

Rapid adjustment of leaf angle explains how the desert moss, *Syntrichia caninervis*, copes with multiple resource limitations during rehydration

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Abstract. Although the desert moss *Syntrichia caninervis* Mitt. is extremely desiccation tolerant, it still requires water and photosynthates for growth. The ecological significance of the leaf angle in maintaining a balance between water and light availability is critical to its survival. Active leaf repositioning balances water and light availability following rehydration. *S. caninervis* can adjust leaf angles from a steep (84–69°) to a stable level at 30° within 7 s after rehydration, obtaining maximum net photosynthetic gain at a shoot relative water content of ~60%. Leaf morphological characters, (leaf hair points, surface papillae and costal anatomy) and ultrastructural changes (chloroplast reordering and loss of lipid reserves as shown by changes in osmiophilic globules) were linked to rapid leaf spreading, water gain and sunlight reflectivity of leaves during rehydration. The high 377.20 ± 91.69 (cm² g⁻¹) surface area to mass ratio was a major factor in facilitating the rapid response to rewetting. Hyaline cells of the leaf base absorbed water, swelled and forced the leaf away from the stem as soon as rehydration commenced. Loss of leaf hair points retards leaf angle adjustment during rehydration.

Additional keywords: desiccation, leaf angle adjustment, leaf hair points (LHPs).

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Introduction

The xerophytic moss *Syntrichia caninervis* Mitt. (Pottiaceae) is dominant component of biological soil crusts (BSCs), which are frequently exposed to high levels of ultraviolet (UV) radiation, atmospheric nitrogen (N) deposition, rising temperatures, and changes in patterns of precipitation in desert ecosystems (Reed *et al.* 2012). *S. caninervis* has developed special structural (Zhang *et al.* 2009), physiological (Li *et al.* 2010) and molecular (Yang *et al.* 2012) characteristics as a result of compromises that optimise resource capture and utilisation (e.g. light for photosynthesis), minimise resource loss (e.g. water from transpiring leaves) and ultimately enhance its survival and fitness in the desert environment. All of these adaptations help *S. caninervis* ‘resurrect’ when in suitable conditions. However, its ability to cope with multiple resource limitations during resurrection remains poorly understood.

The angle between the leaf surface and the horizontal (θ) is an important component of plant architecture and affects both photosynthesis and water use (van Zanten *et al.* 2010). Given a particular radiation stream, changes in leaf angle can strongly influence the amount of sunlight intercepted and absorbed by leaves. Maximum interception occurs when sunrays are

perpendicular to a horizontal leaf (Knapp and Smith 1997). Leaf angle may also affect the way in which water droplets are channelled off leaf surfaces. Steeper leaf angles decrease rainfall interception and increase through-fall to the soil (Holder 2012). The relationship between leaf angle and canopy hydrophobicity may have a significant influence on moss hydrological processes (Holder 2012) and photosynthesis (Waite and Sack 2010) in arid and semiarid regions.

Plants can have constitutive leaf angles that are fixed and do not vary much in different environments. However, *S. caninervis* has a particular ability to actively adjust its leaf angles. Active leaf repositioning can contribute to avoidance of detrimental environmental conditions such as heat stress (van Zanten *et al.* 2010). For example, leaves closely inserted at high angles along the stem enabled *S. caninervis* to avoid high solar radiation interception and, thus, survive in a desiccated state for 12 months or more (Wu *et al.* 2012). The vertical orientation of desiccated leaves reduces the extinction coefficient of a canopy resulting in a more homogeneous distribution of light over the leaves and higher carbon gain at the whole canopy level (van Zanten *et al.* 2010). During resurrection, *S. caninervis* absorbs water from a variety of sources, including dew and fog, and can

demonstrate an immediate change in turgor. Leaves spread out from the stem, taking up their normal hydrated position (Wu *et al.* 2012), presenting the greatest surface area to the light and atmospheric CO₂. However, during rehydration, as the angles of the rapidly reviving leaves are reduced, intercepted solar radiation, leaf temperature and water loss increase. The capture of light energy to fuel the photosynthetic machinery within leaves unavoidably involves the conversion of solar energy to thermal energy, although a potential trade-off between energy gain through photosynthesis and water loss (and water stress) through increased transpiration would be expected (Knapp and Smith 1997). Few data are presently available to assess the relative potential of alternative architectural properties, for example optimum leaf angle and leaf size of *S. caninervis*, to facilitate rapid recovery after rewetting.

Photosynthesis and water absorption are both important processes in *S. caninervis* and, thus, research on the mechanisms of coping with multiple resource limitations is of great scientific significance when exploring its survival strategies during rehydration. Zhang *et al.* (2011) used modulated chlorophyll fluorescence to determine the speed of recovery of photosynthetic activity of *S. caninervis* after remoistening, and found that when shoots were remoistened in the dark, minimum and maximum fluorescence (F_0 and F_m) and photosynthetic yield (F_v/F_m) of PSII recovered rapidly. This was particularly so for F_v/F_m , which, within the first minute of remoistening, achieved 90% of the value measured 30 min after remoistening. In the Gubantunggut Desert of north-western China, overnight dew deposition is essential to enable mosses in BSCs to gain maximum benefit from first light, when F_v/F_m approaches the maximum for photosynthetic activity. As daytime temperatures rise, F_v/F_m levels decrease together with plant water content (Zhang *et al.* 2011).

The main purpose of this study was to investigate the importance of *S. caninervis* leaf angles and their function during rehydration. We speculated that changes in leaf angle would be influenced by leaf hair points (LHPs) because LHPs have the potential to increase absolute water content (AWC) by ~25% and to increase the amount of dew deposited on soil crusts by ~10% (Tao and Zhang 2012). Also, the ability to rapidly vary leaf angle will maintain a stable level to balance light and water availability during rehydration.

Materials and methods

Study area

Our study site was located in the Gurbantunggut Desert located in the centre of the Jungger Basin, of the Xinjiang Uygur Autonomous Region of China. The Gurbantunggut is the second largest desert in China, with an area of 48 800 km² (44°11'–46°20'N, 84°31'–90°00'E). Because of the 'rain shadow' effect caused by the Himalayan uplift, moist air currents from the Indian Ocean fail to reach the area, resulting in a vast arid terrain. Mean annual precipitation is ~80 mm and occurs predominantly in the spring; mean annual pan evaporation is 2607 mm. The average temperature is 7.3°C. Prevailing winds are from the WNW, NW, and N and average 11.2 m s⁻¹ annually. The highest wind speeds occur in the late spring. Natural vegetation in the area is dominated by

Haloxylon ammodendron (C.A.Mey.) Bunge and *Haloxylon persicum* Bunge *ex* Boiss. and Buhse (Amaranthaceae), with a vegetation cover of less than 30% (Zhang *et al.* 2007).

Specifically, we selected a site in the southern part of the Gurbantunggut Desert because it contains characteristic BSCs found throughout the desert: cyanobacteria-dominated crust (including *Microcoleus vaginatus* (Vauch.) Gom., *Microcoleus paludosus* (Kutz.) Gom., *Anabaena azotica* Ley and *Lyngbya martensiana* Meneghini; lichen-dominated crust (including *Collema tenax* (Sw.) Ach., *Psora decipiens* (Hedw.) Hoffm., *Xanthoparmelia desertorum* (Elenkin) Hale and *Diploschistes muscorum* (Scop.) R. Sant); moss-dominated crust (including *Syntrichia caninervis* Mitt., *Bryum argenteum* Hedw. and *Tortula muralis* Hedw.) (Zhang *et al.* 2007; Wu *et al.* 2012). The site is a typical, longitudinal sand dune (44°32'30"N, 88°6'42"E) and acts as a permanent research site for the Gurbantunggut Desert and Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences.

Plant material

In 2011, 20 replicated contiguous patches of moss crusts (each with diameter >10 cm) containing *S. caninervis* gametophytes were collected using polyvinyl chloride (PVC) tubes (5.7 cm in diameter and 3.5 cm in height) from the permanent site. The moss clumps were air-dried and then stored at room temperature in black polyethylene bags for 1 week. Gametophyte tissues were hydrated for 48 h to enable full recovery from dry storage before experiments as described previously. Voucher specimens were in the Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences.

Rehydration

To avoid potential variation due to leaf age, individual shoots comparable in colour and size were chosen for subsequent experiments. Rhizoids kept on all of those ~7 mm shoots. To determine the most efficient method of water absorption by *S. caninervis*, shoots were rehydrated by adding distilled water to the soil (group 1, the rhizoids were then rehydrated by connecting the filter paper to another Petri dish containing distilled water) and by precipitation from the air (group 2, the aerial leaves were then rehydrated using mini watering can from the top of the shoots).

A further comparative investigation was conducted to determine the influence of LHPs on changes in leaf angle. Shoots with LHPs (group 3) and without LHPs (group 4, LHPs were manually removed using fine forceps under an anatomical lens.) were both measured under the precipitation rehydration method. A total of 105 shoots were divided into five replications for each group, and placed in 20 Petri dishes. Shoots were rehydrated in Petri dishes in the natural laboratory atmosphere (19–21°C; 25–27% RH) (Li *et al.* 2010; Wu *et al.* 2012).

Leaf angle

Shoots were placed under a dissecting microscope and photographs were taken using a digital camera (Powershot

A650 IS, Canon Inc., Zhuhai, China) to record the leaf movement. Using the time-lapse function of the camera, photographs were taken every 1 s and used for the measurement of leaf angle (Fujita *et al.* 2008). Adobe Photoshop software (ver. 6.01, Adobe Systems Inc., San Jose, CA, USA) was used to edit the digital images. We randomly selected and sampled 10 plants of similar size and obtained measurements from the four previously mentioned groups. Two upper leaves and two lower leaves were chosen for determining the angle between the leaf surface and the horizontal, to compare the effect of the position of the point of insertion of leaves along the stem on the leaf angle change (θ). This was measured using a commercially available protractor with a plumb line degree indicator (Mineola, New York, NY, USA). A ruler was held up to each leaf and the angle between the leaf and the horizontal was measured to the nearest 0.5°.

Shoot relative water content

The relative water content (RWC) of shoots during rehydration was determined by the weight change of the gametophyte apex as calculated according to the following formula (Oldenhof *et al.* 2006):

$$\text{Relative water content (RWC)} = \frac{\text{fresh weight} - \text{dry weight}}{\text{saturated weight} - \text{dry weight}} \times 100\% \quad (1)$$

The results were expressed as the mean value of five replicates for each treatment. FW was measured at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 45 and 60 s after rehydration. Saturated weight was measured after immersion of the leaves in distilled water for 24 h at room temperature. Redundant external capillary water on shoot surfaces was carefully removing using a piece of filter paper. DW was measured following oven drying at 95–105°C until a constant weight was obtained. The electronic balance (FA1604N; Minqiao Co. Ltd, Shanghai, China) used for all weighing was accurate to 0.0001 g (Wu *et al.* 2012).

Estimation of potential photosynthetic area

Quantifying the effect that a change in leaf angle (θ) and area of cells containing chloroplasts (I_{ch}) may have on intercepted sunlight relative to when the sun is perpendicular to a horizontal leaf is straightforward using Lambert's cosine law. The amount of light incident on a (leaf) surface (potential photosynthetic area, I_{p}) depends on the orientation of the surface relative to the radiant beam, according to: $I_{\text{p}} = I_{\text{ch}} \cos\theta$. Using Adobe Photoshop software (ver. 6.01, Adobe Systems Inc.), we measured the area of LHPs (I_{LHPs}), base transparent zone (I_{t}) (full of enlarged, hyaline, basal cells but no chloroplasts), chloroplast zone (I_{ch}) and whole-leaf area (I_{w}), both desiccated and rehydrated. These were described as I_{p0} , I_{ch0} , θ_0 , I_{LHPs0} , I_{t0} and I_{w0} for the desiccated state as control and I_{p1} , I_{ch1} , θ_1 , I_{LHPs1} , I_{t1} and I_{w1} upon rehydration.

Measurement of maximal quantum efficiency of PSII

Chlorophyll fluorescence was measured with a pulse-modulated fluorometer (Mini-Pam, Heinz, Walz, Germany). The optic fibre was pointed at each sample from a distance of 2–4 mm and at an

angle of 60°. To minimise scattering of data, the initial position of the optic fibre was not changed during the experiment.

To determine maximum quantum yields, leaves were dark-adapted for 30 min then subjected to a pulse of low level of radiation (i.e. $<1 \mu\text{mol m}^{-2} \text{s}^{-1}$) to determine the dark-adapted minimum fluorescence (F_0), and pulsed again at saturation (i.e. $8000 \mu\text{mol m}^{-2} \text{s}^{-1}$) applied to obtain the dark-adapted maximal fluorescence (F_m). The maximum quantum yield of PSII (F_v/F_m) was calculated as $(F_m - F_0)/F_m$ (Zhang *et al.* 2011).

Cytological protocols

Cryo-dried, detached moss leaves on the upper part of shoots with long LHPs were mounted on carbon tape and covered with a 15–20 nm gold layer using a Cressington (Ted Pella, Inc., Redding, CA, USA) sputter 108 auto coater. Images of the fine outer structures of LHPs, leaf surfaces and cross-sections were obtained by scanning electron microscopy with an FESEM (SUPRA 55VP, Carl Zeiss Inc., Oberkochen, Germany), using a secondary electron detector at a working distance of 7–10 mm and 10–20 KV of electron energy.

Each dried shoot was rapidly frozen in liquid nitrogen and put onto the SEM Cryo Transfer (Gatan Alto 2100, Gatan Inc., Abingdon, UK). The images of LHPs capturing water from the air and reserving water internally were taken using a Hitachi-3000N (Hitachi Inc., Tokyo, Japan) scanning electron microscope at a working distance of 11 mm and 15 KV of electron energy.

High pressure freezing (HPF) and freeze substitution (FS) methods (Sosinsky *et al.* 2008) were used to observe the transition of chloroplast ultrastructure upon rehydration. The desiccated leaves and fully rehydrated leaves were put into a high-pressure freezer (Leica EM PACT2, Leica Microsystems, Vienna, Austria) to fast vitrify samples up to 200 μm in thickness without the artefacts of chemical fixation. After freezing, leaf samples were placed into a freeze substitution machine (Leica EM AFS2, Leica Microsystems, Bannockburn, IL, USA) and conventionally incubated and fixed. Epon812 (Shell Chemical. Co., Norco, LA, USA) resin was used for infiltration and embedding.

Fourier transform infrared spectroscopy studies

Using Fourier transform infrared spectroscopy (FTIR) methods described by Oldenhof *et al.* (2006), individual dry and wet LHPs (~2 mm) were conducted to compare the components. Infrared absorption measurements were conducted with a Bruker Equinox 55 remote sensing FTIR spectrometer (Bruker Optics, Ettlingen, Germany). The emission spectra of the combustion flame were recorded in the range of 4000 to 900 cm^{-1} with a spectral resolution of 4 cm^{-1} . Band positions and transition temperatures were calculated according to the methods previously described (Oldenhof *et al.* 2006).

Statistical analysis

SPSS statistical software package (ver. 13.0, Chicago, IL, USA) was used to process data. The levels of variation among samples and levels of significance, if present, were estimated and compared by Fisher's least significant difference (l.s.d.) multiple range tests at the 0.05 level. Correlation procedures were performed to determine the influence of desiccation on measured physiological indices.

Results

Leaf angle changes influenced by rehydration

Leaf angle was related to the point of leaf insertion on the stem. Leaf angle differences between upper leaves and lower leaves were apparent in the desiccated state (Fig. 1, $P < 0.01$). The inclination of upper leaves was in a narrow range of insertion angles ($69\text{--}84^\circ$), whereas the inclination of lower leaves was in a range from 54 to 62° . Leaf spreading following rewetting was rapid in *S. caninervis*, with the first leaves spreading within 1 s of receiving water. To reach the same leaf angle (around 30°), leaves spread more quickly (7 s) when they obtained water from the air than when they obtained water from the soil (30 s) (Fig. 1, $P < 0.01$). Compared with lower leaves, upper leaf angles decreased quickly with lower shoot RWC (60%) (Fig. 2) and stabilised at the same level as lower leaves (Figs 1, 2). Leaves obtaining water from the air can remain at lower angles while drying than those deriving water from the soil (Fig. 2) at any RWC 90% or less.

Leaf angle changes influenced by LHPs

Leaves on the upper part of shoots had longer LHPs than those below. Based on results from the air rehydration studies, LHPs affected the upper leaf angles during rehydration. Upper leaves without LHPs reached a constant state with greater leaf angles, around 50° (Fig. 1), than those with LHPs ($\sim 30^\circ$). For leaves with LHPs, leaf angles of upper leaves could significantly decrease and reached stability around 30° with a lower shoot RWC (60%) (Fig. 2). For leaves without LHPs, there was a significant leaf angle difference between the upper and lower leaves (Fig. 1, $P < 0.01$).

Relationship between leaf angle and photosynthetic recovery

To understand the changes in photosynthetic recovery following leaf angle changes, chlorophyll fluorescence was investigated at different leaf angles. During the rewetting, when the leaf angle reached minimum values, the LHP area (I_{LHPs}) and leaf base transparent zone area (I_t) (full of enlarged, hyaline, basal cells but no chloroplasts) were slightly altered; however, both the area of the chloroplast zone (I_{ch}) and the whole leaf (I_w) reached twice the size previously reached at maximum leaf angle. Influenced by both leaf angle (θ) and chloroplast zone area I_{ch} , the potential photosynthetic area I_p also significantly increased, up to 5-fold upon rehydration (Fig. 3a). During leaf rewetting, F_v/F_m also recovered quickly. When the leaf angle decreased to around 30° , F_v/F_m recovered to twice that of max leaf angle. In contrast to the remaining constant leaf angle, F_v/F_m continued to increase slightly, reaching its maximal value 36 s after leaves were remoistened (Fig. 3b).

Leaf cytological transition towards active metabolism upon rewetting

Morphological characteristics pertinent to recovery

Syntrichia caninervis is able to furl its leaves longitudinally (recurving leaf margins) to reduce evaporation by minimising the amount of exposed area during the desiccated state. Adaxial (upper or facing towards the stem) and abaxial (lower or facing away from the stem) leaf surfaces showed some common morphological features. Both upper and lower surfaces of the leaf cells were covered with numerous papillae composed of four or five divarications (Fig. 4a–d), which might aid water movement and possibly create more internal capillary spaces among the fibrils of the cell wall (Crandall-Stotler and Bozzola

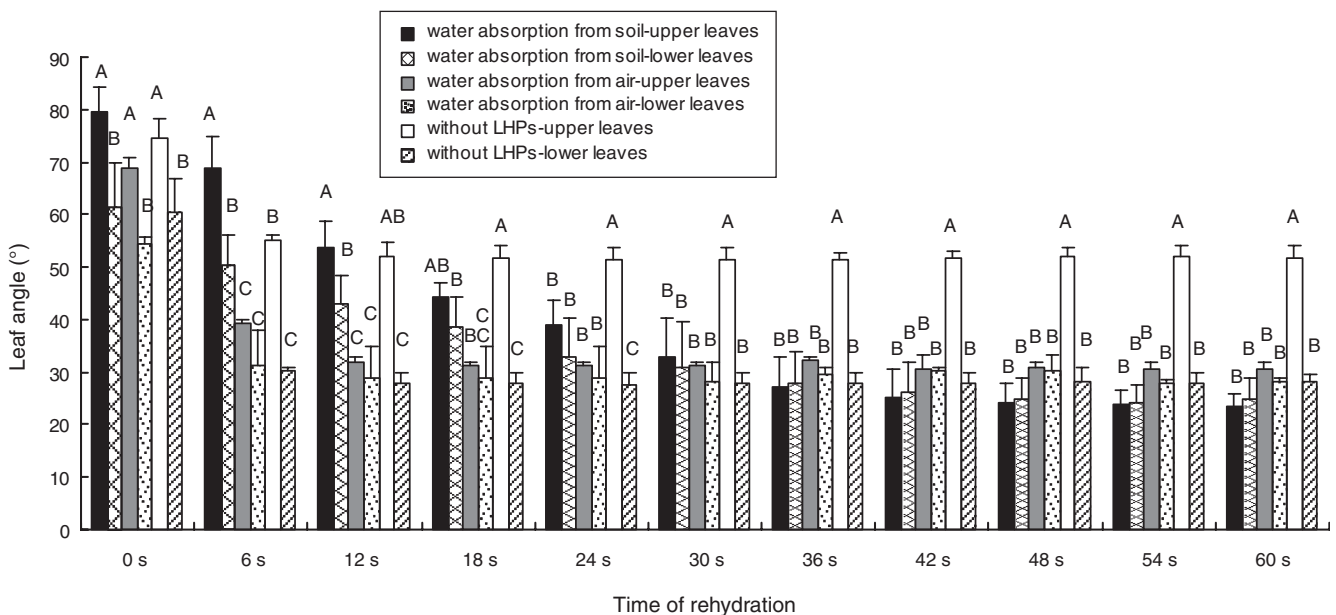


Fig. 1. Changes in *Syntrichia caninervis* leaf angle after different periods of rehydration. Leaves spread more rapidly when water was obtained from the air than when it was obtained from the soil. Different letters indicate significant differences with different treatments included under the same rehydration time ($P < 0.01$ in all cases, $n = 20$).

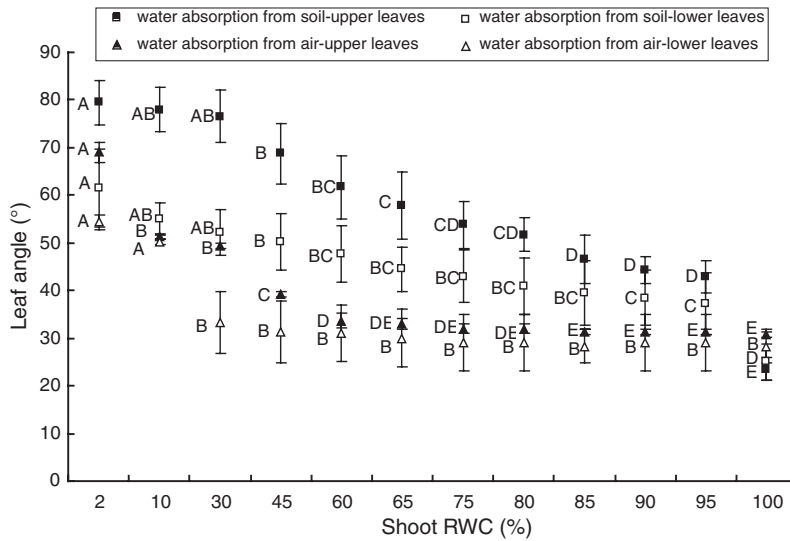


Fig. 2. Effect of *Syntrichia caninervis* shoot RWC on changes to leaf angles. Different letters indicate significant differences within same treatment under different shoot RWC % ($P < 0.05$ in all cases, $n = 20$, RWC and leaf angles in this figure were under same time during rehydration).

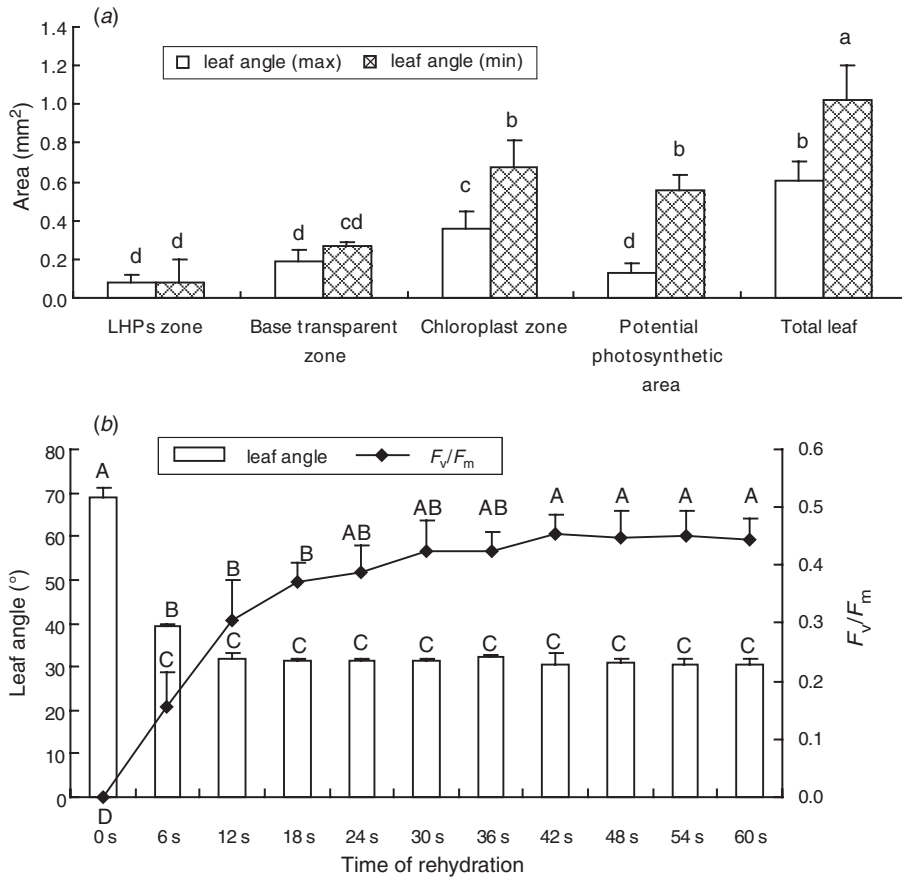


Fig. 3. Relationship between *Syntrichia caninervis* leaf angle and photosynthetic recovery. (a) Comparison of potential photosynthetic area under minimum and maximum leaf angle. Different letters indicate significant differences among different areas ($P < 0.01$ in all cases, $n = 10$). (b) Relationship between leaf angles and maximal quantum efficiency of PSII. Different letters indicate significant differences of leaf angles and F_v/F_m during rehydration ($P < 0.01$ in all cases, $n = 20$ for leaf angle, $n = 20$ for F_v/F_m).

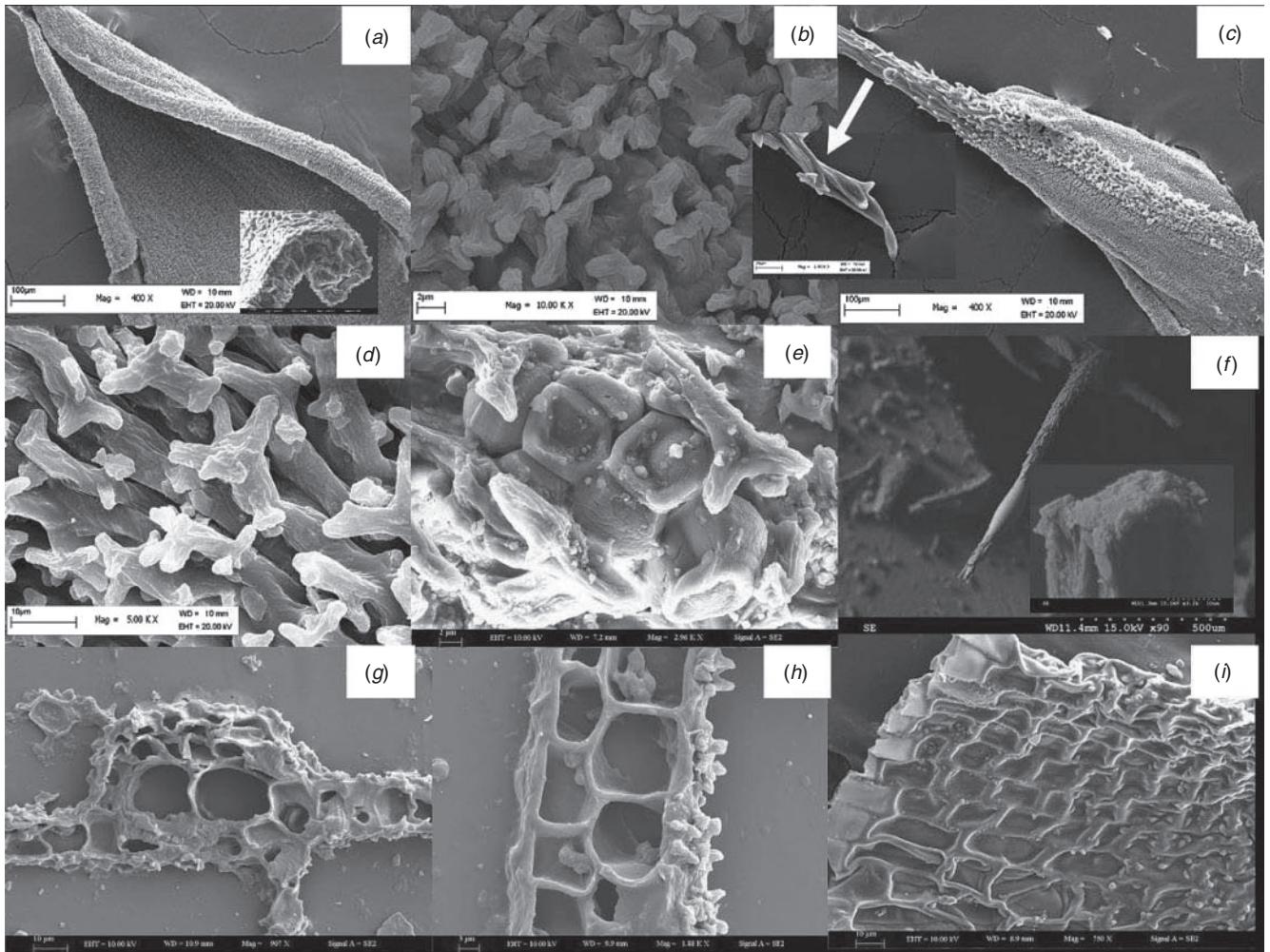


Fig. 4. Morphological and anatomical characteristics of *Syntrichia caninervis* before rewetting. (a, b, i) Densely papillose adaxial (upper) leaf surface showing papillae with four or five divarications. (c–e) Abaxial surface of leaf showing much larger interstitial spaces between papillae on the leaf costa and many cubic wax particles (5–7 μm in diameter). (g, h) Cross-section of a leaf blade showing the costa (g) with supporting stereids and adjacent lamina cells (h). Scale bars: (f) = 500 μm ; (a, c) = 100 μm ; (d, g, i) = 10 μm ; (h) = 3 μm ; (b, e) = 2 μm .

1990; Crandall-Stotler and Bozzola 1991). However, the papillae and the interstitial spaces between papillae were much larger on the abaxial surface of the costa than on the leaf lamina (Fig. 4b, d). The larger papillae may play an important role in altering the boundary layer and reducing water loss (Proctor 1984). Many cubic wax particles with diameters \sim 5–7 μm were deposited on the costal surface (Fig. 4e).

The leaf surface features of *S. caninervis* function to enhance water absorption, conduction and retention. In the dry state, LHPs (hyaline, hair-like extensions to the leaf tips) were closely appressed to the stem. LHPs seemed to act as minute collectors that capture moisture from the air. Minuscule spinulae covering LHPs increased the surface area of LHPs for water absorption and also increased the volume of water that was captured by LHPs. Stored water was visible inside transverse sections of LHP spinulae (Fig. 4f). Additionally, the two large cells (deuter cells) in the costa supported rapid water conduction during rehydration (Fig. 4g). Elongate, thick-walled, slender and fibre-like stereid cells on one or both sides of the costa appear to

function as protection against desiccation. The bistratose upper leaf lamina containing chloroplasts has a thickness of 15–20 μm in the mid to upper part of leaf (Fig. 4h) and may protect the chloroplasts against high intensity UV radiation and drought. In the lower part of the leaf, the basal angle cells were usually thin-walled and unistratose (thickness no more than 5 μm). The shrinkage of desiccated alar cells in the lower, outer corners of the leaves created gas pockets on the leaf surface (Fig. 4i). Structures such as papillae and LHPs become more transparent when wet, facilitating light transmission.

Transition of ultrastructure upon rewetting

Based on our previous conclusion that membranes of *S. caninervis* remained intact during a drying–rewetting cycle (Wu *et al.* 2012), we found the following transition of ultrastructure towards active metabolism upon rewetting (Fig. 5). First, chloroplasts with disordered thylakoids reverted to normal structure after 2 h of recovery. The main body of each

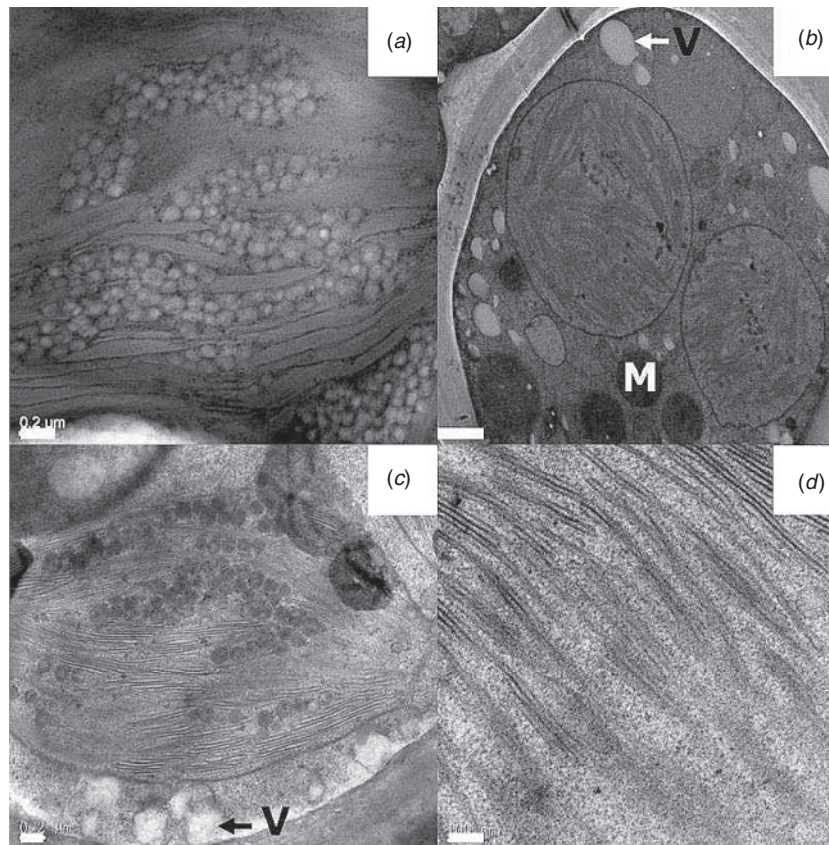


Fig. 5. Transition of *Syntrichia caninervis* chloroplast upon rewetting. (a, b) Disordered thylakoids with many osmiophilic globules during desiccation. (c, d) Reorganisation of thylakoids and reduced osmiophilic globules during rehydration. Abbreviations: M, mitochondria; V, vacuole. Scale bars: (b) = 0.5 μm ; (a, c) = 0.2 μm ; (d) = 100 nm.

chloroplast was packed with well organised grana. The thylakoids with numerous lamellae were interconnected by parallel, interregnal thylakoids. This rapid structural reorganisation supported the fast restoration of PSII. Second, the number of osmiophilic globules (lipid-containing bodies in chloroplasts) decreased as active metabolism was restored upon rewetting. This suggests that lipid reserves are used during recovery from desiccation.

Component of LHPs towards active metabolism upon rewetting

The composition of LHPs appeared not to change upon rehydration; however, the absorbance differed (Fig. 6). The absorption peak at 3330 cm^{-1} corresponds to hydroxy stretching vibrations mainly arising from carbohydrates, cell wall material and proteins. The absorption peak at 2880 cm^{-1} in the methyne stretching region of the spectra reflected the contribution of cell wall material including cellulose, pectin and protein. The absence of an absorption peak between 1780 and 1720 cm^{-1} reflects a degree of damage to the membranes. This suggests that LHP cells may be empty. The increasing absorption at 3400 , 2880 and 1650 – 1620 cm^{-1} (reflecting the stability of protein secondary structure) together indicates protein and pectin accumulation in the desiccated LHPs. This suggests that under

drought conditions the flexibility and extensibility of LHPs may be enhanced and serve to prevent mechanical injury to the underlying leaf.

Absorption peaks at 3450 , 2932 , 1154 cm^{-1} and 1062 cm^{-1} together reflects the contribution of chemical compounds including fatty acids, alcohols and alkanes. It confirmed the existence of wax covering LHPs (Figs 4f, 6). No absorption was observed in the wave number range between 2000 and 2400 cm^{-1} , suggesting that the thickness of the wax layer did not exceed $3\text{ }\mu\text{m}$. The amount of wax components in desiccated LHPs was higher than that in the hydrated LHPs.

Discussion

Syntrichia caninervis is extremely desiccation tolerant, but still needs water and photosynthates for growth. The ecological significance of leaf angle in maintaining a balance between water and light availability is critical to its survival. In this study, the rate of leaf spreading within 7 s of rewetting was consistent with morphological (Zheng *et al.* 2010), physiological (Li *et al.* 2010) and molecular parameters (Yang *et al.* 2012) reported previously. Our results indicated a slight trend for both rapid optimum use of water (Figs 1, 2) and light (Fig. 3) to be coupled with this rapid leaf spreading. Although the relationship was not straightforward, the results demonstrated

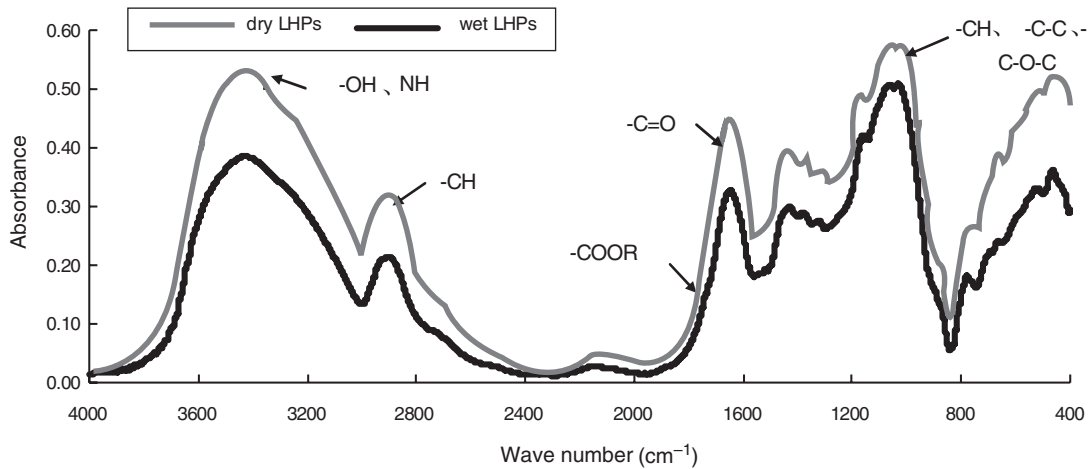


Fig. 6. Contribution made by *Syntrichia caninervis* leaf hair points to active metabolism upon rewetting.

that *S. caninervis* has the potential to attain maximum net photosynthetic gain at shoot relative water content around 60% by rapidly reducing leaf angle from steep 84° to stable 30° angles upon rehydration (Figs 1, 2). Morphological structures (LHPs, papillae and costae) and transition of ultrastructure (chloroplast reorder and lipid reserves) were closely coupled with rapid leaf spreading, water gain and sunlight reflectivity of leaves during rehydration.

Zhang *et al.* (2011) determined that the *S. caninervis* photosynthetic responses of fully desiccated leaves to remoistening involved an extremely rapid increase in F_0 and F_v/F_m within the first minute, followed by a more gradual increase over the next 6–8 h. This fits well with our results, which show that the leaf angle decreased quickly to interception of light within 12 s following rehydration and F_v/F_m recovered quickly with the decreasing leaf angle. Dark-adapted F_v/F_m ratios can be used to provide an estimate of maximum quantum efficiency of PSII. Proctor (2003) used an F_v/F_m from 0.76 to 0.83 to indicate the chlorophyll fluorescence as an index of recovery of desiccation tolerant bryophytes (Proctor 2003). However, Zhang *et al.* (2011) reported F_v/F_m of *S. caninervis* rapidly reached 0.6 after remoistening, and then remained steady at 0.7 for 8 h. At this stage, it may not be feasible to measure fluorescence levels within the first few second (e.g. at 5, 10, 15, 55 s) of rehydration (Zhang *et al.* 2011). If the leaf angle reaches its optimum for light interception (~30°) within 7 s, the photosynthetic ‘platform’ is in place well before 1 min, or the time at which the rapid rise in photosynthetic activity has been measured.

Regulation of leaf structure on water potential availability

Syntrichia caninervis is a typical poikilohydric bryophyte, so has a limited ability to regulate water content by utilising anatomical and morphological structures (Fig. 4). The angle of leaf insertion on the stem is one of the major factors determining water storage capacity and stem water flow (Garcia-Estringana *et al.* 2010). In vascular plants, steep leaf angles result in reduced radiation absorption, higher water potentials, lower temperatures and transpiration rates, all of which are crucial for survival in arid

environments (Valiente-Banuet *et al.* 2010). Vascular plants with vertically orientated leaves with insertion angles >45° promote water flow towards the stem and the narrow range of insertion angles (69–84°) of upper leaves produces conical or funnel shapes that enhance stem water flow when the plant is desiccated (Garcia-Estringana *et al.* 2010). Steep leaf angles in vascular plants are regarded as an important functional trait to shed water from leaves to root systems (Valiente-Banuet *et al.* 2010; Holder 2012); however, desert mosses rely on the absorption efficiency of aerial gametophytes (Fig. 1) to capture rain, fog or dew rather than absorption of water from the soil. Moss rhizoids at the base of the stems serve primarily for anchorage while the absorption of moisture occurs over the entire surface of the plant. Leaf angles of *S. caninervis* change almost as soon as rehydration begins, reaching and stabilising at an angle of around 30°, an angle that appears to be optimal for leaf water storage (Figs 1, 2). Changes in anatomical structures also support rapid, active leaf spreading and interstitial spaces between papillae form a capillary conducting system responsible for rapid water movement (Fig. 4d).

LHPs are important desiccation-tolerant features of xerophytic mosses. Some common ecological functions of LHPs include reduction of mechanical abrasion by sand particles, reflection of visible light (Koch *et al.* 2008) and they play a role in water retention and dew nucleation (Tao and Zhang 2012). Our data enhance the understanding of the relationship between LHPs and water use efficiency, thus, the presence of LHPs was closely linked to the rewetting responses and loss of LHPs may threaten leaf recovery (Fig. 2). Rainfall events in arid and semiarid regions in summer are characterised as being of high intensity and of short duration. During these events, it is speculated that LHPs of *S. caninervis* maximise interception of water. We propose that LHP apical cells with little or no surface wax or papillae may provide a starting-point for rehydration from LHPs to the chloroplast zone and that the minute spiculae and cuticular wax layer may facilitate uptake of water (Figs 4f, 5).

The role of cell surface papillae (Fig. 4a–d) cannot be ignored, but their role in relation to water absorption has long been controversial. Together with wax crystals, papillae significantly influence the hydrophobicity of a leaf surface.

A surface with a high density of smaller papillae is more effective for water repellence than one with a lower density of larger papillae (Koch *et al.* 2008). Both upper and lower leaf surfaces of *S. caninervis* should demonstrate water repellence, especially leaves which have a high density of smaller papillae (Fig. 4*b, d*). However, we observed the opposite behaviour under a dissecting microscope. When 0.15 μL of DNA free water was dropped onto upper and lower leaf surfaces using an Eppendorf pipette, contact angles could not be determined on either surface as water droplets were immediately adsorbed by leaf surface, indicating that leaf surface provide the most efficient water absorption mechanism (superhydrophilic). The interstitial spaces between papillae formed a microcapillary conducting system for rapid movement of water across the leaf surface. The primary ecological significance of surface hydrophilicity is to facilitate water uptake in arid regions (Hauck *et al.* 2008). Leaf superhydrophilicity is the basis for uptake of water and nutrients from the environment, greatly benefiting non-vascular plants, which lack both roots for water uptake and vessels for water transport (Koch *et al.* 2008).

The relationship between papillae and waxy deposits over the surface of the costa (Fig. 4*e*) remains to be investigated, although wax particles and papillae may retard water loss and regulate gas exchange in desert moss (Xu *et al.* 2009).

During rehydration, *S. caninervis* may well maintain shoot water content by keeping leaf angles at a stable level around 30° (Fig. 1), the point at which shoot relative water content reached more than 60% (Fig. 2). Evaporation rates in arid and semiarid regions remain very high even during rainfall events, because of low humidity levels and high vapour pressure deficits so that the ability to control intercepted water loss becomes a selective advantage (Holder 2012). Apart from mosses that live in the coastal regions, moss leaves are typically one cell thick and lack any development of vascular tissue and stomata with which to control water loss (Waite and Sack 2010). A combination of factors contributes to higher shoot water content, including small leaf size, rapid leaf movement, wax on LHPs and coastal regions (Figs 4*f, 6*), boundary layer effect and dead space created by leaf surface papillae (Fig. 4*b, d*).

Regulation of leaf structure on light interception

Numerous studies, including moss studies, have investigated leaf angle as a functional trait that influences light interception (Waite and Sack 2010). Our results demonstrate the relationship between leaf angle, potential photosynthetic area and transition of chloroplasts upon rewetting (Figs 3, 5). Steep leaf angles minimise light interception in the desiccated state, but on rewetting, leaf spreading significantly increases the area of the chloroplast zone and the potential photosynthetic area and, ultimately, light interception (Fig. 3). These findings support the argument that there is a co-ordinated suite of architectural and physiological characteristics maintaining the photosynthetic integrity of xerophytic mosses (Hamerlynck *et al.* 2000).

Absorbance or reflectance of light determined by the state of moss hydration and chlorophyll fluorescence levels following rehydration show the photosynthetic apparatus of *S. caninervis* to be fully functional (Li *et al.* 2010). In dry moss, there may be internal or external scattering of light whereas the more

homogeneous surface and interior of wet moss allows light to travel with less scattering (Lovelock and Robinson 2002), optimising photosynthetic yield gain. Papillae on both leaf surfaces (Fig. 4*a–d*) may play a role in scattering light in dry mosses while allowing transmission in wet ones (Glime 2007).

Preservation of the integrity of the photosynthetic apparatus is shown in the transition of ultrastructure following rewetting (Fig. 5). Membranes are 'porous' when dehydrated and carbohydrate leakage occurs rapidly through these membrane gaps on rehydration. However, membrane integrity is rapidly restored and the pool of intracellular carbohydrates is rapidly replenished (Melick and Seppelt 1992, 1994). During each rehydration event, cell membranes require energy to repair damage (Glime 2007). In a significant contrast to this membrane repair scenario, membranes of *S. caninervis* have been shown to remain intact during a drying–rewetting cycle (Wu *et al.* 2012). If energy is not needed for membrane repair, why are osmiophilic globules lost during rehydration (Fig. 5), and what is the relationship between lipid reserves and leaf movement?

Mechanism of regulation of leaf movement

Leaf angle adjustment is considered to be an adaptive strategy for enhancing photosynthetic performance and water use efficiency although mechanisms by which mosses regulate leaf movement remain obscure. In contrast with many plants that adjust their leaves to track the sun (Mullen *et al.* 2006), regulation of leaf movement of desert mosses is induced by water.

Petiole curvature is considered to be a key factor in changes to the direction of individual leaves of tracheophytes (Fujita *et al.* 2008). Moss leaves do not have petioles, but they may reorient their leaves by means of biophysical turgor-driven reversible changes in the swelling and shrinkage of specialised strategically-localised leaf cells. Leaves of *S. caninervis* are closely appressed to the stem when dry, but enlarged hyaline basal cells (Fig. 4*i*) absorb water readily, swelling and forcing leaves away from the stem. Although most bryophytes demonstrate rapid spreading upon re-moistening, only two moss taxa, *Ptilium crista-castrensis* and *Dicranum polysetum* are known to spread the first leaf within 2 s after receiving water (Y. Li, unpubl. data; Glime 2007). Leaf surface area to mass ratio is important in determining speed of rewetting. The surface area to mass ratios of some moss taxa (e.g. *Polytrichum piliferum*) that are slow to absorb moisture, were no more than 50 (Larson 1981). It should be noted that the ratio of *S. caninervis* was as high as 377.20 ± 91.69 ($\text{cm}^2 \cdot \text{g}^{-1}$) ($n=20$), and this difference may explain its water absorption efficiency.

The leaf hair points of the desert moss *S. caninervis* are a critical part of adaptation to, and survival in, the extremely arid environment of the Gurbantunggut Desert, facilitating the capture of minimal quantities of water (rain, fog and/or dew), increasing the speed at which water can be absorbed into the leaf, and altering the leaf angle to effect optimum photosynthesis and carbon gain.

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