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Gas exchange and hydraulics in seedlings of *Hevea brasiliensis* during water stress and recovery

JUN-WEN CHEN,[1,2,4] QIANG ZHANG,[1,3] XIAO-SHUANG LI[1] and KUN-FANG CAO[1]

[1] *Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, 88 Xuefu Road, Kunming, 650223, Yunnan, People's Republic of China*

[2] *Department of Crop Science, College of Agronomy and Biotechnology, Yunnan Agricultural University, Kunming, 650201, Yunnan, People's Republic of China*

[3] *College of Life Science, South China Normal University, Guangzhou, 510631, Guangdong, People's Republic of China*

[4] *Corresponding author (cjw31412@hotmail.com)*

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Summary The response of plants to drought has received significant attention, but far less attention has been given to the dynamic response of plants during recovery from drought. Photosynthetic performance and hydraulic capacity were monitored in seedlings of *Hevea brasiliensis* under water stress and during recovery following rewatering. Leaf water relation, gas exchange rate and hydraulic conductivity decreased gradually after water stress fell below a threshold, whereas instantaneous water use efficiency and osmolytes increased significantly. After 5 days of rewatering, leaf water relation, maximum stomatal conductance ($g_{\text{s-max}}$) and plant hydraulic conductivity had recovered to the control levels except for sapwood area-specific hydraulic conductivity, photosynthetic assimilation rate and osmolytes. During the phase of water stress, stomata were almost completely closed before water transport efficiency decreased substantially, and moreover, the leaf hydraulic pathway was more vulnerable to water stress-induced embolism than the stem hydraulic pathway. Meanwhile, $g_{\text{s-max}}$ was linearly correlated with hydraulic capacity when water stress exceeded a threshold. In addition, a positive relationship was shown to occur between the recovery of $g_{\text{s-max}}$ and of hydraulic capacity during the phase of rewatering. Our results suggest (i) that stomatal closure effectively reduces the risk of xylem dysfunction in water-stressed plants at the cost of gas exchange, (ii) that the leaf functions as a safety valve to protect the hydraulic pathway from water stress-induced dysfunction to a larger extent than does the stem and (iii) that the full drought recovery of gas exchange is restricted by not only hydraulic factors but also non-hydraulic factors.

Keywords: drought, embolism, hydraulic conductivity, photosynthesis, recovery.

Introduction

Drought is considered the main environmental factor adversely limiting plant growth (Boyer 1982) and strongly influencing plant distribution (Engelbrecht et al. 2007, Kursar et al. 2009). The water stress-induced limitation to plant growth is mainly caused by reductions in plant carbon balance, which is largely dependent on photosynthesis. Plants initially respond to water stress by closing stomata, which can in part reduce water loss by transpiration. Stomatal closure inevitably lowers CO_2 availability in the chloroplasts. Consequently, photosynthetic capacity is progressively suppressed by enhanced diffusive resistances within the leaf and by drought-induced impairments of metabolic processes (Tezara et al. 1999, Lawlor and Cornic 2002, Flexas et al. 2004, 2006). Numerous efforts have been made to address whether stomatal closure or metabolic impairment constitute a primary limitation to gas exchange under a limited water supply.

However, the suppression of leaf gas exchange by water stress is closely correlated with the loss of xylem hydraulic conductivity (Sperry and Pockman 1993, Hubbard et al. 2001, Cochard et al. 2002). Furthermore, there is evidence showing a link between the water potential that induces stomatal closure and triggers xylem cavitation (Jones and Sutherland 1991, Sperry and Saliendra 1994, Brodribb and Cochard 2009). In some species, earlier stomatal closure provides a large safety margin against embolism formation (Pockman and Sperry 2000, Froux et al. 2002, Martinez-Vilalta et al. 2002). Rather, plants are capable of pre-empting the water stress-induced xylem cavitation by closing their stomata (Nardini and Salleo 2000, Cochard et al. 2002, Brodribb and Holbrook 2003). Correspondingly, in other species, the loss of xylem hydraulic conductivity directly leads to stomatal closure (Cochard 2002, Brodribb et al. 2003, Nardini et al. 2003). Thus, photosynthetic performance and hydraulic capacity should be synchronously monitored in water-stressed plants, and this would significantly improve our understanding of plant desiccation tolerance.

The water supply to leaves depends on maintaining an intact water column within the vascular system. Under drought conditions, the water potential becomes more negative throughout the plant and the hydraulic pathway is under increasing tension. Elevated hydraulic tension largely increases the risk of xylem dysfunction once species-specific thresholds are transgressed (Sperry and Tyree 1988; Tyree and Sperry 1989). Unexpectedly, many plants in the natural environment regularly operate at a water potential very close to their cavitation threshold (Lo Gullo et al. 2003, Brodribb and Holbrook 2004), and furthermore, quickly recover lost hydraulic conductivity as the water potential increases up to a threshold (Holbrook and Zwieniecki 1999, Bucci et al. 2003, Brodribb and Holbrook 2004).

The response of gas exchange to water stress has received considerable attention in the past, but relatively few studies have explored the underlying mechanism of recovery from drought stress (Flexas et al. 2006). To our knowledge, only few studies have been conducted in an effort to understand the recovery of gas exchange from water stress after rewatering (e.g., Kirschbaum 1988, Souza et al. 2004, Cai et al. 2005, Miyashita et al. 2005, Gallé et al. 2007, Galmés et al. 2007). Meanwhile, there is a paucity of research examining the recovery of both gas exchange and hydraulic capacity (e.g., Lo Gullo et al. 2003, Pou et al. 2008, Brodribb and Cochard 2009, Blackman et al. 2009, Resco et al. 2009); however, such a small amount of research has identified plant hydraulics as one of the principal governors of gas exchange under water stress as well as during the recovery period following rewatering.

Here, we focus on the changes in gas exchange and hydraulic capacity in seedlings of rubber tree (*Hevea brasiliensis* (Willd. ex A.Juss.) Muell. Arg.) with respect to soil drought stress and recovery. Rubber tree is a commercially important woody species, originating from Amazonia tropical rainforest (Wycherley 1992). Interestingly, it is an evergreen tree in its native range, but drought deciduous in the tropical margin, such as in Xishuangbanna of Southwest China (Chen and Cao 2008). Such a change in leaf habit of rubber tree still remains poorly understood. The objectives of this study were to (i) examine the responses of photosynthetic performance and hydraulic capacity to water stress and recovery, (ii) analyse a possible link between photosynthetic performance and hydraulic capacity during water stress and recovery and (iii) assess the limiting mechanism in the recovery of physiological function from water stress after rewatering.

Materials and methods

Study site

The study site was located in Xishuangbanna Tropical Botanical Garden (21°41′ N, 101°25′ E, 600 m a.s.l.), Chinese Academy of Sciences in southern Yunnan, China. The average annual temperature is 21.7 °C and annual precipitation is ~1560 mm. There is a distinct dry season from November to April and a well-defined wet season from May to October; ~85% of the annual rainfall occurs in the wet season.

Plant material and experimental design

Two-year-old seedlings of rubber tree from a local commercial nursery were planted in pots (30 cm in diameter × 35 cm in depth) on 12 March 2007. Potted soils were collected from the surface layer (0–20 cm) of a local rubber tree plantation. All seedlings were well watered and kept under natural conditions. After 1 month of transplanting, all pots received 30 g NPK slow-release compound fertilizer (Osmocote, Scotts, Marysville, OH). Some healthy seedlings were then transferred to a greenhouse on 10 October 2007. In the greenhouse, all plants were well irrigated until the onset of drought treatment, and control plants were then continuously watered to field capacity every 2–3 days throughout the experimental period. The height of seedlings was ~75–85 cm and the diameter at breast height was ~11–13 mm before the drought treatment was started on 10 November 2007. The seedlings were subjected to two different treatments, well watered (control plants) and withholding water (water-stressed plants), and arranged in a completely randomized design. Water-stressed seedlings were rewatered to field capacity when leaf water potential (Ψ_{leaf}) reached approximately −3.0 MPa, at which a catastrophic hydraulic failure occurred in the detached stems of the rubber tree (Chen et al. 2009*a*). The surface of each pot was covered with gas-impermeable opaque plastic film to avoid water evaporation loss. Over the experimental period, maximum air temperature was 27 ± 2 °C during the day and minimum air temperature was 19 ± 2 °C during the night, air relative humidity was 85 ± 3% and maximum diurnal photosynthetic photon flux density (PFD) was ~800 μmol photon m^{-2} s^{-1} in the greenhouse. During the periods of water stress and recovery, soil and plant water status and photosynthetic- and hydraulic-related traits were periodically monitored in water-stressed and control plants.

Soil and leaf water status

Fresh weight of soil at a depth of 20–25 cm from the surface was determined shortly after sampling early in the morning, and then the soil was oven-dried at 105 °C for 24 h and dry weight of soil was determined. Soil water content (SWC) was calculated as: SWC = 100 × (fresh weight − dry weight)/dry weight. Meanwhile, Ψ_{leaf} was measured with a pressure chamber (PMS1000; PMS, Corvallis, OR); fully expanded leaves were cut with a sharp razor blade and the balancing pressures were immediately determined. In addition, leaf relative water content (RWC) was calculated as: RWC = 100 × (fresh weight − dry weight)/(turgid weight − dry weight). Turgid leaf weight was determined after keeping the leaf in distilled water in darkness at 4 °C to minimize respiration losses until they reached a constant weight (fully turgid, typically after 12 h). Leaf dry weight was obtained 48 h after keeping the turgid leaf at 70 °C in an oven.

Stem hydraulic conductivity

Stems of water-stressed and control plants were collected early in the morning, recut immediately under water and returned to the laboratory shortly. Stem segments of more than 30 cm in length were cut under water, trimmed with a fresh razor blade and connected to the hydraulic apparatus. The segment was then perfused with filtered, 10-mmol oxalic acid solution. The perfusing solution was supplied from a reservoir raised above the flow meter to generate a gravity-induced pressure head of ~5 kPa. The segment was perfused with solution until a steady-state flow was attained, at which point the hydraulic conductivity (K_h) of the segment was calculated as $K_h = FL/\triangle P$ where F is the flow rate (in kilograms per second), $\triangle P$ is the pressure drop (in megapascals) along the stem segment and L is the length of stem segment (in metres). Afterwards, the segment was perfused with 0.01% Safranin dye to determine conductive sapwood area. Leaf surface area distal to the stem segment was measured using a leaf area meter (LI-COR, Lincoln, NE). Sapwood and leaf area were used to calculate K_S and K_L. Sapwood area-specific hydraulic conductivity (K_S, in kilograms per metre per second per megapascal) and leaf area-specific hydraulic conductivity (K_L, in kilograms per metre per second per megapascal) was calculated as K_h divided by sapwood and leaf area, respectively.

Stem percentage loss of conductivity and vulnerability curves

Water-stressed and control plants were collected early in the morning when stem xylem water potential is thought to approximate Ψ_{leaf}. To minimize the possible differences between different parts of the soil-to-leaf water potential, the canopy of seedlings was covered with gas-impermeable opaque plastic film overnight to decrease water transpiration loss until the onset of sampling measurement. In the laboratory, a stem segment (more than 30 cm in length) was then cut under water and connected to the hydraulic apparatus. For a fixed pressure head, K_h is proportional to volumetric flow rate (J_v) of water through stem segments, and thus in practice, the calculations of percentage loss of conductivity (PLC) were based on J_v rather than K_h. After the initial measurement of flow rate (J_i), the segments were flushed using filtered, 10-mmol oxalic acid solution under a constant pressure of 0.15–0.18 MPa for 10–30 min to remove embolism until stable readings of flow rate were reached. Afterwards, the maximum flow rate (J_{max}) was measured by using the same pressure head as before. PLC was then calculated as: $\mathrm{PLC} = 100(J_{max} - J_i)/J_{max}$. As expected, PLC in control plants did not change significantly throughout the experimental period. However, PLC increased and Ψ_{leaf} decreased gradually in water-stressed plants as drought proceeded. The hydraulic vulnerability curves in water-stressed plants were generated by fitting curves for PLC against stem xylem tension (Pammenter and Vander Willigen 1998): $\mathrm{PLC} = 100/(1 + \exp(a(\Psi - b)))$ where Ψ is the stem xylem water potential, a is the slope of the line and b is the stem xylem water potential at which a 50% loss of hydraulic conductivity occurs ($\mathrm{P50}_{stem}$). $\mathrm{P20}_{stem}$ was also calculated from the fitted curves, and the $\mathrm{P20}_{stem}$ is an estimate of the xylem tension at which the incipient xylem cavitation and embolism occur (Brodribb et al. 2003).

Leaf hydraulic conductivity and vulnerability curves

The branches of water-stressed and control plants were collected early in the morning and then leaf hydraulic conductivity (K_{leaf}) was shortly measured according to the method described by Franks (2006). In brief, $K_{leaf} = \triangle v/(10 \times A_{leaf} \times (\Psi_1 - \Psi_2))$ where $\triangle v$ is the volume of sap expressed in the first 10 s following the step increase in pressure from Ψ_1 to Ψ_2 and A_{leaf} is the leaf area (in square metres). The chamber pressure was increased to the balance pressure (Ψ_1) and allowed to equilibrate for ~2 min. The chamber pressure was then rapidly increased to Ψ_2, and the sap expressed in the first 10 s was collected and weighed on an analytical balance to obtain $\triangle v$. Ψ_2 was 0.5 MPa more than Ψ_1 (accurately measured to ±0.01 MPa), and Ψ_1 was defined to be equal to Ψ_{leaf}. Leaf area was measured with a leaf area meter (LI-COR). A series of values of K_{leaf} and Ψ_{leaf} would be obtained with progressive drought. The leaf hydraulic vulnerability curves in water-stressed plants were generated by plotting K_{leaf} against Ψ_{leaf} with an exponential sigmoid equation (Chen et al. 2009*b*): $K_{leaf} = a/(1 + \exp(-b(\Psi_{leaf} - c)))$ where a is the maximum K_{leaf}, b is the slope of the line and c is Ψ_{leaf} at which a 50% loss of maximum K_{leaf} occurs ($\mathrm{P50}_{leaf}$). Here, it should be emphasized that this method used to measure K_{leaf} involves applying a positive pressure to squeeze water out of the leaf, and this may refill embolism in the leaf xylem when the water is pushed through the leaf at substantial pressure. Accordingly, this might lead to an overestimation of leaf hydraulic conductivity for dehydrated leaves, and thus to an underestimation of P50. Therefore, caution should be used when comparing the values of K_{leaf} and P50 given here with values determined using different methods in other studies.

Biochemical analysis

Mature and fully sun-exposed leaves were harvested early in the morning for the assays of soluble sugar and proline. The sampled leaves were kept immediately at −80 °C until analysis. Soluble sugar was analysed according to the methods described by David et al. (1998), and proline was quantified by the acid–ninhydrin method and the procedure was carried out as detailed by Monreal et al. (2007).

Leaf gas exchange

Photosynthesis-related traits were examined in situ on the mature and fully sun-exposed leaves in the late morning using a portable photosynthesis apparatus (LI-6400; LI-COR). Throughout the experiment, vapour pressure deficit remained at 1.0–1.5 kPa between 09:00 and 10:00. CO_2 concentration and temperature in the chamber were 360 μmol mol^{-1} and 25 °C, respectively. PFD was controlled by an internal LED light source (LI-6400-2B). In well-watered plants, PFD was kept at 800–1500 μmol photon m^{-2} s^{-1} to obtain maximum net photosynthetic assimilation rate at light saturation (A_{max}). Considering the effects of drought on the light saturation point, PFD applied to water-stressed and subsequently rehydrated plants was stepwise increased from 150 μmol photon m^{-2} s^{-1} to an optimal intensity to obtain A_{max}. The recorded PFD fluctuated between 1500 and 150 μmol photon m^{-2} s^{-1}, depending on the drought intensity. Measurements were logged once rates of gas exchange become stable. In addition, maximum stomatal conductance ($g_{s\text{-}max}$) and instantaneous water use efficiency (WUE), calculated as A/g_s, were simultaneously obtained. Upon completion of the measurement of gas exchange, the water potential was measured promptly as before.

Statistical analysis

Seven to nine replicates in water-stressed and control plants were performed. Data were subjected to analysis of variance based on a completely randomized experimental design, and a *t*-test was then used for testing significant differences between measurements of water-stressed and control plants.

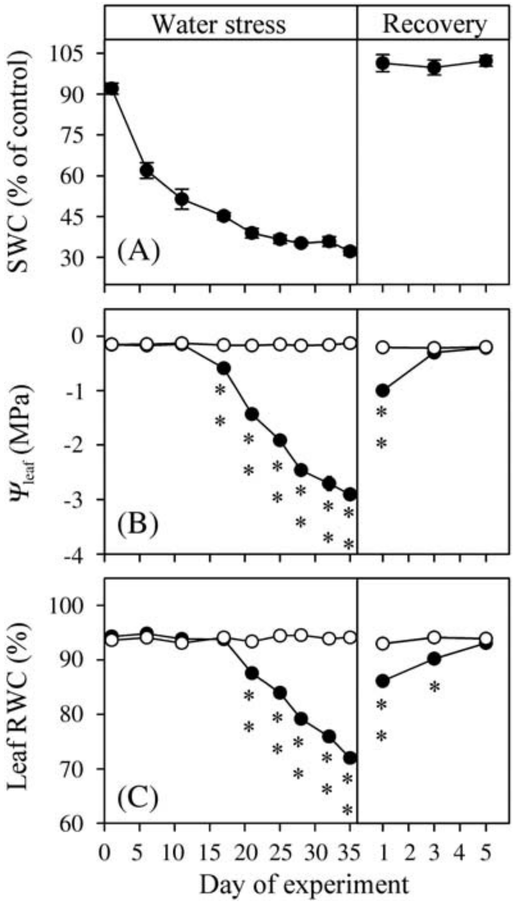

Figure 1. Changes in water status of *H. brasiliensis* tree seedlings during the periods of water stress and recovery. Soil water content (SWC, A), leaf water potential (Ψ_{leaf}, B) and leaf relative water content (RWC, C) were determined in both well-watered (open circles) and water-stressed (closed circles) plants. Data are presented as the means ± SD (n = 7–9). Significant difference between control and stressed plants at each date of the experimental periods: *$P \leq 0.05$; **$P \leq 0.01$.

Results

In drought-treated pots, SWC dropped sharply to ~50% and <40% of field capacity after 11 and 21 days of withholding water, respectively (Figure 1A). Afterwards, SWC slowly declined until recovery upon rewatering. Ψ_{leaf} in water-stressed plants decreased gradually with ongoing drought stress after 11 days of withholding water and was approximately −3.0 MPa at the end of drought stress (Figure 1B). Leaf RWC significantly declined after 17 days of withholding water and approached ~70% before rewatering (Figure 1C). After 3–5 days of rewatering, the values of Ψ_{leaf} and leaf RWC in previously water-stressed plants almost recovered to the values of control plants. As control plants were well watered to field capacity, Ψ_{leaf} and leaf RWC were not significantly changed throughout the experimental period (Figure 1B and C).

The losses of soil and leaf water under progressive drought were followed by a gradual decline in A_{max} and $g_{s\text{-}max}$ (Figure 2A and B). The value of A_{max} in water-stressed plants dropped steeply from ~12.0 to <3.0 μmol CO_2 m^{-2} s^{-1} after 11–35 days of withholding water; meanwhile, the value of $g_{s\text{-}max}$ decreased rapidly from ~480 to 50 mmol H_2O m^{-2} s^{-1}. In contrast, the value of WUE increased sharply from ~40 to 140 μmol mol^{-1} (Figure 2C). After 5 days of rewatering, the value of A_{max} in previously water-stressed plants was still significantly lower than the control value, while the value of $g_{s\text{-}max}$ was almost restored to the control value after 3 days of rewatering, thus leading to a significant decline in WUE after recovery from water stress.

PLC significantly increased until 17 days of withholding water, its value being close to 70% before recovery from water stress (Figure 3A), while K_{leaf} significantly decreased until 21 days of withholding water (Figure 3B). The value of PLC dropped to ~20% after 1 day of rewatering, and embolism in stem xylem was completely refilled after 3 days of rewatering. In addition, the value of K_{leaf} nearly recovered to the control value after 1 day of rewatering.

The value of K_S remained at ~4.5 kg m^{-1} s^{-1} MPa^{-1} until 11 days of withholding water; thereafter, it significantly decreased to 1.0 kg m^{-1} s^{-1} MPa^{-1} at the end of water stress (Figure 4A). Similarly, the value of K_L was significantly reduced after 11 days of withholding water and was <2.0 × 10^{-4} kg m^{-1} s^{-1} MPa^{-1} before the recovery from water stress (Figure 4B). After 3 days of rewatering, the value of K_S recovered to ~3.5 kg m^{-1} s^{-1} MPa^{-1}, which was still significantly lower than the value of K_S in control plants. In contrast, after

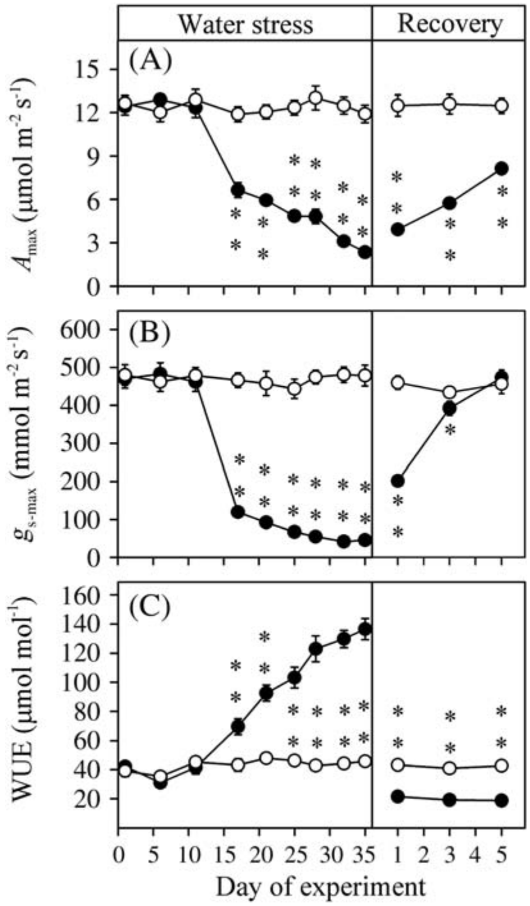

Figure 2. Gas exchange in *H. brasiliensis* tree seedlings during periods of water stress and recovery. Maximum net photosynthetic assimilation rate (A_{max}, A), stomatal conductance ($g_{s\text{-}max}$, B) and water use efficiency (WUE, C) were measured in both well-watered (open circles) and water-stressed (closed circles) plants. Data are presented as the means ± SD ($n = 7$–9). Significant difference between control and stressed plants at each date of the experimental periods: $*P \leq 0.05$; $**P \leq 0.01$.

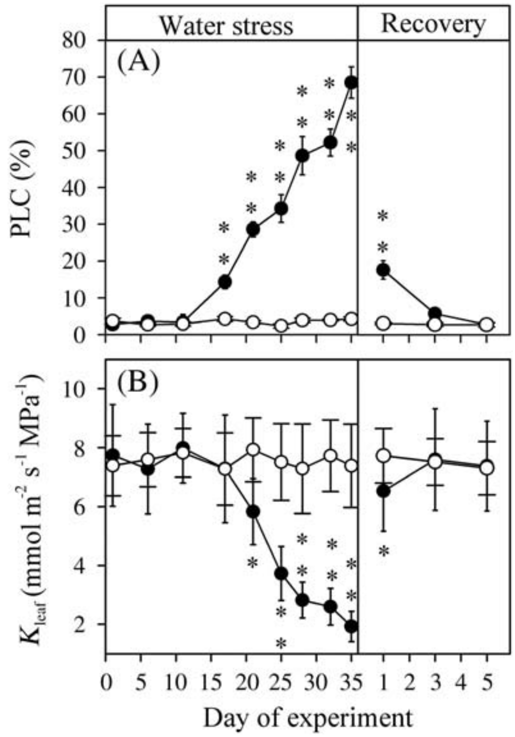

Figure 3. Stem percentage loss of conductivity (PLC, A) and leaf hydraulic conductivity (K_{leaf}, B) in *H. brasiliensis* tree seedlings during periods of water stress and recovery. PLC and K_{leaf} were measured in well-watered (open circles) and water-stressed (closed circles) plants. Data are presented as the means ± SD ($n = 7$–9). Significant difference between control and stressed plants at each date of the experimental periods: $*P \leq 0.05$; $**P \leq 0.01$.

3 days of rewatering, the value of K_L completely restored to the control value. On the other hand, the shedding of a few leaves was observed in water-stressed plants after 25 days of withholding water (personal observation).

The contents of soluble sugar and proline significantly increased after 17 days of withholding water and were still significantly higher than the control values after the recovery from water stress (Figure 5A and B). The responses of $g_{s\text{-}max}$, PLC and K_{leaf} to the water potential in water-stressed plants with ongoing drought stress are shown in Figure 6. The water potential at 50 and 20% of maximum g_s was −1.27 and −1.93 MPa, respectively; the water potential at 50% loss of maximum K_{leaf} was −1.68 MPa; and the water potential at 20 and 50% PLC was −1.27 and −2.42 MPa, respectively. As shown in Figure 7, during the phase of water stress, $g_{s\text{-}max}$ was linearly correlated ($P < 0.01$) with hydraulic capacity (K_{leaf}, K_S and K_L) when water stress exceeded a threshold; furthermore, a positive relationship was also shown to occur between the recovery of $g_{s\text{-}max}$ and of hydraulic capacity during the phase of rewatering.

Discussion

Since water relation and gas exchange were not significantly changed in control plants, soil and leaf water shortage in water-withheld plants is uniquely responsible for the observed changes in gas exchange and hydraulic capacity. When water stress exceeded a threshold, $g_{s\text{-}max}$ sharply decreased in parallel with A_{max} (Figure 2). Stomatal limitations account mainly for this initial reduction in A_{max}. Nevertheless, the suppression of photosynthesis by water shortage does not arise from stomatal closure alone. Stomatal and non-stomatal limitations jointly contribute to the reduction of photosynthesis under moderate to severe drought conditions (Gimenez et al. 1992, Gulías et al. 2002, Flexas et al. 2004). Before stomata are completely closed or $g_{s\text{-}max}$ falls below a threshold, elevated WUE values are commonly observed in water-stressed plants (Brodribb 1996, Rouhi et al. 2007, Pou et al. 2008); this is also evidenced in our experiment (Figure 2C).

While stomatal closure may postpone the occurrence of embolism during drought stress (Tyree and Ewers 1991, Cochard 1992, Sparks and Black 1999, Brodribb et al.

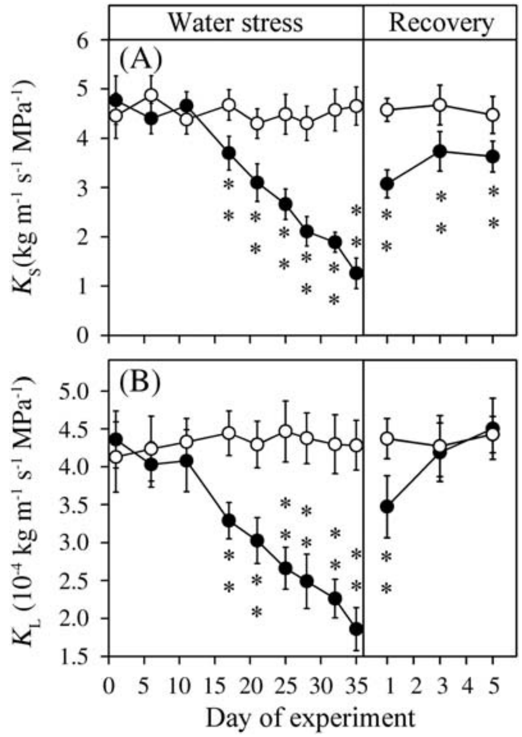

Figure 4. Sapwood area-specific hydraulic conductivity (K_S, A) and leaf area-specific hydraulic conductivity (K_L, B) in *H. brasiliensis* tree seedlings during the periods of water stress and recovery. K_S and K_L were measured in well-watered (open circles) and water-stressed (closed circles) plants. Data are presented as the means ± SD (n = 7–9). Significant difference between control and stressed plants at each date of the experimental periods: *$P \leq 0.05$; **$P \leq 0.01$.

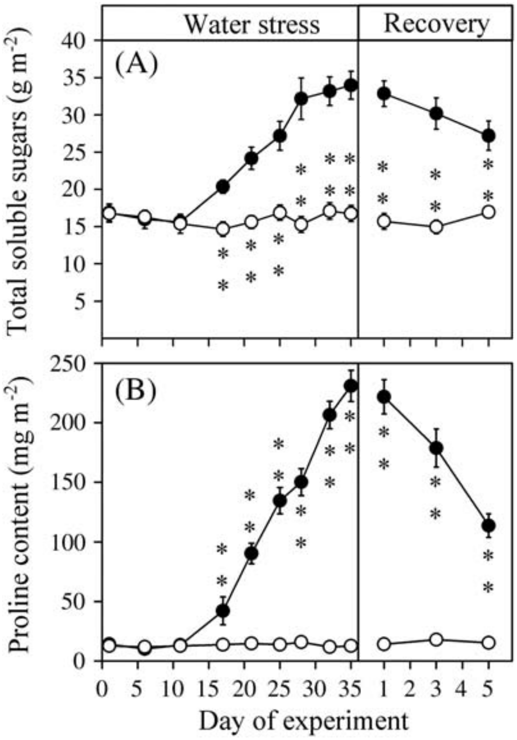

Figure 5. Changes in contents of total soluble sugar and proline in leaves of *H. brasiliensis* tree seedlings during the periods of water stress and recovery. Contents of total soluble sugar (A) and proline (B) were measured in both well-watered (open circles) and water-stressed (closed circles) plants. Data are presented as the means ± SD (n = 7–9). Significant difference between control and stressed plants at each date of the experimental periods: *$P \leq 0.05$; **$P \leq 0.01$.

2003), ultimately, it cannot prevent further cavitation or facilitate repair of embolism during the progression of drought. With a prolonged period of withholding water, water stress inevitably induces xylem cavitation, consequently reducing the hydraulic efficiency of the conductive system (Figures 3 and 4). Besides a link between stomatal closure and the loss of hydraulic conductivity under water stress (Figure 7), we found that the $P20_{stem}$ at which the incipient cavitation occurs in stem xylem vessel (Brodribb et al. 2003) corresponds to the water potential at 50% stomatal closure (Figure 6). This implies that coordination exists between the stomatal and hydraulic responses to water potential. At the same time, the water potential at 80% stomatal closure ($g_{s\text{-max}}$ ~0.10 mol H_2O m^{-2} s^{-1}) was much less negative than the $P50_{stem}$, indicating a broad safety margin for the stem xylem. This also supports the idea that stomatal closure occurs as a protective mechanism against stem xylem cavitation (Tyree and Sperry 1988).

Unexpectedly, stomata were not completely closed before a 50% loss of maximum K_{leaf} had occurred (Figure 6), thus suggesting the lack of a safety margin for the leaf xylem. Rather, stomata even operate at the risk of leaf hydraulic catastrophic failure. However, the fact that the water potential at 50% stomatal closure was less negative than the $P50_{leaf}$ further confirms the notion that the stomatal response to water potential is extremely rapid and stomatal closure is the earlier event in water-stressed plants (Cornic 2000). Given that the leaf represents a large resistor in the hydraulic pathway through the plant, it is surprising that this resistor was more vulnerable to water stress-induced cavitation ($P50_{leaf} > P50_{stem}$). However, this is consistent with the findings of Choat et al. (2005). It is plausible that, to minimize leaf hydraulic resistance and maximize water supply to the leaf for photosynthesis, the leaf xylem is constructed with large pores in inter-conduit pit membranes enhancing hydraulic conductivity, but increasing the risk of air seeding through pit membranes (Sperry and Tyree 1988). Overall, our findings indicate that the leaf functions as a safety valve to mitigate cavitation damage to a greater extent than does the stem.

The Ψ_{leaf} and leaf RWC rapidly recovered to the control level after rewatering (Figure 1). This is consistent with previously reported results (Sánchez-Blanco et al. 2002, Gallé et al. 2007). The reason for the complete recovery of leaf water relation may be that drought-induced embolism is promptly refilled after rewatering (Milburn and McLaughlin 1974, Holbrook et al. 2001). In fact, the leaf provides probably the best environment for refilling of embolized conduits (Salleo et al. 2000, 2001) due to the relative abundance of inor-

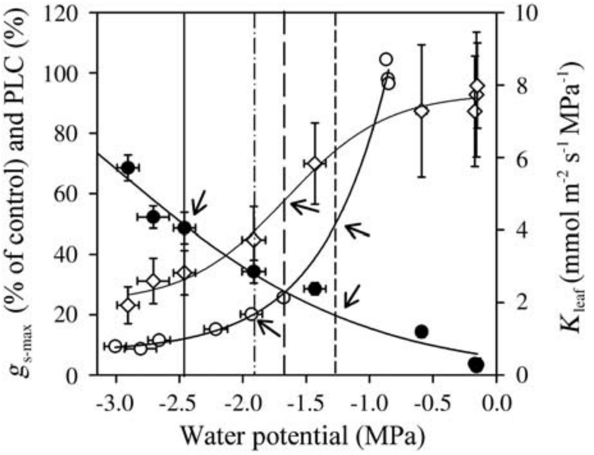

Figure 6. Relationships between the water potential and maximum stomatal conductance ($g_{s\text{-max}}$, open circles), stem percentage loss of conductivity (PLC, closed circles) and leaf hydraulic conductivity (K_{leaf}, open diamonds) in *H. brasiliensis* tree seedlings during the period of water stress. Values for each point are the means ± SD of more than 10 measurements on water-stressed plants from seven to nine individual trees (n = 7–9). The vertical short-dashed line indicates the water potential at 50% stomatal closure and 20% loss of stem hydraulic conductivity, the vertical long-dashed line indicates the water potential at 50% loss of maximum K_{leaf}, the vertical dash-dotted line indicates the water potential at 80% stomatal closure and the solid line indicates the water potential at 50% loss of stem hydraulic conductivity.

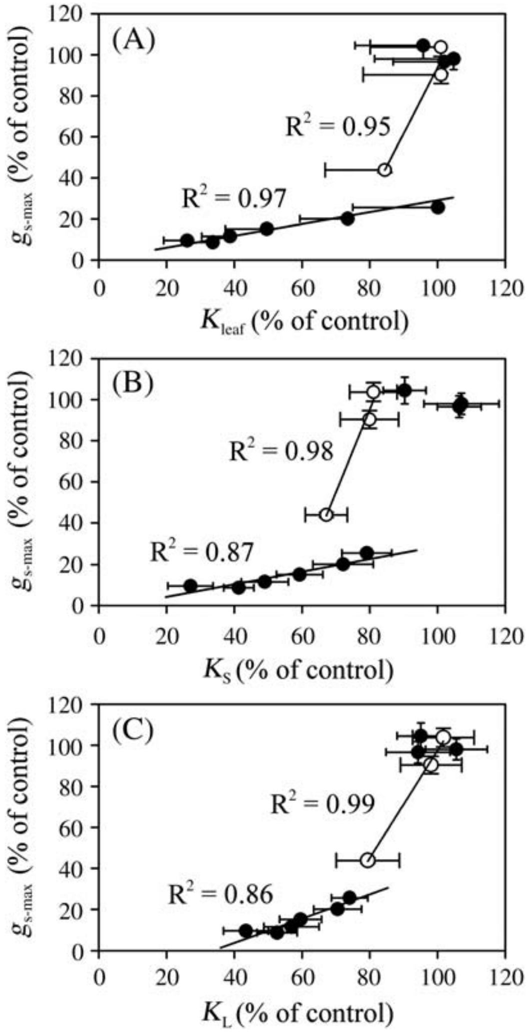

Figure 7. Relationships between maximum stomatal conductance ($g_{s\text{-max}}$) and leaf hydraulic conductivity (K_{leaf}, A), sapwood area-specific hydraulic conductivity (K_S, B) and leaf area-specific hydraulic conductivity (K_L, C) during the periods of water stress (closed circles) and recovery from drought (open circles). Some points ($g_{s\text{-max}}$ > 80% of control) were excluded from a regression relationship between $g_{s\text{-max}}$ and hydraulic capacity under water stress.

ganic ions and other osmolytes that could be used to generate positive pressures (Holbrook and Zwieniecki 1999) as well as possessing large amounts of metabolic energy to drive ion movement. Understandably, the observed accumulation of net solutes (Figure 5) not only contributes to drought resistance under water stress but also promotes refilling of embolism after rewatering. Thus, not surprisingly, the rapid recovery of K_{leaf} was observed in previously water-stressed plants following rewatering (Figure 3B). Furthermore, good evidence exists to suggest that xylem tissue collapse (Cochard et al. 2004, Brodribb and Holbrook 2005, Brodribb and Cochard 2009) and leaf turgor loss (Brodribb and Holbrook 2006, Kim and Steudle 2007) may both play a part in the loss of K_{leaf} in a variety of plants. Therefore, the rapid recovery of K_{leaf} could also be attributed to the possible fact that the initial loss of K_{leaf} is not associated with xylem cavitation. Leaf water relation might rapidly recover even though the value of stem PLC reached ~70% in our experiments. Our results indicate that leaf hydraulics is not the factor limiting the final recovery of gas exchange.

However, the K_S could not fully recover to the control level (Figure 4A) even if embolisms were completely refilled (Figure 3A). Presumably, this incomplete recovery of K_S is the result of drought-induced reduction in function or active vessel size. Wider xylem vessels are more vulnerable to water stress-induced embolism than narrower xylem vessels (Maherali et al. 2006, Rosner et al. 2007, Chen et al. 2009*a*, 2009*b*). Thus, it is quite likely that drought treatment irreversibly damaged the conductive system of larger-sized vessels and just temporarily weakened the water transport efficiency of smaller-sized vessels. Once water-stressed plants are rewatered, embolisms are refilled immediately and hydraulic capacity recovers completely in vessels of smaller size, but not in vessels of larger size. Consequently, incomplete recovery of K_S was observed in previously water-stressed plants after rewatering since the absolute value of K_S is positively correlated with vessel size (Zhang and Cao 2009, Chen et al. 2009*a*, 2009*b*). Alternatively, it is also more likely that drought stress resulted in permanent dysfunction of some vessels via tyloses, mucilage or resins that were released in response to drought.

In contrast, the K_L recovered to the control level after 3 days of rewatering (Figure 4B). At first thought, this seems unreasonable since the complete recovery of K_S was not achieved and the different trends in K_S and K_L are only reconciled by a change in Huber value. A reduction in leaf area is a commonly observed response to water deficit, especially in facultatively deciduous plants. Such a response to water stress was also found in our experiment (personal observation). Thus, we thought that the reduction in total leaf area may, at least in part, contribute to the complete recovery of K_L. Provided that all the leaves distal to stems were taken into account in calculating the value of K_L in previously water-stressed plants after rewatering, it is possible that the K_L would not recover to the control level as the K_S did. Similarly, the incomplete recovery of K_S and complete recovery of K_L were simultaneously observed in previously water-stressed black spruce (*Picea mariana*) after recovery from drought (Blake and Li 2003). Our results raised such a question of what plants benefit from the shedding of a few leaves during the period of drought. Clearly, the shedding of a few leaves prevents a sharp decline in K_L, which facilitates the complete recovery of K_L in the presence of incomplete recovery of K_S after rewatering. A maintained relatively high K_L lowers the soil-to-leaf pressure gradient (Tyree and Ewers 1991, Choat et al. 2007), thus in return reducing the risk of xylem cavitation.

Recently, a body of research has highlighted the control that hydraulic limitations impose on gas exchange recovery in plants rehydrated from a range of drought severities (Lo Gullo et al. 2003, Brodribb and Cochard 2009, Blackman et al. 2009, Resco et al. 2009). Evidently, our results showed that, besides a close link between the loss of hydraulic conductivity and the reduction of $g_{\text{s-max}}$ under water stress, the recovery of $g_{\text{s-max}}$ is also related to the recovery of hydraulic capacity during recovery following rewatering (Figure 7). This suggests that plant hydraulics plays an important role in steering the dynamic response of g_s during water stress and recovery. Indeed, additional factors contributing to the dynamic response of g_s are not ruled out. A possible candidate is the accumulation of plant hormone (abscisic acid [ABA]) in leaves, which is commonly associated with stomatal closure (Davies and Zhang 1991, Wilkinson and Davies 2002, Lovisolo et al. 2008). However, high ABA concentrations are not likely to persist for >3 days after water stress is relieved (Davies and Zhang 1991). Furthermore, there is evidence showing that woody plants rely more on hydraulic signalling than chemical signalling, whereas ABA is probably more common in herbaceous plants (Fuchs and Livingston 1996). Considering the rapid recovery of $g_{\text{s-max}}$ (Figure 2B) and a close link between the recovery of $g_{\text{s-max}}$ and of hydraulic capacity after rewatering (Figure 7), it might be speculated that the effects of ABA on g_s is minor and plant hydraulics predominates the dynamic response of g_s during recovery in our experiment. Thus, our results also partially support the idea that the recovery of gas exchange depends strongly on the recovery of plant hydraulic capacity after drought relief (Brodribb and Cochard 2009, Blackman et al. 2009, Resco et al. 2009).

Despite the complete recovery of $g_{\text{s-max}}$, the value of $A_{\max}$ did not completely recover to the control level (Figure 2). Apparently, this incomplete recovery of $A_{\max}$ results primarily from non-stomatal limitations. Previous studies have suggested a threshold value ($g_{\text{s-max}} < 0.10$ mol H_2O m^{-2} s^{-1}) for incomplete drought recovery in terms of a minimum stomatal conductance (Flexas et al. 2004, 2006, Galmés et al. 2007). In the current study, $g_{\text{s-max}}$ fell to 10% of the control ($g_{\text{s-max}} =$ 0.05 mol H_2O m^{-2} s^{-1}) and below such a threshold (Figures 2 and 6). Thus, we considered that irreversible damage to the photosynthetic apparatus really takes place during drought treatment, but such damage does not affect reopening of stomata after rewatering. Indeed, a variety of factors including injury to electron transfer in PSII, damage to the chloroplast and mitochondrial structure and limitation of mesophyll conductance are possibly involved in the incomplete recovery of $A_{\max}$ after drought relief (Lopez et al. 1987, Miyashita et al. 2005, Galmés et al. 2007). Alternatively, end-product inhibition of photosynthesis resulting from an elevated pool of soluble carbohydrates, as observed in our study (Figure 5A), may also contribute to the incomplete recovery of $A_{\max}$ (Souza et al. 2004). In addition, reduced WUE was observed in previously water-stressed plants after rewatering (Figure 2C). Miyashita et al. (2005) and Gallé et al. (2007) thought that photosynthesis reacts faster than stomata after drought-induced suppression, which results in enhanced WUE. Nevertheless, elevated WUE was not observed in other studies (Cai et al. 2005, Ennahli and Earl 2005). One possible reason for this discrepancy in the recovery of WUE might be species-specific or stress-specific. Anyway, our results suggest that a combination of hydraulic and non-hydraulic factors limit the drought recovery of gas exchange in previously water-stressed plants after rewatering.

Conclusion

In conclusion, the initial suppression of photosynthesis in water-stressed plants might arise mainly from stomatal closure. However, stomatal closure, possibly triggered by hydraulic signalling, could effectively reduce the risk of xylem dysfunction. Also, we found that the leaf functions as a safety valve to protect the hydraulic pathway from water stress-induced cavitation to a larger extent than does the stem during the period of water stress. After rewatering, leaf water relation could rapidly recover to the control level, thus leaf hydraulics would not limit the final drought recovery of gas exchange. Meanwhile, the complete recovery of K_L facilitates lowering the soil-to-leaf pressure gradient, thus in return reducing the risk of xylem cavitation in the presence of incomplete recovery of K_S. Considering the complete recovery of $g_{\text{s-max}}$ and incomplete recovery of $A_{\max}$, our results revealed that the full drought recovery of gas exchange is restricted by not only hydraulic factors but also non-hydraulic factors.

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