65.2 ± 4.1 |
| Leaf-weight ratio | LWR | g·g ⁻¹ | <0.001 | <0.001 | 0.14 | <u>0.045</u> | 0.13 ± 0.02 | <i>a</i> | 0.26 ± 0.02 | <i>b</i> | 0.33 ± 0.02 |
| Relative Growth Rate | RGR _{TP} | g·g ⁻¹ ·d ⁻¹ | <0.001 | <0.001 | 0.62 | 0.44 | / | | 0.016 ± 0.001 | <i>a</i> | 0.025 ± 0.001 |
| Transpiration efficiency | TE | mg·g ⁻¹ | 0.069 | | | | / | | 8.65 ± 0.54 | <i>a</i> | 6.53 ± 0.47 |
| b) Leaf traits | | | | | | | | | | | |
| Leaf Size | A _l | cm ² | <0.001 | <0.001 | 0.54 | 0.86 | 60.9 ± 9.5 | <i>a</i> | 100.5 ± 9.5 | <i>b</i> | 136.1 ± 9.5 |
| Leaf water content | WC _l | % | <0.001 | 0.09 | 0.28 | <0.001 | 83.35 ± 0.36 | <i>a</i> | 83.75 ± 0.36 | <i>a</i> | 84.11 ± 0.36 |
| Specific Leaf Area | SLA | cm ² ·g ⁻¹ | 0.53 | | | | 169 ± 8 | <i>a</i> | 178 ± 8 | <i>a</i> | 183 ± 8 |
| Leaf water potential | Ψ _{pd} | MPa | <0.001 | <0.001 | <u>0.012</u> | 0.67 | -0.24 ± 0.03 | <i>b</i> | -0.35 ± 0.03 | <i>a</i> [†] | -0.36 ± 0.03 |
| | Ψ _{md} | MPa | 0.06 | | | | -0.37 ± 0.05 | <i>a</i> | -0.48 ± 0.05 | <i>a</i> | -0.48 ± 0.05 |
| | ΔΨ | MPa | 0.63 | | | | 0.13 ± 0.04 | <i>a</i> | 0.13 ± 0.04 | <i>a</i> | 0.12 ± 0.04 |

| | | | | | | | | | | | |
|---|----------------------|-------------------|------------------|------------------|------------------|------|---------------|----------|---------------|----------|---------------|
| Stomata (abaxial) | SD _{ab} | #·mm ² | <0.001 | <0.001 | 0.68 | 0.50 | 415 ± 21 | <i>a</i> | 241 ± 21 | <i>b</i> | 196 ± 21 |
| | SI _{ab} | / | 0.19 | | | | 13.2 ± 0.7 | <i>a</i> | 12.6 ± 0.7 | <i>a</i> | 11.9 ± 0.7 |
| | L _{stom,ab} | mm | <0.001 | <0.001 | 0.61 | 0.70 | 26 ± 0.8 | <i>a</i> | 29.5 ± 0.8 | <i>b</i> | 31.7 ± 0.8 |
| c) Stem and root water content and density | | | | | | | | | | | |
| Root water content | WC _r | % | <0.001 | <0.001 | 0.37 | 0.42 | 72.8 ± 1.4 | <i>a</i> | 78.0 ± 1.4 | <i>b</i> | 79.4 ± 1.4 |
| Stem water content | WC ₁ | % | 0.002 | <0.001 | <u>0.048</u> | 0.26 | 80.1 ± 0.4 | <i>a</i> | 81.2 ± 0.4 | <i>b</i> | 81.2 ± 0.4 |
| Wood water content | WC _w | % | 0.001 | <0.001 | <0.001 | 0.33 | 77.1 ± 0.4 | <i>a</i> | 78.2 ± 0.4 | <i>b</i> | 78.8 ± 0.4 |
| Wood density | WD | g·cm ₃ | 0.90 | | | | 0.252 ± 0.031 | <i>a</i> | 0.266 ± 0.031 | <i>a</i> | 0.259 ± 0.031 |

615 Values in bold indicate a P-value of 0.001 or less, underlined values a P-value of 0.05 or less. Different characters in the right columns indicate significant differences ($P < 0.05$) between factor
616 levels, using Tukey post-hoc testing.

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618

619