Journal of Arid Environments 128 (2016) 17–29

Contents lists available at ScienceDirect

Journal of Arid Environments

journal homepage: www.elsevier.com/locate/jaridenv

Leaf epidermal water-absorbing scales and their absorption of unsaturated atmospheric water in *Reaumuria soongorica*, a desert plant from the northwest arid region of China

Xiaohua Wang*, Honglang Xiao, Yiben Cheng, Juan Ren

Key Laboratory of Ecohydrology and of Inland River Basin, Division of Hydrology Water-Land Resources in Cold and Arid Regions, Cold and Arid Regions Environmental and Engineering Research Institute, Chinese Academy of Sciences, Lanzhou 730000, People's Republic of China

ARTICLE INFO

Article history:
Received 8 July 2015
Received in revised form
23 December 2015
Accepted 12 January 2016
Available online xxx

Keywords:
Water-absorbing scales
Unsaturated atmospheric water
Fluorescence tracer
Drought environments

ABSTRACT

This study found water-absorbing scales (WASs), which could absorb unsaturated atmospheric water (UAW), in the leaves of *Reaumuria soongorica*, a super-xerophytic desert plant. WASs were a complex cellular structure surmounted by four to seven valves that formed an inverted cone pore. The base of the cone comprised three or four flat, thin-walled, living cells. Under low humidity, the valves of WASs contracted, becoming nearly closed and leaving a small central opening, thus forming a more or less impermeable lid. Under high humidity, the valves of WASs absorbed UAW from the atmosphere and swelled, gradually expanding and forming a larger central opening. The basal cells raised the lid of the scale and allowed water to flow by capillary action over the cuticle. At a relative humidity of 75–95% with a wetting period of 3 h in duration, the valve opened fully, the central opening expanded to form a polygonal structure, and the water content of the leaves increased from 47.8% to 54.2%. An EDS analysis with a scanning electron microscope found more chloride, sulfate and potassium in the valves of WASs than in normal leaf epidermis. This chemical composition may facilitate the absorption of water from unsaturated air by WASs.

1. Introduction

Since the 1950s, many studies have reported the absorption and utilization of atmospheric water by the aerial parts of plants. This ability has been found primarily in xerophytes and halophytes such as Coulter pine (*Pinus coulteri*) and *Pinus ponderos* (Stone et al., 1950), *Stipagrostis sabulicola* (Ebner et al., 2011), *Lavandula stoechas*) (Munné-Bosch et al., 1999), and the Coast Redwoods (Limm et al., 2009; Limm and Dawson, 2010), which can survive in extremely arid habitats by extracting water from the air through their foliar structures or tender stems. Atmospheric water vapor, fog, dew and condensation water absorbed by leaves or tender stems become important sources of water for the growth of plants or for drought avoidance by plants (Sveshnikova, 1972; Bruijnzeel et al., 1993; Eller et al., 2013; Oliveira et al., 2005; Hao et al., 2012).

The surface of the leaf is a key ecological interface for material exchange between the plant and the atmosphere. Leaf surfaces are covered by a waxy cuticle which is, in general, hydrophobic. The surface cuticle of the leaf functions as the main barrier to transport and as an interface with the environment, limiting ion transport and the loss or leaching of water from the leaf interior as well as controlling foliar uptake (Kerstiens 1996; Riederer and Schreiber, 1995; Koch et al., 2009).

However, over evolutionary time, structural and chemical modifications have induced variation in surface wetting, ranging from hydrophobic to hydrophilic. Hydrophilic surfaces exist on many flower leaves that have a papillar cell morphology and cuticular folding with smooth 2-D wax films on their surface. Superhydrophilic surfaces are found in land plants, of which the leaf surfaces have hydrophilic compounds on their surface including the secretion of salt by salt glands, the secretion by nectaries of sugars or of other substances such as mucus, and the deposition of pollutants and aerosols from the atmosphere (Burkhardt et al., 2012). As these substances are hydrophilic and may change the hydrophobicity and water surface tension on the leaves of plants (Burkhardt et al., 2012), they can effectively adsorb water on the leaf surface or condense water molecules on its leaves from an unsaturated atmosphere (Mooney et al., 1980).

* Corresponding author.
E-mail address: wangxiaohua950@126.com (X. Wang).

http://dx.doi.org/10.1016/j.jaridenv.2016.01.005
0140-1963/

In general, the foliar uptake of water (FUW) from atmospheric air consists of two processes. One is that the water molecules in the air are concentrated on the surfaces of leaves. The other is that the condensed water on the surface of the leaves is transported into the mesophyll cells. The process of condensation of atmospheric water molecules is related to the wettability of the leaf surface, which can be facilitated by micro-morphological structures and by the chemistry of the surface, as explained in detail above. For the second process, FUW, the transport path of solutes through the cuticle is thought to be composed of two routes, namely, the lipophilic and the hydrophilic pathway (Schreiber, 2005). The hydrophilic pathway for polar or ionic solutes consists of 'aqueous polar pores', which are supposed to result from water adsorbed to less hydrophobic constituents of the cuticles (Schönherr, 2000; Chamel et al., 1991).

In addition to the cuticular pathway, there is evidence for an uptake pathway located in openings such as stomata (Burkhardt et al., 2012), hydathodes (Martin and Willert, 2000) and the porous surfaces of thorns (Schill and Barthlott, 1973) or trichomes (Nowak and Martin, 1997; Ohrui et al., 2007) or the WASs of epidermis (Dolzman, 1964, 1965), enabling the uptake of hydrophilic substances (Eichert and Burkhardt, 2001; Eichert and Goldbach, 2008; Eichert et al., 1998). Overall, the structures and chemical compositions of plant leaves are expected to have an important role in FUW.

Desert plants are widely distributed in the arid region of northwest China. Their long history of living in arid or saline habitats has frequently resulted in unique morphological or anatomical characteristics on their leaves or stems, such as leaves that are generally degraded; some leaves have many trichomes or verruca or papillae; some have many salt glands, some have lenticels, and some have epidermal hydathodes on the leaves or specialized pores on the leaf surface. These specialized features of the leaf surface of desert plants facilitate the absorption of more water from the atmosphere, as reported by a few previous studies. Fahn (1986) found that the trichomes of the xeromorphic leaves of 12 plant species could absorb water from the leaf surface. Grammatikopoulos and Manetas (1994) reviewed the hairy leaves of *Phlomis fruticosa* contributed to avoid drought by the direct absorption of water. *Bassia dasyphylla*, a desert plant from China with a highly fluffy leaf surface, can absorb dew from the surface of the leaves (Zhuang and Ratchiffe, 2012). In 27 species of *Crassula* from Namib Desert in southern Africa, epidermal hydathodes might allow the absorption of surface water (Martin and Willert, 2000). Several reports have indicated that the stomatal uptake of water can occur without the use of surfactants (Burgess and Dawson, 2004; Breshears et al., 2008; Limm et al., 2009; Simonin et al., 2009). Salt glands on the leaves of *Nolana mollis*, a shrub of the Atacama Desert, contribute to the foliar uptake of atmospheric water by this species (Mooney et al., 1980).

The findings discussed above only confirm that aerial parts of the studied plants can absorb leaf surface water, intercept precipitation, and absorb fog, dew and condensation water. The source of all these inputs is an atmosphere that is saturated with water. A few plants can absorb UAW (Lange et al., 1986; Virzo De Santo et al., 1976). Prominent among these species are a few desert xerophytes occurring in China. It is not yet possible to identify the particular leaf surface structure that plays the main role in water absorption, nor is it possible to identify the specific site at which absorption occurs or the process that gives rise to FUW. These matters have not yet been experimentally investigated. They have only been addressed through indirect evidence or speculation.

R. soongorica, considered an archaic and relic species, is an extreme xeric semishrub belonging to the genus *Reaumuria* Linn and the family Tamaricaceae. It is resistant to extreme drought and barrenness and is a dominant and architecturally important constituent species in desert shrub vegetation. The species is distributed widely in arid and semiarid regions throughout a large area spanned by a drought gradient, from western Erdos, Alashan (Helan Shan), the Hexi Corridor, and the Qaidam Basin to the Tarim Basin and the Dzungar Basin (from the east to the west) (Liu and Liu, 1996), where the average annual rainfall is less than 400 mm. *R. soongorica* can grow well under these conditions, requiring an annual rainfall of only 7–101.1 mm in the Ejinaqi Region (Wang et al., 2011). The distribution of the roots of mature plants of this species in the Gobi Desert region is shown in supplementary Fig. 1. As this figure indicates, the depth of the main root of the plant is approximately 1 m, whereas the water table at this location is at a depth of 2–3 m, and deep groundwater is generally at a depth of 10–15 m. Therefore, it is difficult for *R. soongorica* to absorb soil water or groundwater.

R. soongorica is so drought resistant that many researchers have studied its mechanisms of drought adaptation. These studies have focused mainly on protective mechanisms that confer stress tolerance and on strategies that are adaptive in the face of abiotic stress. These mechanisms in *R. soongorica* include photosynthesis (Xu et al., 2008, 2010; Chong et al., 2010a; Yan et al., 2012), transpiration (Chong et al., 2010b), osmotic adjustment (Liu et al., 2007a; Li et al., 2012), and antioxidant enzymes (Bai et al., 2009, 2010). Several studies have also addressed foliar stable carbon isotope ratios (Ma et al., 2007), the chromosomes of the plant (Wang et al., 2011) and its genome (Shi et al., 2013). These studies found that *R. soongorica* adapts to its stressful environment by lowering leaf water potential, increasing water use efficiency, increasing photosynthesis, copiously accumulating osmotic substances that facilitate osmosis and increasing the enzyme activities responsible for the response of the anti-oxidative system (Xu et al., 2008; Liu et al., 2007a,b; Bai et al., 2009; Xu et al., 2010). In addition, all the C4 photosynthesis genes are present and active in *R. soongorica*, which had been considered a typical C3 plant (Shi et al., 2013). These genes may help the plant to adapt to abiotic stressors, without the need for a large, complex genome (Wang et al., 2011).

Although the results reviewed above help explain the strong drought resistance of *R. soongorica*, it is unclear how *R. Soongorica* is able to perform its life cycle, from spring-sprouting leaves to blossoms and fruit in autumn under such conditions, requiring an annual rainfall of only 7 mm (it cannot absorb soil water or groundwater) in the Ejinaqi region in extreme drought years. In addition to the above mentioned drought resistance mechanisms, can *R. Soongorica* absorb atmospheric water through its leaves to supplement its water requirements? In general, when plants are growing in desert regions, there is a strong temperature difference between day and night, and the humidity can be as much as 60% at night and in the morning on a clear summer day. In the light of the above findings and reasoning, we investigated whether the leaves of *R. soongorica* can absorb UAW to facilitate survival in its extreme arid habitat. Furthermore, we asked whether the leaves of *R. soongorica* have special structures that can absorb moisture, even UAW. To answer these questions, we observed the microscopic structure of the leaves of *R. soongorica*, and we conducted a series of experiments involving fluorescence methods and the humidification of an unsaturated atmosphere and scanning electron microscope observation. These experiments showed that there were numerous WASs in the leaf surface of *R. soongorica*, that they could absorb water from unsaturated air, and that the micro-morphologic structures of the leaf changed during the process of absorption.

2. Materials and methods

2.1. Study area

The research was conducted in a desert steppe area near the town of Luanjintan (105°22′ E, 37°57′ N; elevation 1332 m), Left Alxa County, Alxa League, Inner Mongolia autonomous region, northwest China, during the growing season of July–September 2014. The climate in this region is arid, with a mean annual precipitation of 197.7 ± 57.1 mm (1961–2010), nearly 70% of which occurs during the growing season. The mean annual evaporation is 3000–4100 mm, the mean annual temperature is approximately 9.8 °C, and the mean annual humidity is 36–47%. The mean annual wind velocity ranges from 3.44 to 4.74 m s^{-1} (weather data were from Left Alxa County, Alxa League Weather Bureau.). The zonal soil is classified as typic calciorthid according to the Chinese Soil Classification System (CSTCRG, 1995), characterized by a coarse texture and loose structure and highly susceptible to wind erosion. The dominant plant species in this region are shrubs, half-shrubs and forbs, including *R. soongarica*, *Zygophyllum xanthoxylum*, *Haloxylon ammodendron*, *Nitraria tangutorum*, *Peganum harmala*, *Ammopiptanthus mongolicus*, and *Hedysarum scoparium*. We selected sampling sites approximately 10 km away from Luanjintan. *R. soongorica* is part of the natural vegetation of these sites, where the adults of this species are approximately 100 years old (the data of plant age is from tree ring experiments determined by ourselves) and have an average crown diameter of 0.7 × 0.9 m and a height of 0.5–0.8 m. Adult *R. soongorica* showing consistent growth were selected as the plants to be sampled.

2.2. Field fluorescence humidifying experiment and sampling

To evaluate the anatomical pathways involved in foliar water uptake in the field experimental plot, we conducted atmospheric fluorescence experiments by wetting the aboveground parts of the plants in a sealed glasshouse using an ultrasonic humidifier.

During the wetting experiment, a sample plant was sealed in a glasshouse and wetted by an ultrasonic humidifier. The glasshouse was assembled from several Plexiglas plates that were connected and attached with bolts and screw caps. One of the plates was not fixed and could be opened to collect leaf samples. Gaps between plates were sealed with transparent adhesive tape. A portable thermo-hygrometer MicroLog PRO-EC750 (Fourier Systems Ltd., Israel) was hung on a branch to monitor the change of the temperature and humidity in the glasshouse. At the same time, another portable thermo-hygrometer was hung at the same height above the ground to monitor the change in the temperature and humidity of the natural environment. The portable thermo-hygrometer recorded the temperature and humidity once a minute.

Humidifiers loaded with 0.1% Fluorescent Brightener (FB) aqueous solution were used to humidify the sampled plants of *R. soongorica*. FB is an apoplastic tracer that binds to polysaccharides of the cell wall and emits a strong, pure-blue fluorescence under an excitation wavelength of 350 nm. The design of the wetting experiment is shown in supplementary Fig. 2. The atmospheric fluorescent wetting experiment was carried out in August with the changes in relative humidity kept at approximately 75–95%. This range of relative humidity was produced by manually switching the humidifier on and off as necessary. Wetting in the field experiment was performed from 21:00 to 24:00.

Branches with mature leaves were collected at 0 h, 1 h, 2 h, and 3 h. Before removing them from the chamber, the plants were exposed in the air for a while to make a thin water film on the leaves dried. For each collection, some leaves from the branches were placed in a self-sealing bag and put in a car refrigerator at approximately 4 °C. Other leaves were fixed in FAA (formaldehyde, acetic acid, 70% ethanol; 5:5:90 [v/v]). The samples were then taken to the laboratory for analysis.

2.3. Determination of leaf water content

In the sampling stage of the procedure, 6–10 new branches that had new leaves and that were of a consistent length and size were cut to investigate the absorption of water by leaves of *R. soongorica*. The new branches were promptly weighed ($Mass_{sample0}$, g) in the field tent, using an electronic balance with an accuracy of 0.0001 g. After weighing, the branches were put into an envelope filled with silica gel desiccant and taken back to the laboratory to further process the samples by oven-drying them at 105 °C for 0.5 h, then further drying them at 85 °C for 48 h to complete the drying of the branches and the leaves. The dry weight of the fresh branches with leaves was then determined ($Mass_{sample\ n}$, g). For this purpose, the formula $LWC_{sample\ n}(\%) = (Mass_{sample\ 0} - Mass_{sample\ n}) \times 100\%/Mass_{sample0}$ was used to evaluate the water content of the leaves on the new branches that had been treated by humidifying the atmosphere. Three replicates were used for each sample.

2.4. Detection of fluorescence by microscope

The collected samples were kept in a 4 °C refrigerator in the laboratory for 2–3 days for fluorescence detection. First, 10–15 mature leaves were taken from the middle of the branches and washed three times with distilled water. The picked leaves were further processed. The epidermis was removed from some of the leaves. The other leaves were sectioned with a Small Plant Tissue Special Slicer MTH-1(DL Nature Gene Life Sciences, Inc, Japan) at a position in the middle of the leaf. All of the preparations were then mounted in glycerol and observed under an Olympus BX53 fluorescence microscope (Olympus Corporation, Tokyo, Japan) under a bright field and under fluorescent conditions with an excitation wavelength of 350 nm. Each sample was repeated three times, including the control and the treated samples, using fluorescent humidifying at different times.

2.5. Analytical scanning electron microscope (SEM)

The samples fixed in FAA were examined with a SEM (Quanta 200, FEI) equipped with energy dispersive X-ray spectroscopy (EDS; X flash 3001 Brucker). The leaf samples were dehydrated, oven-dried at 45 °C for 2 days and then mounted on the sample holder without further preparation. The SEM was operated at 15 kV in low vacuum (133 Pa) and in back scattered electron mode. EDS analyses were performed either on spots of interest or in scanning mode.

3. Results

3.1. The morphological and anatomical characteristics of WASs

Fig. 1a–e represented the structure of the leaf epidermis. The epidermis was removed from the leaf, dried, gilded and photographed by SEM. Fig. 1a shows that the leaf epidermis was interspersed with stomata, WASs and salt glands (Fig. 1a). The middle parts of the salt glands contained much crystalline salt, (Fig. 1d,e), whereas the middle parts of the stomata (Fig. 1b) and the WASs did not (Fig. 1c). The stomata of the dried leaves were all closed (Fig. 1a,b), while the WASs were open. The valves of cells were all fully open, and the pore aperture attained its maximum size (Fig. 1c).

Fig. 2a–g show the structure of the surface of the leaves whose

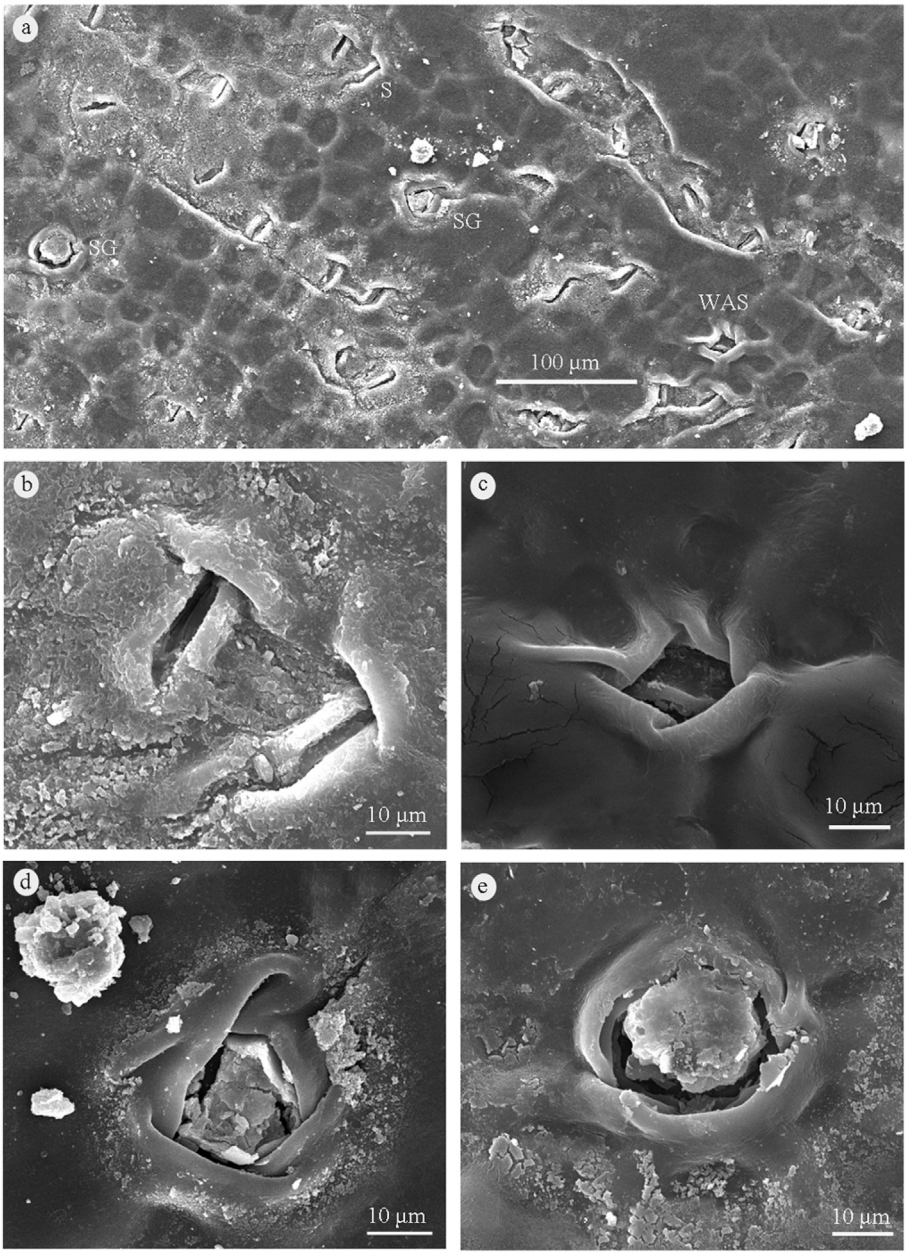

Fig. 1. The SEM structure of the leaf surface of *R. soongorica* with the epidermis removed. a, Leaf epidermis, photo at ×150; b, Stomata, photo at ×1500; c, WAS taken photo at ×1500; d, e, Salt glands shown in photos at ×1500; S, stomata, SG, salt glands, WAS, water-absorbing scale.

epidermis was allowed to remain on the leaf. The leaves were scanned after they were dried. These figures show that the leaf epidermis of *R. soongorica* consisted of many papillae interspersed with stomata, salt glands and WASs (Fig. 2a). The WASs were composed of four to seven valves and formed polygonal structures with a small hole in the center (Fig. 2d–g). The wrinkled epidermis caused the closed stomata to be depressed and difficult to observe (Fig. 2a). Similarly, the salt glands secreted much salt, and masses of salt crystals covered the salt glands. These masses often masked the salt glands and their two pores (Fig. 2b). A salt gland bears two secretion pores through which salts are discharged to the leaf surface (Fig. 2c). In addition, we found that WASs and salt glands were distributed inconsistently and non-uniformly over the leaf surface, usually with more WASs and salt glands on the adaxial leaf surface than on the abaxial leaf surface.

Living leaves of *R. soongorica* were observed under fluorescent light and the bright field of a fluorescence microscope. If the epidermis that had been removed was clean and free of adhering mesophyll cells, it was penetrated by a type of wrinkled structure. These structures were WASs, surrounded by valves to forma polygonal structure with a hole in the center. Observed at 100x magnification under a microscope, this type of wrinkled structure appeared to be closed and to lack a small hole in the center (Fig. 3a). Under 400x magnification, a small hole approximately 5 μm in diameter was apparent in the center (Fig. 3b and Fig. 3d on the left), and the wrinkled structures were not closed or nearly closed (Fig. 3b). The structure of WASs was easier to see under fluorescence (Fig. 3b) than under the bright field (Fig. 3c), i.e., it was hard to find WASs in the bright field. When the epidermis strip that had been removed had adhering mesophyll cells, the WASs resembled a

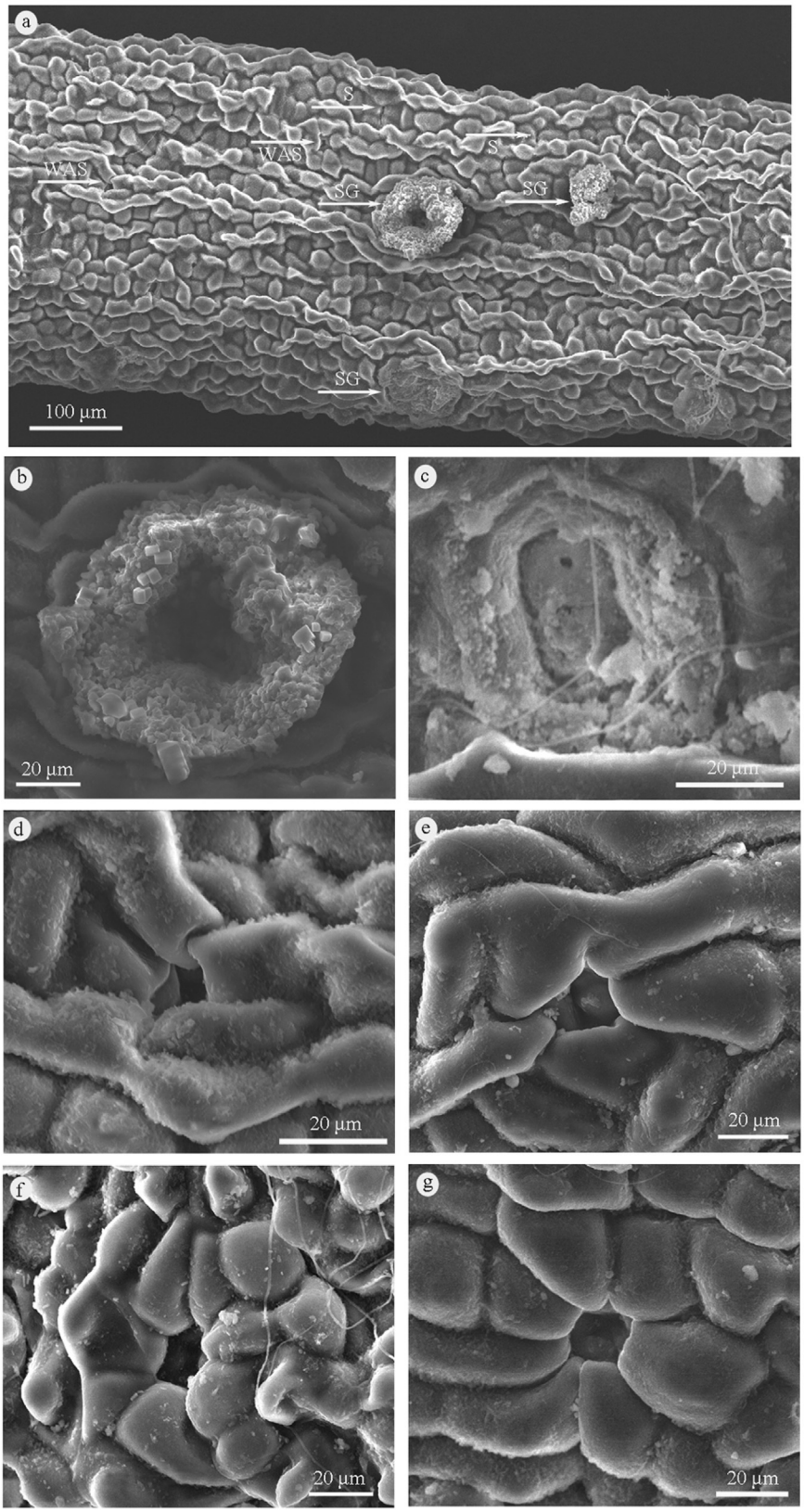

Fig. 2. The SEM structure of leaf surface of *R. soongorica* that did not have the epidermis removed. a, Leaf epidermis, photo at ×150; b, Salt gland amplified; c, The base of salt gland with two pores in the middle; d, WAS amplified with four valve cells; e, WAS amplified with five valve cells; f, WAS amplified with six valve cells; g, WAS amplified with seven valve cells.

manhole cover placed on the plant leaf epidermis, and the valves of the WASs were wrinkled and were surrounded by a circle on the

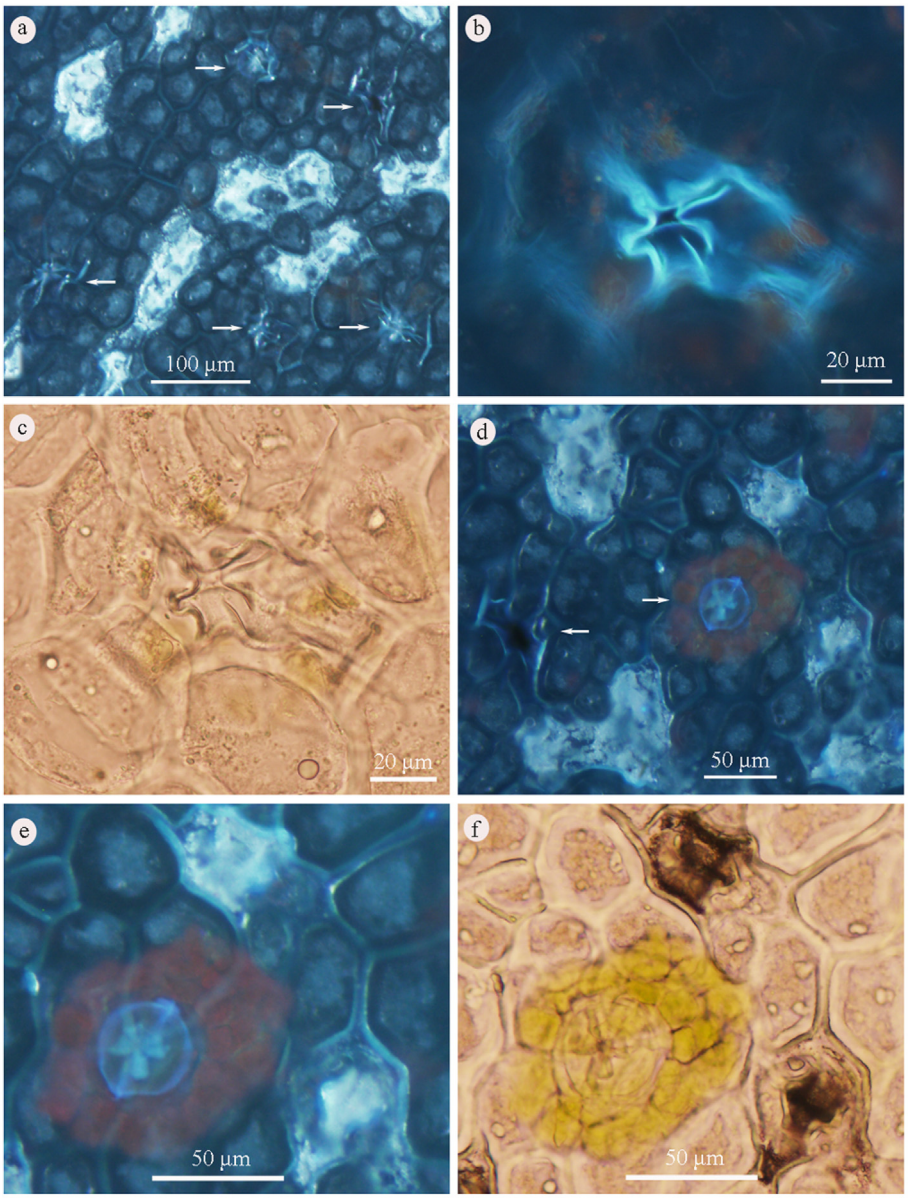

Fig. 3. Structures of WASs of leaf epidermal strips of *R. soongorica* observed under different conditions. a, Leaf surface, photo at ×150; b, WAS, photo at ×400 under fluorescence; c, WAS of b under bright field; d, WAS with adhering mesophyll cells on the right and WAS without adhering mesophyll cells on the left; e, WAS with adhering mesophyll cells amplified, resembling a manhole cover; f, WAS of e under bright field; arrows indicate the locations of WASs.

periphery (Fig. 3d on the right; Fig. 3e). Fig. 3f corresponds to Fig. 3e but represents observations under the bright field. As Fig. 3c and Fig. 3f show, it was not easy to find WASs under the bright field, and this may be one reason why WASs were seldom observed. Under the 340 nm fluorescence excitation, the valves of the WASs emitted light blue fluorescence (Fig. 3b,e), while mesophyll cells emitted red fluorescence (Fig. 3e), which was produced by chloroplasts in the mesophyll cell. This indicated that WASs contained no chloroplasts.

The cross-sections of the WAS showed it was a complex cellular structure, the whole unit forming an effective water-absorbing area and resembling a scale in shape (Fig. 4a). Haberlandt (1914) called it a scale. The base of the WAS resembled a ring (Fig. 4a). If the cross-section was made at the central position, the base of the WAS had a cone-like shape (Fig. 4b). From the central cross-cut section, it is evident that the cone was composed of three flat, thin-walled living cells and a rod cell in the center (Fig. 4b).

The WASs, stomata and salt glands differed in two ways. First, the WASs sank deeper than the stomata and salt glands. They sank into the mesophyll cells (Fig. 4a,b), while the stomata and salt glands only sank into the epidermis, not into the mesophyll cells (Fig. 4c,e). Second, the stomata had a substomatic cavity (Fig. 4e,f), while the WASs and salt glands did not (Fig. 4a–d). WASs, stomata and salt glands were three leaf surface structures embedded in the epidermis of leaves of *R. soongorica*. However, their morphology and their function differed.

3.2. The water-absorbing process and morphological changes in WASs

Fluorescence detection found that during the absorption of water by WASs, changes occurred not only in the shapes of their

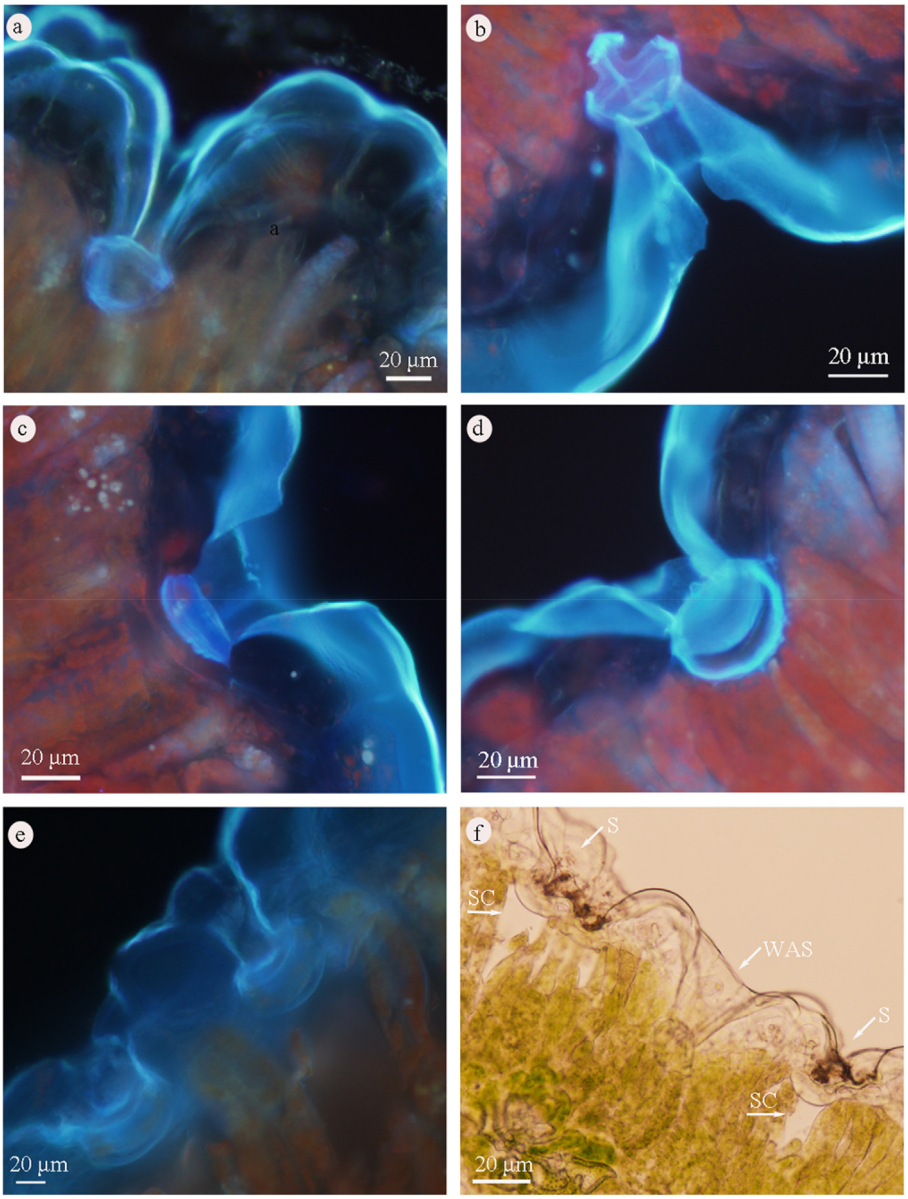

Fig. 4. Cross-sections of WASs, salt glands and stomata of *R. soongorica* leaves. a, b, WASs; c, d, salt glands; e, Stomata under fluorescence; f, Stomata under bright light. SC represents substomatic cavity.

base cells but also in their morphology and fluorescence intensity.

Fig. 5 shows the process by which changes occurred in the base cells as the WASs absorbed water. When dry or not absorbing water, the base cells of the WASs assumed a ring structure, as shown in Fig. 5a and Fig. 5c (on the left). When wetted or absorbing water, the rod cell in the middle lifted the living cells in the base with a half-lift into a cup-shaped structure (Fig. 5b) and with a total lift into a corner T-shaped structure (Fig. 5c, on the right) when the WASs were full of water (Fig. 5c, on the right).

During fluorescence wetting, the valve cells of many WASs began to turn blue (Fig. 6a) after approximately 1 h at relative humidity 85–95% (see Fig. 8), while the stomata (Fig. 6a,b; Fig. 7b) and salt glands (Fig. 6a,b; Fig. 7b) did not change to a blue color but emitting a light milky white fluorescence consistent with the color of the controls (Fig. 3a). This showed that WASs could first absorb UAW, while stomata and salt glands could not.

Fig. 6c–f show the characteristics of the changes in the morphology of the WASs when they were absorbing unsaturated water from the air under fluorescence wetting conditions (Fig. 8). When wetting had not been performed (i.e., wetting at 0 h) with the relative humidity at 30.11%, the WASs were basically in a closed state with the valves contracted, with only a small hole in the middle and without blue fluorescence in the valve (Fig. 6c). When wetting lasted 1 h at relative humidity 85–95% and average temperature approximately 25 °C (Fig. 8), the valves of the WASs stretched partly, emitting a slight blue fluorescence, while the middle hole became slightly larger (Fig. 6d). When wetting continued for another about 1 h, the valves were fully open, the middle hole further enlarged, and the valves issued a faint blue fluorescence. The color did not intensify (Fig. 6e). When wetting continued to 23:50 (lasting about 3 h), the valves of the WASs were fully open, the middle hole became the largest and formed a polygonal structure, and all the valves became completely blue and emitted a deep-blue fluorescence (Fig. 6f). Thus, in drought, WASs

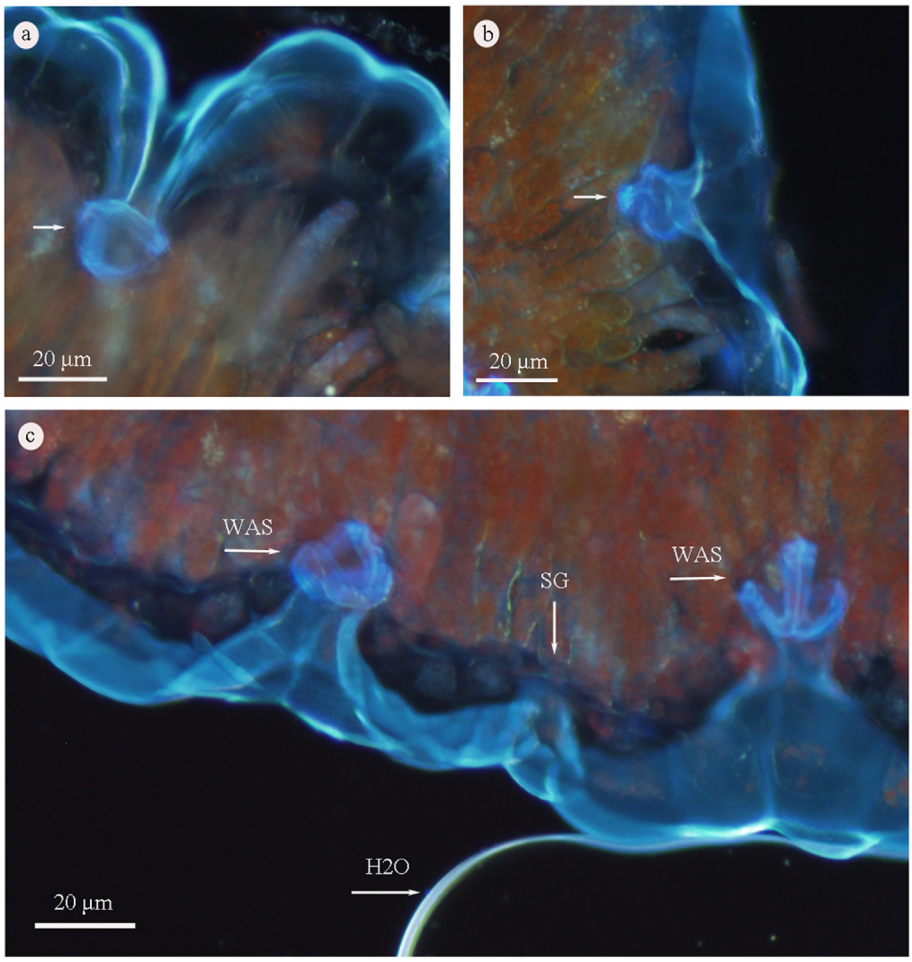

Fig. 5. Change processes of the base cells of WASs when they were absorbing water. a, When dry or not absorbing water; b, When wetted at the beginning, the base of the WAS half lifted into a cup-shaped structure; c, When wetted, the base of WAS on the right totally lifted into a corner T-shaped structure, when not wetted, the base of WAS on the left remained in a ring structure.

were closed and the middle holes were the smallest; under wetting conditions, WASs absorbed water, swelled, and formed a polygonal structure with a large hole in the middle.

Similarly, as seen from the fluorescent cross-sections of the WASs, there was also an obvious change in the color of the WASs. It first began to turn light blue (Fig. 6g) when fluorescent wetting lasted 1 h at the relative humidity and temperature changes shown in Fig. 8; when the wetting lasted approximately 3 h, the entire valve of the WAS turned deep blue (Fig. 6h).

The above results show that with increases in the humidity and the wetting time, the fluorescence intensity of the WASs became stronger. This indicated that more and more water was absorbed by the WASs.

3.3. Variation in the water content of the leaves

To further demonstrate the occurrence of foliar unsaturated water uptake, we compared the variation in the sample with that in the control using the weighing method. Fig. 8 shows that the water content of wetted new branches was higher than that of the control (before being wetted) and increased as the wetting continued. This further confirmed that the leaves of *R. soongorica* absorbed unsaturated water from the air.

3.4. Compositional differences among WAS, normal EP and SG

We identified the component of the normal leaf epidermis (EP), the valves of WASs and salt gland (SG) of *R. soongorica* to investigate the reasons that WASs could absorb water. The main results are shown in as Fig. 9 and Table 1. As shown in Fig. 9 and Table 1, EP was mainly composed of the atomic elements C (83.91%) and O (15.54%), which accounted for 99.45% of the entire content; WASs were also composed of C (81.05%) and O (15.38%), but they contained small amounts of Cl (1.07%), K (0.91), S (0.44%), and Na (0.39%). Compared to EP and WASs, SG contained a relatively low amount of C, only 39.71%, while it contained higher O (44.87%), Ca (8.6%) and Cl (2.95%). These results showed that EP and WAS were mainly composed of organic compounds, whereas SG was mainly composed of inorganic salt crystals. The percent content of chloride, potassium, sulfate, sodium and magnesium was greater in WASs than in EP (Fig. 9 a,b).

4. Discussion

As these samples were all collected from the field, many environmental factors would affect the leaf structure, which would lead to differences in the water-absorbing capacity of leaves from the same parts of different branches and even of leaves from different sites on a given branch. We detected that the same older or tender

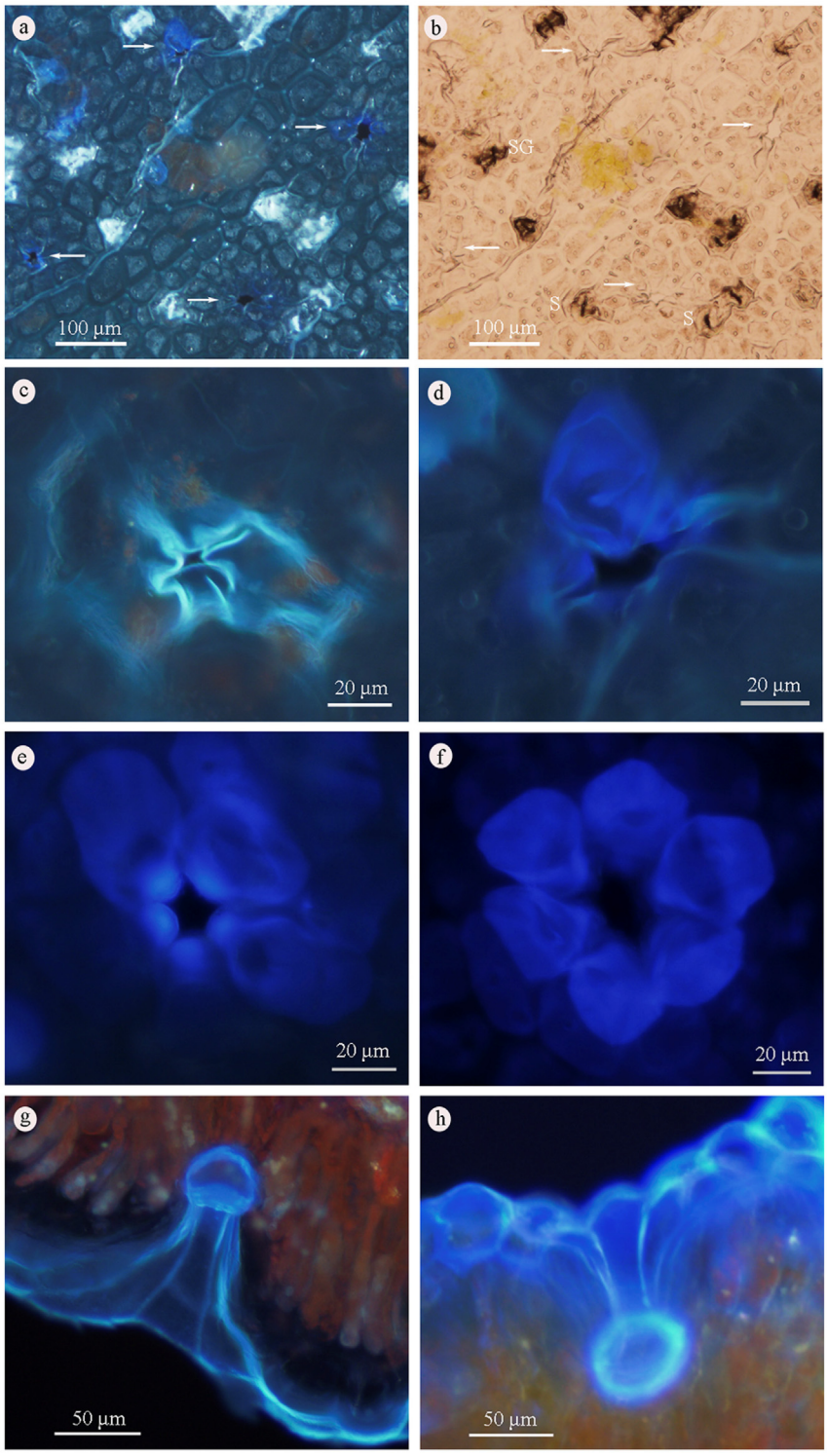

Fig. 6. Change processes of morphology and fluorescence intensity of WASs at different times during fluorescence wetting. a, Wetting at 1 h, and photos at ×100; b, Corresponding photo of a observed under bright field; c, WAS wetting at 0 h (i.e., the control) photos at ×400; d, WAS wetting at 1 h photos at ×400; e, WAS wetting at 2 h, photos at ×400; f, WAS wetting at 3 h, photos at ×400; g, The cross-section of WAS wetting at 1 h; h, The cross-section of WAS wetting at 3. Arrows point to the sites of WAS.

leaves from the different branches treated with the same processing emitted different intensities of fluorescence. Sometimes even the fluorescence intensity of the same older or tender leaves from the same sites of a branch was not consistent. Thus, Fig. 6 represents the majority of cases but does not represent all cases or special cases.

Few previous papers have addressed the WASs of leaves, mainly in species of the Bromeliaceae, as reported by Haberlandt (1914) and by Dolzman (1964, 1965). The Bromeliaceae are a group of epiphytes and xerophytes widely distributed in tropical America and the West Indies, possessing vestigial roots and almost completely dependent upon rain falling on their leaves to maintain

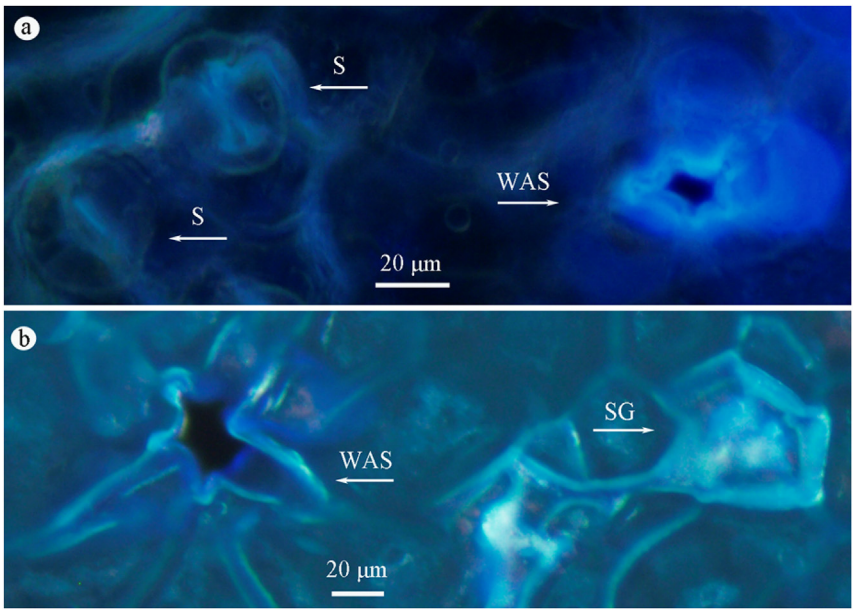

Fig. 7. Comparison of the fluorescence colors of S, SG and WASs during fluorescence wetting. a, Comparison of S and WASs at wetting 2 h; b, Comparison of SG and WASs at wetting 1 h. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

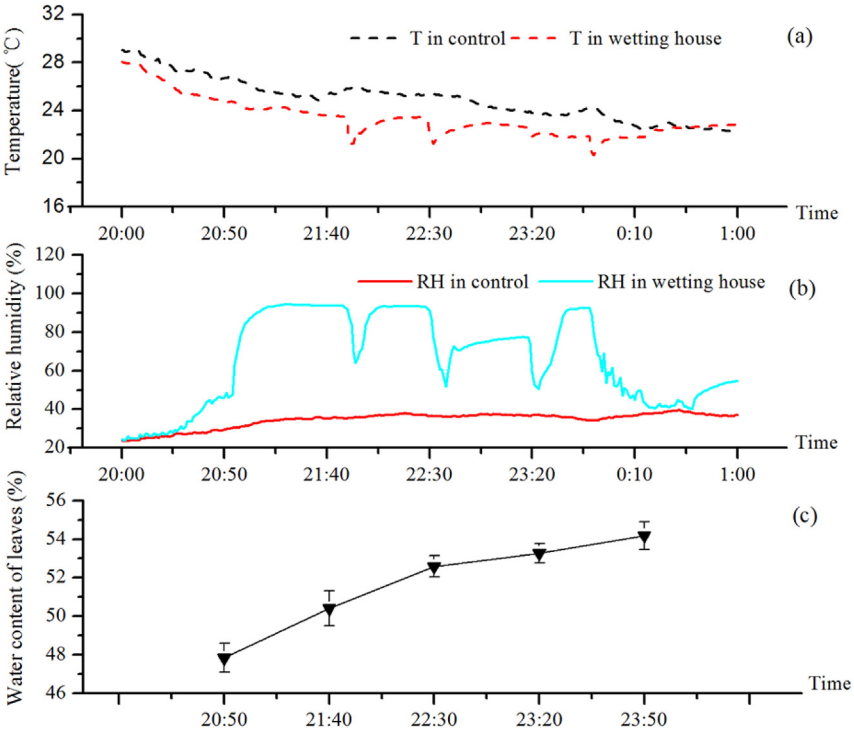

Fig. 8. Variation of leaf water content with changes in the relative humidity and temperature when fluorescence wetting. a, The temperature change in the glasshouse and in natural environment; b, The relative humidity change in the glasshouse and in nature environment; c, Changes in leaf water content at different fluorescence wetting time. T in control represented the temperature in natural environment ; T in wetting represented the temperature in the glasshouse. RH in control represented the relative humidity in nature environment; RH in wetting represented the relative humidity in glasshouse.

their water balance. The leaves of *Vriesea psittacina* (Bromeliaceae) are penetrated by a number of pores and that each pore is topped by a complex cellular structure, the whole unit forming an effective water-absorbing area and non-return valve (Martin and Juniper, 1970). When the leaf is dry, the large number of scales on the leaf surface causes the whole leaf to appear white. When the leaf is wet, it appears green. This generalization is consistent with our observations of *R. soongorica*. In the field, when it rained, we found that the leaves of *R. soongorica* became green. If it had not rained for a long time or if the site was very dry, the leaves of *R. soongorica* looked white, which perhaps resulted from the presence of WASs in the leaf epidermis of this species. Dolzman (1964, 1965) observed

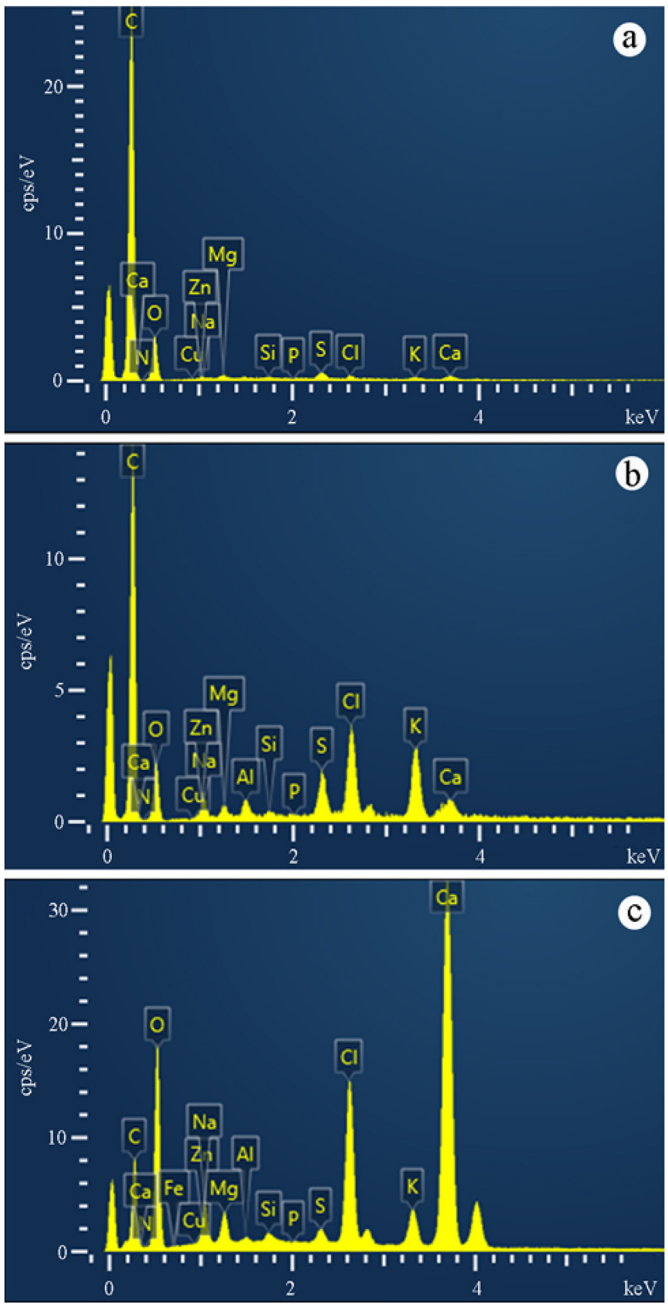

Fig. 9. Compositional differences of WAS, normal EP and SG. a, The components of EP; b, The components of WAS; c, The components of SG.

in Fig. 5. When dry, the cells on the top of the scale flattened and formed a more or less impermeable lid for the valve (Fig. 5c on the left). When wetted, they rapidly absorbed water, swell, raised the lid of the scale and allowed water to flow by capillarity over the cuticle (Fig. 5c on the right and Fig. 6h). These phenomena, observed in WASs in *R. soongorica* in the present study, were also demonstrated previously in species of Bromeliaceae (Haberlandt, 1914). In addition, we observed that the valve cells on the top of the WASs of the leaves of *R. soongorica* have a function similar to that of the dead cells in species of *Tillandsia*: atmospheric water vapor was first captured by the valve cells, then activated all the epidermal cells and was taken into the mesophyll cells (Fig. 6h).

However, we think that unsaturated water absorbed by WASs was not directly transmitted to the mesophyll cells below but was transmitted to the sides of the epidermal cells and then transmitted to the vicinity of the mesophyll cells or through the epidermal cells. Dolzman (1964, 1965) speculated that those highly hydrophilic living cells had an extraordinarily fine structure that might be connected with their absorbing powers and were also connected to each other by large numbers of plasmodesmata, a possible route for the passage of water. This speculation appears correct according to Fig. 6h, as all the leaf epidermal cells around WASs turned green. As the mesophyll cells near the bottom of WASs didn't turn green (Fig. 6h), we resumed that the bottom of the WASs was impermeable to water. This view is inconsistent with Dolzman (1964, 1965), who considered that these structures provided a continuous water-absorbing path between the dead cells above and the epidermal and palisade tissue below.

Based on a comparison of the components of the WASs with those of the normal leaf epidermis of *R. soongorica*, we reviewed that salts on or in the valve cells should help WASs absorb unsaturated water from the air. The layer of thin salt on the valve cells should help WASs first capture water molecules from the atmosphere. In air with high humidity, more and more water molecules are adsorbed on the valves, eventually forming a continuous water pathway between the valve cells and the living cells in the bottom of the WASs.

Water is attracted by deposited aerosols, many of which consist partially or entirely of hygroscopic salts (Burkhardt et al., 2012). Salts suddenly become deliquescent (liquid) at a salt-specific relative humidity (RH), the deliquescence relative humidity (DRH; approximately 33% for KCO_3 and $NaCO_3$ (Schönherr, 2006), 75% for NaCl or NaClO3, 40% for NH4HSO4 and 80% for $(NH_4)_2SO_4$ (Burkhardt et al., 2012)). The salts absorb exponentially more water with further increases in humidity (Pilinis et al., 1989; Zhao et al., 2008; Mauer and Taylor, 2010). The salts in or on the WASs are mainly KCO_3 or $NaCO_3$, NaCl or KCl and Na_2SO_4 or K_2SO_4, whose deliquescence points are less than or equal to 80%. These compounds with water vapor will first lead to capillary condensation

Table 1
Compare the percent content of element gradient of EP, WAS and SG in leaves of *R. soongorica*.

	C	O	Na	Mg	Al	Si	P	S	Cl	K	Ca
EP	83.91	15.54	0.04	0.1	0	0.02	0	0.14	0.07	0.06	0.14
WAS	81.05	15.38	0.39	0.19	0.23	0.05	0	0.44	1.07	0.91	0.29
SG	39.71	44.87	1.79	0.88	0.1	0.13	0.01	0.24	2.95	0.74	8.6

that the base of the WASs in the leaves of *V. psittacina* (Bromeliaceae) was composed of three or four flat, thin-walled, living cells. This structure was also confirmed by our observations of the leaves of *R. soongorica* (Fig. 4b).

The water-absorbing process of WASs in *R. soongorica* is showed (Eiden et al., 1994) and then to the deliquescence of salts (Burkhardt, 2010) and following to form a thin water film connection across WASs on the leaf surface, i.e. “microscopic leaf wetness” (Burkhardt and Hunsche, 2013), which are invisible to the naked eye and can reduce the saturation vapor pressure and

promote condensation, and in species acts as similar to surfactants. Once established, a liquid water connection across the WASs will provide a continuous pathway for the flow of water and solutes, similarly to the function of HAS (hydraulic activation of stomata) on the leaf surface (Burkhardt, 2010). Thus these compounds caused the leaves of *R. soongorica* to absorb atmospheric water at a low humidity, i.e., a humidity less than 80%. As we found in the fluorescence experiment, humidifying only to 55–65% could also cause foliar water uptake in *R. soongorica*.

The salt glands on the leaf surface of *R. soongorica* did not first absorb atmospheric water. We consider that this lack of absorption is caused by the thick layer of crystallized salt on the salt gland (Fig. 2b) because salts secreted by salt glands as well as atmospheric aerosols may cause the coalescence and the formation of salt crusts by deliquescence and subsequent efflorescence of the salts (Burkhardt, 2010). As SG contained higher O (44.87%), Ca (8.6%) and Cl (2.95%), and it also contained a certain amount of Mg (0.88) (Table 1 and Fig. 9.), the salt crusts in or on the SG may be formed some water-insoluble $CaCO_3$ and $MgCO_3$, which typically reduces or delays the water penetrating into mesophyll cell. It is similar to the possibility of particles forming crusts on the leaf surface, which contained both fine particles and coarse particles like cement (Peirce, 1909; Burkhardt et al., 1999), typically reduces the washing efficiency (Burkhardt, 2010).

In fact, the highest amount of K in the WAS could contribute WASs to absorb atmospheric water because K could have a function similar to its function in stomatal guard cells, i.e. an active uptake and subsequently swelling of cells by osmosis caused water uptake (Burkhardt et al., 2001).

This study is the first report that there exist many WASs in the leaf epidermis of desert sub-shrub *R. soongorica*. These WASs can absorb unsaturated water from the air. It is assumed to occur based on the hydration of inorganic salts in or on the valves of WASs as a physical phenomenon, i.e. inorganic salts in or on the valves of WASs can contribute the atmospheric water molecules to condense onto the leaf surface and penetrate them into the leaves of *R. soongorica*. It may be an adaptive strategy for *R. soongorica* to grow in extreme drought environments by using special leaf surface structure WASs to absorb UAW.

Acknowledgments

This study was supported by The National Natural Science Foundation of China (grant no. 31400319) and the Major Program of Heihe River Research Plan of The National Natural Science Foundation of China: The utilization mechanisms and adaptive processing of desert plants to absorb atmospheric water vapor (grant no. 91125025).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jaridenv.2016.01.005.

References

Bai, J., Gong, C.M., Chen, K., Kang, H.M., Wang, G., 2009. Examination of antioxidative system's responses in the different phases of drought stress and during recovery in desert plant. *Reaumuria soongorica* (Pall.) Maxim. J. Plant Biol. 54, 417–425.

Bai, J., Gong, C.M., Wang, C., Kang, H.M., 2010. Antioxidative characteristics of *Reaumuria soongorica* under drought stress. Acta Bot. Boreali-Occident. Sin. 30, 2444–2450.

Breshears, D.D., McDowell, N.G., Goddard, K.L., Dayem, K.E., Martens, S.N., Meyer, C.W., Brown, K.M., 2008. Foliar absorption of intercepted rainfall improves woody plant water status most during drought. Ecology 89, 41–47.

Bruijnzeel, L.A., Waterloo, M.J., Proctor, J., Kuiters, A.T., Kotterink, B., 1993. Hydrological observations in montane rain forests on Gunung Silam, Sabah, Malaysia, with special reference to the Massenerhebung effect. J. Ecol. 81, 145–167.

Burgess, S.S.O., Dawson, T.E., 2004. The contribution of fog to the water relations of Sequoia sempervirens (D. Don): foliar uptake and prevention of dehydration. Plant Cell Environ. 27, 1023–1034.

Burkhardt, J., 2010. Hygroscopic particles on leaves: nutrients or desiccants? Ecological Monographs 80, 369–399.

Burkhardt, J., Hunsche, M., 2013. "Breath figures" on leaf surfaces – formation and effects of microscopic leaf wetness. Front. Plant Sci. 4, 1–9.

Burkhardt, J., Basi, S., Pariyar, S., Hunsche, M., 2012. Stomatal penetration by aqueous solutions – an update involving leaf surface particles. New Phytol. 196, 774–787.

Burkhardt, J., Kaiser, H., Kappen, L., Goldbach, H.E., 2001. The possible role of aerosols on stomatal conductivity for water vapour. Basic Appl. Ecol. 2, 351–364.

Burkhardt, J., Kaiser, H., Goldbach, H., Kappen, L., 1999. Measurements of electrical leaf surface conductance reveal recondensation of transpired water vapour on leaf surfaces. Plant Cell Environ. 22, 189–196.

Chamel, A., Pineri, M., Escoubes, M., 1991. Quantitative determination of water sorption by plant cuticles. Plant Cell Environ. 14, 87–95.

Chinese Soil Taxonomy Cooperative Research Group, Institute of Soil Science, Academic Sinica, 1995. Chinese Soil Taxonomy (Revised Proposal). Chinese Agricultural Science and Technology Press, Beijing China.

Chong, P.F., Li, Y., Su, S.P., 2010b. Gas exchange characteristics of *Reaumuria soongorica* from three geographical populations. J. Northwest Agric. For. Univ. 38, 111–118.

Chong, P.F., Li, Y., Su, S.P., Gao, M., Qiu, Z.J., 2010a. Photosynthetic characteristics and their effect factors of *Reaumuria soongorica* on three geographical populations. Acta Ecol. Sin. 30, 914–922.

Dolzman, P., 1964. Elektronen mikroskopische Untersuchungen an den Sanghaaren von *Tillandsia usneoides* (Bromeliaceae). I. Einige Beobachtungen zur Feinstruktur der Plasmodesmen. Planta 60, 461–472.

Dolzman, P., 1965. Elektronen mikroskopische Untersuchungen an den Sanghaaren von *Tillandsia usneoides* (Bromeliaceae). II. Einige Beobachtungen zur Feinstruktur der Plasmodesmen. Planta 64, 76–80.

Ebner, M., Miranda, T., Nebelsick, A.R., 2011. Efficient fog harvesting by Stipagrostis sabulicola (Namib dune bushman grass). J. Arid Environ. 75, 524–531.

Eichert, T., Burkhardt, J., 2001. Quantification of stomatal uptake of ionic solutes using a new model system. J. Exp. Bot. 52, 771–781.

Eichert, T., Goldbach, H.E., 2008. Equivalent pore radii of hydrophilic foliar uptake routes in stomatous and astomatous leaf surfaces – further evidence for a stomatal pathway. Physiol. Plant. 132, 491–502.

Eichert, T., Goldbach, H.E., Burkhardt, J., 1998. Evidence for the uptake of large anions through stomatal pores. Bot. Acta 111, 461–466.

Eiden, R., Burkhardt, J., Burkhardt, O., 1994. Atmospheric aerosol particles and their role in the formation of dew on the surface of plant leaves. J. Aerosol Sci. 25, 367–376.

Eller, C.B., Lima, A.L., Oliveira, R.S., 2013. Foliar uptake of fog water and transport belowground alleviates drought effects in the cloud forest tree species, *Drimys brasilienis* (Winteraceae). New Phytol. 199, 151–162.

Fahn, A., 1986. Structural and functional properties of trichomes of xeromorphic leaves. Ann. Bot. 57, 631–637.

Grammatikopoulos, G., Manetas, Y., 1994. Direct absorption of water by hairy leaves of *Phlomis fruticosa* & its contribution to drought avoidance. Can. J. Bot. 72, 1805–1811.

Haberlandt, G., 1914. Physiology plant anatomy. Macmillan Lond. 75 (86), 218–274.

Hao, X.M., Li, C., Guo, B., Ma, J.X., Ayup, M., Chen, Z.S., 2012. Dew formation and its long–term in a desert forest ecosystem on the eastern edge of the Taklimakan Desert in China. J. Hydrol. 472, 90–98.

Kerstiens, G., 1996. Cuticular water permeability and its physiological significance. J. Exp. Bot. 47, 1813–1832.

Koch, K., Bhushan, B., Barthlott, W., 2009. Multifunctional surface structures of plants: an inspiration for biomimetics. Prog. Mater. Sci. 54, 137–178.

Lange, O.L., Kilian, E., Ziegler, H., 1986. Water vapor uptake and photosynthesis in lichens: performance differences in species with green and blue-green algae as phycobionts. Oecologia 71, 104–110.

Li, P.G., Zhou, H.Y., Chen, C.Y., Zhao, X., Liu, Y., 2012. Seasonal variation of solute sugars in dominant plant species in Alxa Desert of Northwest. Chin. J. Ecol. 31, 3018–3023.

Limm, E.B., Dawson, T.E., 2010. *Polystichum munitum* (Dryopteridaceae) varies geographically in its capacity to absorb fog water by foliar uptake within the redwood forest ecosystem. Am. J. Bot. 97, 1121–1128.

Limm, E.B., Simonin, K.A., Bothman, A.G., Dawson, T.E., 2009. Foliar water uptake: a common water acquisition strategy for plants of the redwood forest. Oecologia 161, 449–459.

Liu, S., Liu, X.Y., 1996. The estimating model of upper plant weight on *Reaumuria soongorica* semishrub. Arid Zone Res. 13, 36–41.

Liu, Y.B., Wang, G., Liu, J., Zhao, X., Tan, H.J., Li, X.R., 2007a. Anatomical, morphological and metabolic acclimation in the resurrection plant *Reaumuria soongorica* during dehydration and rehydration. J. Arid Environ. 70, 183–194.

Liu, Y.B., Zhang, T.G., Li, X.R., Wang, G., 2007b. Protective mechanism of desiccation tolerance in *Reaumuria soongorica*: leaf abscission and sucrose accumulation in the stem. Sci. China Ser. C-Life Sci. 50, 15–21.

Ma, J.Y., Chen, K., Xia, D.S., Wang, G., Chen, F.H., 2007. Variation in foliar stable

carbon isotope among populations of a desert plant, *Reaumuria soongorica* (Pall.) Maxim. in different environments. J. Arid Environ. 69, 365–374.
Martin, C.E., Willert, D.J.V., 2000. Leaf epidermal hydathodes and the ecophysiological consequences of foliar water uptake in species of Crassula from the Namib desert in Southern Africa. Plant Biol. 2, 229–242.
Martin, J.T., Juniper, B.E., 1970. The Cuticles of Plants. Edward Arnold (Publishers) Ltd., pp. 94–95
Mauer, L.J., Taylor, L.S., 2010. Water–solids interactions: deliquescence. Annu. Rev. Food Sci. Technol. 1, 41–63.
Mooney, H.A., Gulmon, S.L., Ehleringer, J., Rundel, P.W., 1980. Atmospheric water uptake by an Atacama desert shrub. Science 209, 693–694.
Munné-Bosch, S., Nogués, S., Alegre, L., 1999. Diurnal variations of photosynthesis and dew absorption by leaves in two evergreen shrubs growing in Mediterranean field conditions. New Phytol. 144, 109–119.
Nowak, E.J., Martin, C.E., 1997. Physiological and anatomical responses to water deficits in the CAM epiphyte *Tillandsia ionantha* (Bromeliaceae). Int. J. Plant Sci. 158, 818–826.
Ohrui, T., Nobira, H., Sakata, Y., Yamamoto, C., Nishida, K., Yamakawa, T., Sasuga, Y., Yaguchi, Y., Takenaga, H., Tanaka, S., 2007. Foliar trichome- and aquaporin-aided water uptake in a drought-resistant epiphyte *Tillandsia ionantha* Planchon. Planta 227, 47–56.
Oliveira, R.S., Dawson, T.E., Burgess, S.S.O., 2005. Evidence for direct water absorption by the shoot of the desiccation–tolerant plant *Vellozia flavicans* in the savannas of central Brazil. J. Trop. Ecol. 21, 585–588.
Peirce, G.J., 1909. The possible effect of cement dust on plants. Science 30, 652–654.
Pilinis, C., Seinfeld, J.H., Grosjean, D., 1989. Water content of atmospheric aerosols. Atmos. Environ. 23, 1601–1606.
Riederer, M., Schreiber, L., 1995. Waxes-the transport barriers of plant cuticles. In: Hamilton, R.J. (Ed.), Waxes: Chemistry Molecular Biology and Functions. The Oily Press, Dundee Scotland, pp. 131–156.
Schill, R., Barthlott, W., 1973. Kakteendornen als wasserabsorbierende Organe. Naturwissenschaften 60, 202–203.
Schönherr, J., 2000. Calcium chloride penetrates pear leaf cuticles via aqueous pores. Planta 212, 112–118.
Schönherr, J., 2006. Characterization of aqueous pores in plant cuticles and permeation of ionic solutes. J. Exp. Bot. 75, 2471–2491.
Schreiber, L., 2005. Polar paths of diffusion across plant cuticles: new evidence for an old hypothesis. Ann. Bot. 95, 1069–1073.
Shi, Y., Yan, X., Zhao, P.S., Zhao, X., Xiao, H.L., Li, X.R., Chen, G.X., Ma, X.F., 2013. Transcriptomic analysis of a tertiary relict plant, extreme xerophyte *Reaumuria soongorica* to identify genes related to drought adaptation. PLOS ONE 8, 1–11.
Simonin, K.A., Santiago, L.S., Dawson, T.E., 2009. Fog interception by Sequoia sempervirens (D. Don) crowns decouples physiology from soil water deficit. Plant Cell Environ. 32, 882–892.
Stone, E.C., Went, F.W., Young, C.L., 1950. Water absorption from the atmosphere by plants growing in dry soil. Science 111, 546–548.
Sveshnikova, V.M., 1972. Absorption of water vapor by the aboveground parts of the Karakum desert plants (in Russian). Bot. ZH 57, 880–888.
Virzo De Santo, A., Alfani, A., Luca, P.D., 1976. Water vapour uptake from the atmosphere by some *Tillandsia* species. Ann. Bot. 40, 391–394.
Wang, X.H., Zhao, T., Wen, Z.N., Xiao, H.L., Yang, Z.J., Chen, G.X., Zhao, X., 2011. The chromosome number, karyotype and genome size of desert diploid plant *Reaumuria soongorica* (Pall.) Maxim. Plant Cell Rep. 30, 955–964.
Xu, D.H., Li, J.H., Fang, X.W., Wang, G., Su, P.X., 2008. Photosynthetic activity of poikilochlorophyllous desiccation tolerant plant *Reaumuria soongorica* during dehydration and re-hydration. Photosynthetica 46, 547–551.
Xu, D.H., Su, P.X., Zhang, R.Y., Li, H.L., Zhao, L., Wang, G., 2010. Photosynthetic parameters and carbon reserves of a resurrection plant *Reaumuria soongorica* during dehydration and rehydration. Plant Growth Regul. 60, 183–190.
Yan, Q.D., Su, P.X., Gao, S., 2012. Response of photosynthetic characteristic of C3 desert plant *Reaumuria soongorica* and C4 desert plant *Salsola passerina* to different drought degress. J. Desert Res. 32, 364–369.
Zhao, L.J., Wang, F., Zhang, K., Zeng, Q.X., Zhang, Y.H., 2008. Deliquescence and efflorescence processes of aerosol particles studied by in situ FTIR and Raman spectroscopy. Chin. J. Chem. Phys. 21, 1–11.
Zhuang, Y.L., Ratchiffe, S., 2012. Relationship between dew presence and *Bassia dasyphylla* plant growth. J. Arid Land 4, 11–18.