Thermoregulation adaptation of *Rheum tanguticum* stereostructure leaf

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Abstract

Background: Leaf is an important plant organ with great variation in shape and size. Leaf size tends to be smaller in plants thrived in the areas of higher elevation and lower annual mean temperature. The Qinghai-Tibetan Plateau is situated at a high altitude of ~4,000 m with low annual average temperature. Leaves for most plants species in Qinghai-Tibetan Plateau are small, however, *Rheum tanguticum* with large leaves is an exception. Here we show that large leaf of *R. tanguticum* with three-dimensional structure is an ideal solution of thermoregulation with little energy consumption.

Results: With increase in age, leaves of *R. tanguticum* transit from a small oval plane to a large palmatipartite stereostructure. Furthermore, temperature variation of different parts is a distinct character in stereostructure leaves of *R. tanguticum*. The temperature of regular leaves under strong solar radiation could be much higher than the ambient temperature. However, the stereostructure leaf could lower leaf temperature by avoiding the direct solar radiation and promoting local airflow to prevent serious scorch.

Conclusions: Our results demonstrate that the robust three-dimensional leaf structure is a formation for *R. tanguticum* to adapt to the strong solar radiation and low temperature in Qinghai-Tibetan Plateau through modulation of local temperature in different parts of the large leaves.

Background

Leaf is an essential photosynthetic organ of all higher plants, and the shape and size of leaf are of great variation. Leaf size varies by over 100,000-fold among plant species worldwide [1]. Leaf area is the most common measure of leaf size. Across the plant kingdom, leaves vary from less than 1 mm² to greater than 1 m² in area [2]. In general, leaf size tends to decrease with higher elevation, lower annual mean temperature and precipitation, and lower soil fertility [3]. In other words, large-leaved species are predominately found in wet, hot, sunny environments; whereas small-leaved species are typically thrived in hot, sunny environments in arid conditions, as well as in high latitudes and elevations [1, 4].

Like leaf size, leaf shape is highly variable between plant species and individuals. However, leaf shape variation has been proven to be more difficult to explain by environmental factors [3]. It is generally assumed that because photosynthetic leaves are critical to plant growth and survival, variation in leaf shape reflects natural selection operating on function [4]. Leaf shape has been shown to vary with plant ontogeny [5, 6], light quality and quantity, ambient temperature, and CO₂ levels. Koyama et al [5] reported that the growth trajectory of *Cardiocrinum cordatum* plant leaves obeyed a dynamic scaling law at the whole-plant level. These patterns have led to the development of an assortment of hypotheses for the adaptive values of leaf shape and its plasticity [7].

The Qinghai-Tibetan Plateau (QTP) is the highest and largest plateau in the world. It is characterized by distinct features such as high altitude, low atmospheric pressure, low air temperature, short growing season, high irradiance, strong winds, and diurnal temperature fluctuations [8, 9]. Maximum viable leaf sizes were shown to be especially small in cold and high elevation regions (such as QTP) [1]. Plants with

large leaves might be more prone to damages by the high temperature under high irradiance [10]. Leaves for most plants in QTP are small, however, *Rheum tanguticum* is an exception. The average plant height of *R. tanguticum* (over 5-year old plants) is about 1.5–2 m tall (Fig. 1a). The basal leaves are orbicular or broadly ovate, large, 30–80 cm long, and deeply palmately 5-lobed [11].

Rheum tanguticum Maxim. ex Balf. (Polygonaceae), is a perennial herb, endangered and endemic to China [12]. The dried rhizomes and roots of *R. tanguticum* are important traditional Chinese medicines. It is mainly distributed in Qinghai and Gansu Provinces and east Tibetan Autonomous Region at altitudes ranging from 2,300 m to 4,200 m and can be found on margins of forests, in valleys or shrubs [11].

Heteroblasty is a phenomenon that results from the temporal development of the shoot apical meristem, creating successive changes in the traits of the lateral organs it produces at each node, including leaf shape [13]. *R. tanguticum* species exhibit dramatic heteroblastic changes in leaf shape (Fig. 1b). The leaves of 1 to 2-year old *R. tanguticum* are small and ovate. The leaves of over 3-year old plants transition to large and palmatilobate. In this report, we study *R. tanguticum* leaf morphology by addressing an important question: how could *R. tanguticum* with large leaves adapt to the strong solar radiation and low temperature in QTP, and what is the underlying adaptive mechanism?

Results

Variation in size and shape among leaves with different ages

The *R. tanguticum* leaves displayed highly significant ontogenetic variations in size and shape (Fig. 1b, Table 1 and Additional file 1: Table S1). The average length and width of leaves in individuals over 5-year old were more than ten times (10.85 and 13.83 respectively) of that in individuals of 1 to 2-year old (Table 1). The average leaf area of over 5-year old plants was about 105 times of that of 1 to 2-year old plants. The average dry leaf mass of over 5-year old plants was nearly 400 times of that of 1 to 2-year old plants (Table 1). For a comparison, the variation in leaf size across ontogeny in *R. tanguticum* showed a similar pattern to the result of *C. cordatum* [6]. According to Wright's [1] prediction, the maximum viable leaf size in QTP was less than 100 cm². However, the largest leaf area of *R. tanguticum* we measured was 3,116 cm². As a result, the leaves of over 5-year old *R. tanguticum* plants can be considered as one of the largest in QTP.

In the juvenile to adult growth transition, leaves of *R. tanguticum* changed from small and ovate to large and palmatipartite (Fig. 1b and Table 1). There was only one leaf plane for 1 to 2-year old plants. In contrast, each leaf of over 5-year old plants had more than ten planes, because the veins had different angles relative to the main plane. In addition, the leaf shape of over 5-year old plants was a three dimensional structure (Fig. 1c and Additional file 3: Figure S2). The angle between the plane of the first main vein and second vein was 30.60±2.44°, whereas the angle between the plane of the first main vein and third vein was 64.20±4.43° (Fig. 1d).

In over 5-year old plants, the ratio of BC to AB+AC was 0.5033 ± 0.0091 , which meant only 50.33% of the sunlight could be absorbed by *R. tanguticum* leaf compared to the same leaf area completely parallel to the ground. The ratio of EF to DE+DF was 0.4977 ± 0.0070 . In 3 to 4-year old plants, the ratio of BC to AB+AC was 0.6779 ± 0.0087 . The 1 to 2-year plants' entire leaf was on the same plane, so the ratio was about 1. With the growth of *R. tanguticum*, the ratios of BC to AB+AC and EF to DE+DF decreased rapidly. In over 5-year old plants, the intersection angle between the blades around the midrib (α) was $60.51 \pm 1.22^\circ$, while the intersection angle between the secondary vein (β) was $59.65 \pm 0.93^\circ$. For 1 to 2-year old plants, the blades between the veins were on the same plane, which indicated the intersection angle was nearly 180.00° . The 3-D leaf structure of 3 to 4-year old plants fell in between the two types mentioned above, with an intersection angle of $85.44 \pm 1.38^\circ$.

Leaf temperature variation

There was significant temperature difference between the two longitudinal halves of the leaf in the tip but not in the middle or basal area (Additional file 4: Figure S3 and Additional file 1: Table S2). The greatest temperature difference at tip was 8.30°C , to the middle and base was 8.10°C and 3.70°C , respectively (Additional file 4: Figure S3). The temperature variation in different parts of *R. tanguticum* leaves could be a result of local airflow in a small scale around the leaves, which could lower the temperature in the warmer part of the leaves.

When the ambient temperature was 14.60°C , the average temperature of sun-exposed part of the leaves was about 24.03°C , while the shaded part was only 17.07°C (Additional file 5: Figure S4). The temperature difference between sun-exposed and shaded parts of leaves was 6.95°C on average, in which 70.00% (21/30) was more than 5.00°C , and 16.67% (5/30) was more than 10.00°C . In total, 10% (3/30) of sun-exposed part of the large leaves reached above 30.00°C . The temperature difference between sun-exposed part of the leaves and ambient temperature was 9.43°C on average, among which 43.33% (13/30) was greater than 10.00°C . When the ambient temperature was 17.80°C , the results were similar to that the ambient temperature of 14.60°C (Additional file 5: Figure S4), and the highest leaf temperature was 35.60°C .

From the thermal imaging of *R. tanguticum* leaf, temperature variation of sun-exposed part and shaded part was obvious (Fig. 2 and Additional file 1: Table S2). When the ambient temperature was 16.00°C , the highest average temperature was $29.27 \pm 0.28^\circ\text{C}$, and the lowest average temperature was $15.91 \pm 0.20^\circ\text{C}$. There were significant differences between the highest temperatures and the lowest temperatures (Additional file 1: Table S2). The difference between the highest temperature and the lowest temperature was $13.36 \pm 0.25^\circ\text{C}$ on average, of which 4.67% (7/150) was less than 10.00°C and 4.67% (7/150) was more than 20.00°C .

Discussion

In general, the leaves of most plants in QTP are small to adapt to the high altitude and dry-cold habitat [1, 9], whereas *R. tanguticum* is an exception. The evolved changes in leaf shape and structure have provided an ideal solution to its adaptive advantage.

In the *R. tanguticum* adult leaves, five lateral veins are produced from the petiole, and the midrib is an extension of the petiole. Lateral veins are inclined above the blade resulting in certain angles between the lateral veins and the plane of the extended midrib. The five veins are not in the same plane, which is the basis for the stereostructure of the leaf blade (Fig. 1d). The most dramatic change of the leaf shape has been the change of a single leaf plane to a three-dimensional structure (Fig. 1c and Additional file 3: Figure S2). In the juvenile to adult transition, the leaf undergoes a transformation from a small oval plane to a giant palmatipartite stereostructure. Due to the 5-vein stereostructure, the whole leaf appears to be divided into more than ten different zones. Because the palmatipartite leaves resemble chicken feet, one of the Chinese common names of this species is “Chicken feet Rheum”. To our knowledge, although this is not the first observation for the three-dimensional structure of Rheum leaves, this has been the first systematic study to explore the underlying mechanism for the adaptive change of *R. tanguticum* leaves.

The leaf size and shape of the same individual varied noticeably along with the life cycle in some plant species is termed as heteroblasty [13, 14]. Heteroblasty usually means leaf morphology change in the same plant; in *R. tanguticum*, it includes both morphological and structural changes. Therefore, *R. tanguticum* is a typical example of heteroblasty, which can be called isomerized heteroblasty.

The stereostructure of *R. tanguticum* appears to be the main reason for the leaf temperature variation. Because of the high altitude and strong solar radiation in QTP [8, 9], the temperature of the large leaves rises quickly under the sun, but more slowly or even decreases in the shade (Fig. 2 and Additional file 5: Figure S4). The highest leaf temperature in our study was 38.00°C, which was 22.00°C higher than ambient temperature of 16.00°C. Previous studies [15–17] showed that broad leaves reached up to 20.00°C above ambient temperature. According to the thermal imaging in our study, the average highest temperature of *R. tanguticum* leaf was up to about 29.27°C, which was 13.27°C above ambient temperature (16.00°C). The heterogeneity of sun exposure on the large leaves is the reason of leaf-to-air temperature differences, rather than the result [1]. In the area of QTP with low temperature, the locally elevated leaf temperature could increase the enzymatic activity and therefore improve the photosynthetic efficiency. With the short growing season, the rapid accumulation of metabolites promotes *R. tanguticum* to grow taller with large leaves. Local leaf temperature could rise to higher than 30.00°C, therefore the photosynthesis efficiency in this part of the *R. tanguticum* leaf could reach the level of tropical plants.

Temperature plays one of the key roles in plant growth. Generally, ambient temperature is used to estimate the growth rate or biomass in most plants species when leaf temperature is close to ambient temperature. However, in our study, there were large leaf-to-air temperature differences between *R. tanguticum* leaves and the ambient (Fig. 2). So if ambient temperature were used to estimate the growth rate of *R. tanguticum*, there would be a large bias. According to the Van't Hoff relationship for monomolecular reactions, variation of leaf temperature for more than 10°C could change the

photosynthesis efficiency for more than two folds. Hence it is more accurate to use leaf temperature rather than ambient air temperature to estimate photosynthesis efficiency of plants with large leaves [18]. The leaf-to-air temperature differences could also explain another outstanding question as why C_4 plants can thrive in QTP or other cold environments [19].

Under the strong light, orbiculate leaves get heated up rapidly if the size of the leaves is large enough. The temperature becomes especially high at the edges of the orbiculate leaves. Consequently, the orbiculate leaves are easily damaged by scorch at the edges of the leaves [10]. However, for large leaves of *R. tanguticum*, the scorched area was usually in the internal part, but not at the leaf edges (Fig. 3). In addition, the scorches of *R. tanguticum* leaf were small patches rather than continuous large regions (Fig. 3). The scorching was inevitable, yet it would not cause fatal damage to the plants. For *R. tanguticum*, the stereostructure of leaf plays an important role in preventing serious leaf scorching. More work is needed in the future to determine if leaf structure (on an anatomical level) of *R. tanguticum* also contributes to the resistance of sun scorching.

In the ecosystem of QTP, even the small leaves of 1 to 2-year old *R. tanguticum* plants are larger than other plants (Fig. 1b and Table 1); hence they have the risk of sun scorch (Fig. 3). Therefore, in order to adapt to the strong solar radiation of plateau ecosystem, *R. tanguticum* has evolved to have changes in the leaf shape and structure during the long period of evolution.

In the stereostructure leaves, there are three possible mechanisms to regulate the leaf temperature. First, compared to the plane leaf with the same leaf area, there is less solar radiation on the stereo-plamati-partite leaf of *R. tanguticum* (about 50% decrease in plants more than 5-year old), and this would prevent the leaf temperature rising too fast or too high. There are angles between the blades and the midrib (Fig. 1c). The older the *R. tanguticum* plants, the larger the leaves, however, the intersection angles between the blades and the midrib also become smaller, hence more apparent of the stereostructure of the leaf blade. This represents a permanent partial leaf fold without energy expenditure, compared to the temporary leaf fold of some plants exposed to high light [20]. Second, temperature variation facilitates the formation of local airflow in the stereostructure leaf. Some part of the leaf is in the sunlight, while other part is in the shadow of the leaf itself (self-shading, Fig. 2) or other leaf. Because the light exposure is opposite in the bilateral sides of the veins, it creates a greater temperature difference, which is in favor of the formation of air circulation around the leaf (Additional file 6: airflow indicated by smoke in the leaf). Third, deep lobing may also reduce leaf temperature. Vogel [17] showed that deep lobing not only improved heat transfer, but notably reduced its dependence on orientation. In addition, it was found that pinnately compound leaves dissipate heat more effectively than simple ones [21].

Transpiration can decrease the leaf temperature, but the process requires energy and water. Besides transpiration, plants use various ways to decrease leaf temperature, especially in dry environments [22]. Leaf can also prevent or decrease scorch by physical structure such as pubescence or even by leaf movement [20, 23], but these processes require energy input. By contrast, *R. tanguticum* takes advantage of leaf physical structure change for thermoregulation. Thus, this mechanism employs the external force

of the infrastructure in exchange of the consumption of energy and water. This may be another reason why *R. tanguticum* has large leaves but adapts well in QTP.

Conclusion

In the juvenile to adult transition of *R. tanguticum*, the leaf has changed from a small oval plane to a giant palmatipartite stereostructure. The stereostructure of *R. tanguticum* is likely the main reason for the leaf temperature variation. The temperature of *R. tanguticum* large leaves is much higher than ambient temperature, which solves the problem of low ambient temperature in Qinghai-Tibetan Plateau. Stereostructure of leaf ensures the leaf temperature staying in a physiological range. Large leaf of *R. tanguticum* with three-dimensional structure is an ideal solution of thermoregulation with little energy consumption. This also explains the relative rarity of giant-leafed species globally [10] because most plants in nature do not have stereostructural leaves. The stereostructure of leaves plays an important role in thermoregulation of plants, and could be a new way to improve leaf area and mass in large simple leaf species, which often suffer from sun scorch easily.

Methods

Study site and plant materials

The study was conducted at the Dawu, Maqin county, Golog Prefecture, Qinghai Province (34°25'50" N, 100°17'3" E, and elevation 3,762 m). This region was at eastern Qinghai-Tibetan Plateau, with an annual mean temperature of 0.64°C and an annual mean precipitation of 561.16 mm. More than 85% of annual precipitation occurred from May to September [24]. The maximum monthly mean temperature was 9.80–12.00°C from 2005–2009 which usually appears in July or August [24]. The annual average sunlight is approximately 2,576 h, and $\geq 0^{\circ}\text{C}$ annual cumulative temperature 914.3°C [25].

According to regulations of the People's Republic of China on wild plants protection, permits are required only for the species included in the list of state-protected plant species. *R. tanguticum* is not on the list of state-protected plant species [26] (Regulations of the People's Republic of China on the protection of wild plants, <http://www.people.com.cn/item/faguiku/zrzyf/U1020.html>). Therefore, no specific permits were required for the described field studies, and the experimental activities had no negative impacts on the land and the environment.

Rheum tanguticum Maxim. ex Balf. plants were identified by Dr. Wenjing Li (Northwest Institute of Plateau Biology, CAS) and voucher specimens (337832) were deposited in the Herbarium of Northwest Institute of Plateau Biology (HNWP), Chinese Academy of Sciences.

Leaf morphology size, leaf area and leaf mass

The sizes of leaves (length and width, Fig. 1b) were measured from 30 individuals of 1 to 2-year old, 3 to 4-year old and over 5-year old plants, respectively using a flexible rule (1 mm). Leaves of 1 to 2-year old plants were scanned to computer by scanner and leaf area was measured using the image analysis software (Image J, National Institutes of Health, Bethesda, MD, USA). Leaves of more than 5-year old plants were large and three-dimensional, and they could not be scanned to computer. Therefore, they were cut and spliced to rectangular and then measured (Additional file 2: Figure S1). Dry mass (oven-drying for 72 h at 50.00°C) were measured by analytical balance (BS224S, Germany).

The intersection angle between the blades around veins

The intersection angle between the blades around the midrib (α) and the intersection angle between blades around the secondary veins (β) were shown in Fig. 1c. Thirty undamaged leaves from 3 to 4-year old plants (for α) and thirty undamaged leaves from over 5-year plants (α and β) were selected. The length of AB, AC, BC (for 3 to 4-year old plants and over 5-year old plants) and DE, DF, EF (over 5-year plants) were measured by a ruler (1 mm) (Fig. 1c). According to the law of cosines, $\cos\alpha = (AB^2 + AC^2 - BC^2) / 2AB \times AC$; $\cos\beta = (DE^2 + DF^2 - EF^2) / 2DE \times DF$.

Measurement of the vein angles relative to the main vein plane

There were five primary veins in over 5-year old *R. tanguticum* leaves (Fig. 1d). Five mature, undamaged leaves were randomly selected for the measurement of vein angles. The blades were removed and only the veins were kept. The first main vein (1 in Fig. 1d) was put on a plane, and the angles between the plane and the other two main veins (2 and 3 in Fig. 1d) were measured by a protractor.

Temperature and thermal imaging of *R. tanguticum* leaf

Leaf temperature (0.1°C) was measured with a thermometer (HOB0 UX100–023 Ext Temp/RH, Onset, USA) at six positions (1 cm from the midrib) along the lamina in the direction of the midrib for fifteen leaves from over 5-year old plants (Additional file 3: Figure S2). In addition, temperatures of the sun-exposed part and shaded part of thirty leaves from thirty individual plants were measured.

Seek Thermal CompactPRO (Seek Thermal Inc., USA) with iPhone 6s (0.2 m from the leaf) was used to take thermal imaging of *R. tanguticum* leaf. A total of 150 thermal imagings of *R. tanguticum* leaf were used to record the highest and lowest temperatures of different parts of the same leaf simultaneously. Measurements were made on sunny days from 11:00 am to 4:00 pm in August.

Statistical analysis

One-way ANOVA (Leaf size) and paired-samples T-tests (Temperature variation) were used for the analysis of variance respectively in SPSS (Version 16.0, SPSS Inc., Chicago, IL, USA).

Abbreviations

QTP: Qinghai-Tibetan Plateau;

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data are fully available without restriction.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

WL conceived the project; YL and WL designed the experiment; YH, QQ, HZ, GL and WL performed the experiments; YH, JW, LW and YL analyzed the data; YH, JC and WL wrote the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 Leaf variation among *R. tanguticum* plants at different ages.

	1 to 2-year old	3 to 4-year old	over 5-year old
Size	Small	Median	Large
Shape	Ovate	Palmatilobate	Palmatipartite
Length of leaf (cm)	7.63±0.39	22.02±1.10	82.79±2.67
Width of leaf (cm)	6.25±0.36	19.36±1.23	86.44±2.81
Intersection angle between midrib (°)	180.00	85.44±1.38	60.51±1.22
Intersection angle between second vein (°)	180.00	-	59.65±0.93
Leaf area (cm ²)	25.50±3.50	173.27±15.51	2677.50±247.01
Dry leaf weight (g)	0.09±0.01	2.07±0.27	35.69±6.19

Data are mean±SE.

Figures

Figure 1

Rheum tanguticum. (a) *R. tanguticum* in Qinghai-Tibetan Plateau. (b) Leaves of *R. tanguticum* transition from juvenile to adult. (c) The intersection angle between the blades around the midrib (α) and the intersection angle between blades around the secondary veins (β). (d) Veins of over 5-year old *R. tanguticum* leaf.

Figure 2

Thermal imaging of *R. tanguticum* leaf. (a) thermal imaging and (b) visible imaging.

Figure 3

Scorch of *R. tanguticum* leaves. (a) over 5-year old plants and (b) 1 to 2-year old plants.

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