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A Study to Detect CAM Plants in Mongolia

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Abstract

In order to discover CAM plants from the Mongolian flora, four species, *Orostachys spinosa* (L.) C. A. Mey., *O. malacophylla* (Pall.) Fisch., *O. thyrsiflora* Fisch. and *Sedum aizoon* L. of *Crassulaceae* D.C. family were examined in terms of their leaf anatomy, photosynthesis and transpiration intensity for a 24-hour cycle. Photosynthesis by these plants has been studied using isotope-discriminate analysis ($\delta^{13}C$) and a special method for CAM. Transpiration was measured by the weight-method and leaf anatomy and stomatal movement by microscopy. $^{13}C/^{12}C$ value of *Orostachys spinosa*, *O. thyrsiflora* C_4-like (-13.44‰,-18.10‰), *O. malacophylla*, *Sedum aizoon* C_3-like (-25.03‰,-26.32‰). CAM plant characters are clearly discovered in two species *Orostachys spinosa* and *O. malacophylla.* Although differences in the acidity value cycle of *Sedum aizoon* in terms of a 24-hour cycle was similar to the previous two plants, stomatal movement was commonly closed during night and day showing that we need to conduct more future studies for this species. *Orostachys thyrsiflora* does not have CAM photosynthetic response.

Key words: leaf anatomy, CAM, photosynthesis, transpiration intensity, stomatal movement

Introduction

During the past several years drought occurrence has increased in Mongolia, and drastic changes have taken place in climate, there has been a significant increase detected in desertification and degradation has occurred in pasturelands. For this reason, there is an essential need to study the eco-physiology of succulent and CAM (Crassulaceaen Acid Metabolism) plants, which have the ability to keep the water in their cells, can endure dry, hot conditions and grow in desert areas. These plants are poorly studied in Mongolia.

CAM plants open their stomata at night and close them during the daytime to survive hot and dry conditions. CAM metabolism was first investigated in members of the family C*rassulaceae* D.C. and called Crassulaceaen Acid Metabolism. The *Crassulaceae* Family has 40 genus, 15,000 species and CAM has been recorded in 25 genus: *Adromischus, Aeonium, Bryophyllum, Cotyledon, Crassula, Cremnophila, Diamorpha, Dudleya, Echeveria, Graptopetalum, Greenovia, Hasseanthus, Hylotelephium, Kalanchoe, Lenophyllum, Monanthes, Nananthus, Pachyphytum, Parvisedum, Rochea, Sedum, Sempervivum, Tylecodon, Umbilicus, Villadia* (″http://biodiversity.uno.edu/delta).

Nowadays, an estimated 15-20,000 CAM species are known in 33 families in terrestrial and also in aquatic plants (Black & Osmond, 2003). From those, 14 families grow in the Mongolian flora including *Asteraceae*, *Chenopodiaceae*, *Caryophyllaceae* which are the largest families in Mongolia's flora.

In crassulacean acid metabolism (CAM), the reactions of photosynthesis and CO_2 uptake are temporally separated; CO_2 uptake and fixation take place at night, and decarboxylation and refixation of the internally released CO_2 occur during the day. CAM is an adaptation primarily to minimize the quantity of water that is lost when stomata are opened to permit the entry of CO_2. In CAM plants, the stomata are opened in the cool of the night. CO_2 is fixed as malic acid, which is stored in the vacuole. As malic acid accumulates, the leaf vacuoles acidify in the dark. Upon illumination, the stomata close, and the leaf deacidifies. The malic acid is recovered from the vacuole and undergoes decarboxylation. The CO_2 that is released is prevented from escaping by stomatal closure and is assimilated via the Calvin cycle using photochemically generated ATP and NADPH (Hatch & Boardman, 1981).

Materials

We have selected the following 4 species of *Crassulaceae*. *Orostachys spinosa, O. malacophylla, O. thyrsiflora, Sedum aizoon* (Table 1). They are all leaf succulent plants and the leaf's water content is as follows: *Orostachys spinosa* (96.17%), *O.malacophylla* (95.32%), *O.thyrsiflora* (89.56%), *Sedum aizoon* (91.38%). The plants were growing in their natural habitats. Plants were taken from near Monostoi am, *Orostachys spinosa, O. malacophylla and Sedum aizoon* grow in steep, rocky places on the southern slopes of mountains and forest edge. *O .thyrsiflora* grows in rocky soil in desert-steppe region.

Methods

Our experiments were conducted in 2003 during a field expedition, mainly in forest steppe and semi-desert areas in the following places: Monostoin am (49°19′N, 106 °35′E) of Shariin gol somon, Darkhan uul province and near Jinst Khairhan mountain (44°32′N, 99°15′E) of Shine Jinst somon, Bayankhongor province.

Photosynthetic types were determined from $\delta^{13}C$ fractionation, $\delta^{13}C$ analysis was performed with a United States National Bureau of Standards bicarbonate sample as a standard. Plant photosynthetic tissue samples were field-collected in natural habitats, sun dried and transported to the

Table 1. Morphology, distribution, habitat and utilization of succulents*

Species	Description	Distribution in botanico-geographical regions of Mongolia	Habitat	Utilization
Orostachys spinosa	5-20cm high, leaves in rosette crowded, oblong, apical spine 2-4mm, stem leaves linear to oblanceolate, 1-3cm, acuminate, flowers in raceme or spike, sepals red variegate, anthers yellow. Flowers greenish-yellow, solitary sessile or on very short pedicels.	Khubsugul, Khentei, Khangai, Mongol-Daurian, Middle khalkha, East Mongolia, Khobdo, Mongol Altai, Depression of Great lakes (Uvs Nuur), Valley of Lakes, East Gobi, Gobi Altai	Stony and debris slopes, rocks, waterside pebbles, sayrs	Medicine, food for small cattle
O. malacophylla	10-30cm high, rosette 4-15cm, leaves oblong to elliptic, entire, without apical spine, flowers in raceme, bracts spathulate-ovate, sepals oblong, petals ovate-oblong, 4-6mm long. Flowers yellow.	Khubsugul, Khentei, Khangai, Mongol-Daurian, Great Khingan, Middle khalkha, East Mongolia	Debris and stony slopes of hills and mountains, rocks, dry stone screes, sandy steppes	Food for small cattle
O. thyrsiflora.	10-20cm high, leaves in rosette green, imbricate, linear-lanceolate, 1-2mm, shortly mucronate, stem leaves oblong, 4-7mm, flowers in raceme, sepals triangular-ovate, anthers dark purple. Flowers white or pinkish, by several on elongated peduncles.	Khentei, Khangai, Mongol-Daurian, Great Khingan, Khobdo, Mongolian Altai Middle khalkha, East Mongolia, Valley of Lakes, Gobi Altai, Dzungarian Gobi, Transaltai Gobi	Rocks, debris and stony slopes, screes, waterside sands and pebbles	Food for small cattle
Sedum aizoon	Flowers yellow; rhizome short, mainly thick, irregularly accrecsent, giving rise to solitary or few straight simple and firm, annually dying off flowering stems 15-40 cm high. Leaves lanceolate, sometimes sublinear, somewhat acute, irregularly serrate-dentate, up to 3-5 (7)	Khubsugul, Khentei, Khangai, Mongol-Daurian, Great Khingan, Middle khalkha, East Mongolia, Khobdo, Mongolian Altai, Valley of Lakes, Gobi Altai	Rocks, stony mountain slopes, screes, sandy-pebble river banks in forest and forest steppe belts	Medicine

*(Ulziikhutag, 1985; Gubanov, 1996; Grubov, 2001)

USA for $\delta^{13}C$ analysis. Microscopic studies of leaf anatomy and daily stomatal movement. For detecting CAM plant were determined daily pH, stomatal movement and transpiration intensity. We did our research in the natural habitats, hourly and using fresh undamaged leaves, including three plants with three repeats. We did research usually on sunny, non-rainy and non-windy days.

To detect acidity we used pH indicator (paper) and titrable acid. Results of these studies correspond with each other so we show acidity by pH value. We used fast weight-method (Vicktorov, 1969): first choose an undamaged leaf positioned at mid-plant height. Take the weight just after isolating and after 3 minutes. During this time the leaf should be kept in a natural environment. Then calculate transpiration intensity by the following formula:

$$T_i=(a-d)*20/a$$

T_i – transpiration intensity
a – first weight
d – weight after 3 minutes

We studied stomatal movement by using the pattern method by Molotkovskii (Vicktorov, 1969). First we prepared an ointment by dissolving a photography film in acetone. Then paint the leaf surface with the ointment. When the acetone is steamed this layer becomes solid, revealing the stomatal pattern and we can count and measure stomatal movement by viewing it through a microscope. This method does not influence opening or closure of stomata. To detect stomatal movement of the leaf we counted the stomata three times every two hours during the experiment under the same magnification after which we calculated the percentage ratio of open and closed stomata.

Results

1. $\delta^{13}C$-analysis

As a result of d[13]C analysis *Orostachys spinosa* and *Orosotachys thyrsiflora* were found to have C_4 photosynthesis; *Orostachys malacophylla* and *Sedum aizoon* have C_3 photosynthesis (Table 2).

C_3 plants have $\delta^{13}C$ of -35-(30-25)-20%o; C_4 plants have $\delta^{13}C$ -20-(15-10)-5%o (Taiz Zeiger 1998). CAM plants can have $\delta^{13}C$ values that are intermediate between those of C_3 and C_4 plants. In CAM plants that fix CO_2 at night via PEP carboxylase, $\delta^{13}C$ is similar to that of C_4 plants.

Table 2. $^{13}C/^{12}C$ value of species

Species	$\delta^{13}C$%o	
	C_3-like	C_4- like
Orostachys spinosa		-13.44
Orostachys malacophylla	-25.03	
Orosotachys thyrsiflora		-18.10
Sedum aizoon	-26.32	

However, when some CAM plants are well watered, they switch to C_3 mode by opening their stomata and fixing CO_2 during the day via rubisco. Under these conditions the isotope composition shifts more toward that of C_3 plants. Thus $^{13}C/^{12}C$ values for CAM plants reflect how much carbon is fixed via the C_3 pathway versus the C_4 pathway (Hatch & Boardman, 1981).

1. For detecting CAM plants

We conducted experiments on the following parameters over a 24-hour cycle at 2 and 3 hour intervals to examine whether these plants show CAM photosynthetic responses. The examined parameters are: acidity and pH values, stomatal movement, transpiration intensity.

a. Orostachys spinosa

pH value: pH value for 24-hour cycle was studied at 2-hourly intervals by testing pH value in the leaf samples. The acidity value was relatively higher (pH = 4.5-4.75) during the night from 1am to 9am, and decreased (pH = 5.0-5.5) in the morning 11am, increased in the evening 11pm and reached its maximum value (pH = 4.5) in the morning from 7am to 9am (Fig. 1).

Stomatal movement: Over a 24-hour cycle, it was detected that a stomata was fully closed from 5am in the morning to 5pm in the evening. From 7pm in evening the numbers of opening and closing stomata was equal. From 11pm to 3am, the number of open stomata increased and the stomata

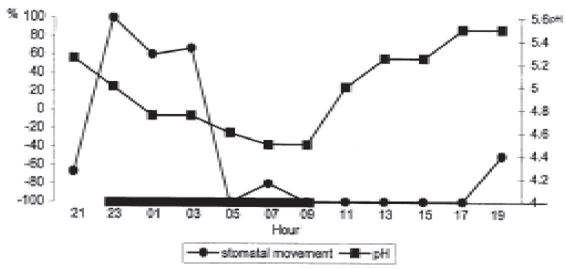

Fig. 1. Daily stomatal movement and pH on *Orostachys spinosa*

was fully opened at 11pm. (Fig. 1).

Transpiration intensity: The cycle of transpiration intensity generally matched the movement of stomata (Fig. 2). The transpiration intensity was highest, 0.34 g/mg per hour, when stomata were fully open in the night 11pm. There was no transpiration when stomata were fully closed at 9am. Although there is a detectable value of transpiration intensity during the daytime i.e. 1-5pm (0.06-0.11 g/mg per hour), it is not stomata transpiration but cuticle transpiration.

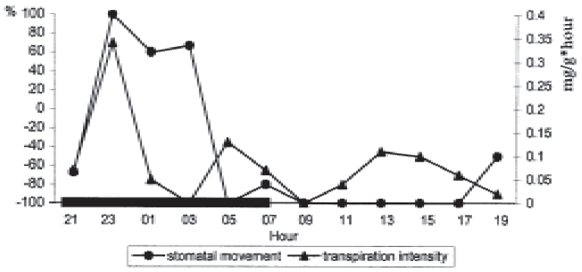

Fig. 2. Daily stomatal movement and transpiration intensity on *Orostachys spinosa*

Since transpiration intensity was relatively small during the daytime, leaf stomata of *Orostachys spinosa* were open during the night, and pH value was higher in the night than during the day, it suggests that this plant has CAM photosynthesis.

b. Orostachys malacophylla

pH value: Acidity value increased (pH = 4.5-5.25) between 1am and 11am in the morning, and decreased (pH =4.7-5.75) from 1pm to 11pm (Fig. 3).

Stomatal movement: The stomata were fully opened at 3am, while 67-100% closed from 5am to 9pm. The number of open stomata increased in the evening 11pm (Fig. 3).

Transpiration intensity: The cycle of transpiration intensity also matches the movement of stomata (Fig. 4). From 11pm to 3am, there was higher transpiration intensity, 0.13 - 0.30 g/mg per hour, while rather low transpiration (0.02-0.08 g/mg per hour) was found during the rest of the time when 80-100% of stomata were closed.

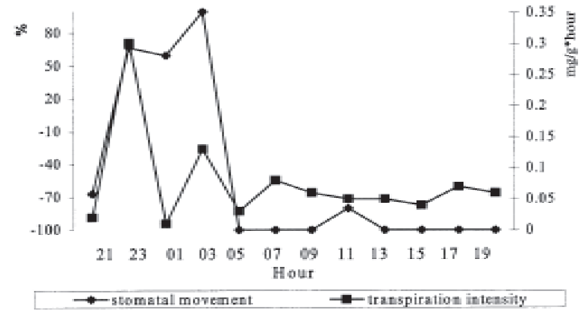

Fig. 4. Daily stomatal movement and transpiration intensity on *Orostachys malacophylla*

The results for *Orostachys malacophylla* were similar to *Orostachys spinosa* suggesting that this plant also has CAM photosynthesis.

c. Orostachys thyrsiflora

pH value: There was an unremarkable difference (pH = 4.5-5.5) between day and night when the cycle of acidity was considered at the 3-hourly interval (Fig. 5).

Stomatal movement: Majority of stomatal cells (67%) were open in the morning 10am; closed numbers were higher (57-75%) during the remaining 24-hours (Fig. 5).

Transpiration intensity: The cycle of transpiration intensity matched the movement of stomata. The transpiration intensity was higher, 0.34 g/mg per hour, at 10am, when more stomata were open. Lower intensity, 0.06-0.11 g/mg per hour, was found during the rest of the time when a large percentage of stomata were closed (Fig. 5).

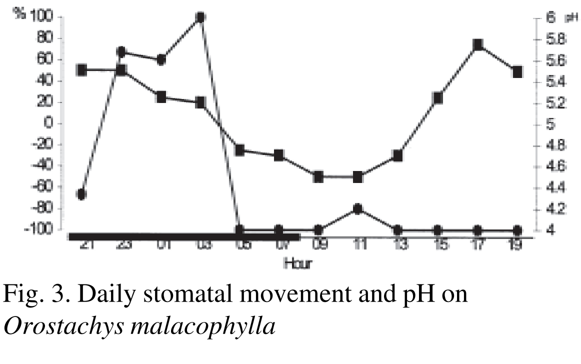

Fig. 3. Daily stomatal movement and pH on *Orostachys malacophylla*

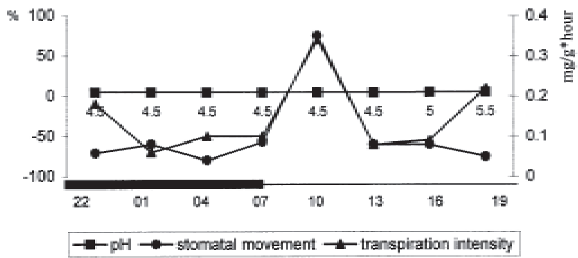

Fig. 5. Daily pH, stomatal movement and transpiration intensity on *Orostachys thyrsiflora*

The result that there was no difference in acid variation during day and night and no open stomata during the night time suggest that *Orosotachys thyrsiflora* does not have CAM photosynthetic response.

d. Sedum aizoon

pH value: Acid value was relatively high (4.75-5.0) from 3am to 9am, but decreased from 11am to 9pm (5.25-5.5) and then increased again at 11pm and 1am (Fig. 6).

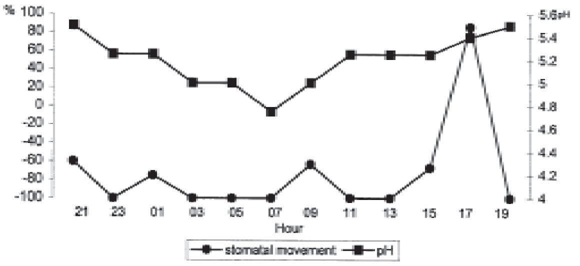

Fig. 6. Daily stomatal movement and pH on *Sedum aizoon*

Stomatal movement: The stomatal cells were more often closed than open apart from at 5pm (Fig. 7).

Transpiration intensity: The transpiration intensity was 0-0.13 g/mg per hour, increases and decreases generally matched the movement of stomatal cells. (Fig. 7).

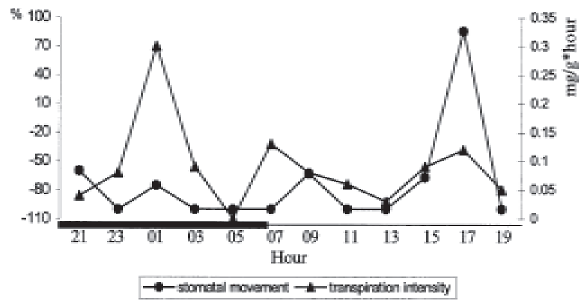

Fig. 7. Daily stomatal movement and transpiration intensity on *Sedum aizoon*

The results for *Sedum aizoon* suggest that it has the same regime as a CAM plant, since the acidity value was high at night and low in the day with the majority of stomatal cells remaining closed for most of the time. Opening and closing times of the stomatal cells corresponded to the increase and decrease of transpiration intensity for this plant.

3. <u>Leaf anatomy</u>

a) Orostachys spinosa (Fig. 8)

Leaf anatomy has a homogenous structure. Mesophyll composed of multi- layered homogenous spongy mesophyll. Anisocytic stomata evenly distributed in the surface of the leaf and located in same level with epidermal cells. Epidermal cells are evenly small, oval in cross section.

b) Orostachys malacophylla (Fig. 9)

Epidermal cell wall slightly undulated and thickened. Stomata are diacytic type.

Stomatal air space is bigger. Cells around the bunds are relatively large and some of them store water.

c) *Orostachys thyrsiflora* (Fig. 10)

Epidermal cell wall thickened unevenly. Stomata are anisocytic type. Air space is smaller. Bunds well-developed and neighboring cells store a water. Mesophyll cells are smaller and composed of more layers. Cross section of leaf is semi-circular

d) *Sedum aizoon* (Fig. 11)

Leaf has homogenous structure. Mesophyll tissue stores water besides photosynthesis. Mesophyll cells are big and have a thin wall. Epidermal cells are big and outer walls are thick. Evenly distributed anisocytic stomata. Bunds are collateral.

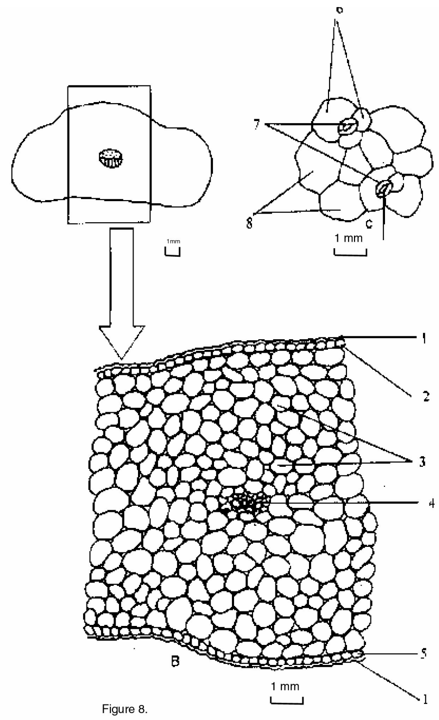

Fig. 8. Leaf anatomy of *Orostachys spinosa* (L.)C.A.Mey. A.Leaf cross section (scheme) B.Leaf cross section (3.2x7(1)) C.Epidermis with stomata (12.5x5(1)) 1.cuticle 2.upper epidermis 3. chlorenchyma 4.bunds 5.lower epidermis 6.subsidiary cells 7.guard cells 8.epidermal cells 9.stomatal pore

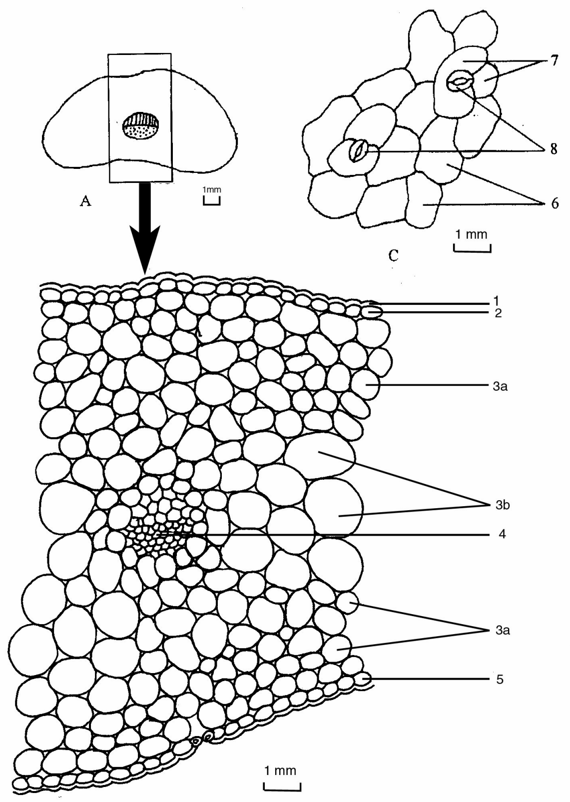

Figure 9.

Fig. 9. Leaf anatomy of *Orostachys malacophylla* (Pall.) Fisch. A.Leaf cross section (scheme) B.Leaf cross section (3.2x7(1)) C.Epidermis with stomata (12.5x5(1)) 1.cuticle 2.upper epidermis 3a. Chlorenchyma 3b.store water cells 4.bunds 5.lower epidermis 6.subsidiary cells 7.guard cells 8.epidermal cells

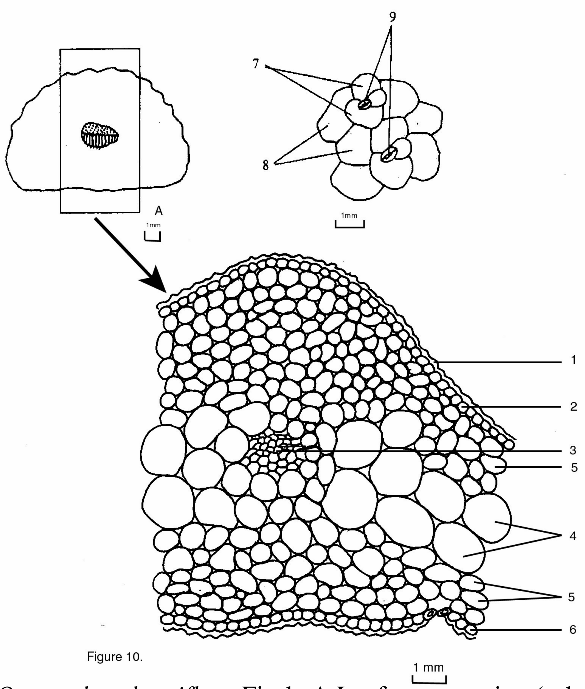

Figure 10.

Fig. 10. Leaf anatomy of *Orostachys thyrsiflora* Fisch. A.Leaf cross section (scheme) B.Leaf cross section (3.2x7(1))C.Epidermis with stomata (12.5x5(1)) 1.cuticle 2.upper epidermis 3.bunds 4.water stored cells 5.chlorenchyma 6.lower epidermis 7.subsidiary cells 8.epidermal cells 9.guard cells

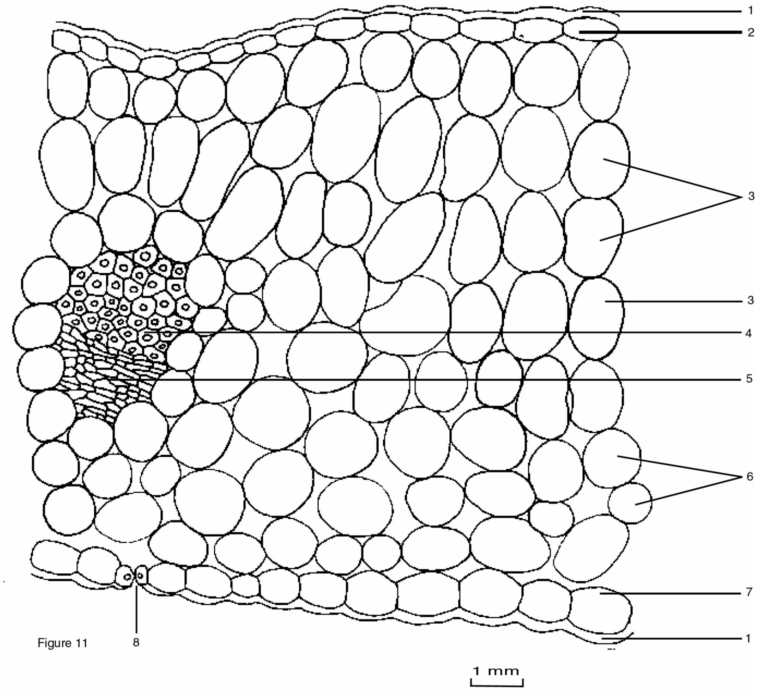

Fig. 11. Leaf anatomy of *Sedum aizoon* L. (12.5x5(0.8)) 1.cuticle 2.upper epidermis 3.water stored cells 4.xylem 5.phloem 6.chlorenchyma 7.lower epidermis 8.guard cells

Discussion

The large group of plants with powerful water conserving types of physiology and biochemistry called Crassulacean Acid Metabolism (CAM) are absent from deserts of Central Asia, but present in other deserts of the world. The deserts of America, Africa and Australia contain large numbers of huge, even tree-like CAM plants such as cactus, Agave, Aloe, and Euphorbs. In fact, for a decade we have searched in Central Asia for CAM plants, but they are absent. (Black *et al*., 2003). However, we discovered CAM in one native Asian genus, *Orostachys* in our experiments. CAM plant characters are clearly discovered in the following two species *Orostachys spinosa* and *O. malacophylla*. The three main characteristics are higher night and lower daytime acidity values, leaf stomata are open during the night, and the higher transpiration intensity at night than during the daytime. Although the cycle of acidity in *Sedum aizoon* was the same as the previous two plant species, the stomatal movement that was commonly closed during both night and day times suggests that we need to conduct further studies on this species.

In contrast to CAM plants, the results for *O. thyrsiflora* which has similar acidity value during day and night, and following the higher number of open cells in the daytime same as other plants, the increases of transpiration intensity in daytime was high, but low at night. However $\delta^{13}C$%o value of *O. thyrsiflora* was similar with C_4 plants but as a result of leaf anatomy *O. thyrsiflora* hasn′t got Kranz anatomy. Therefore we need to conduct further studies on this species. Maybe it is CAM or C_4 plant. Some C_4 species are with and without a Kranz type of leaf anatomy (Pyankov *et al*., 1997).Certainly with so many land and water CAM species, such strong environmental control traits, and being both constitutive and inducible, CAM remains with many useful and informative facets to be discovered! For example, two new types of photosynthesis that are intermediate between CAM and C_4 (Black & Osmond, 2003).

During early $\delta^{13}C$ studies it became clear that CAM photosynthesis was both inducible and constitutive. Numerous species of plants express CAM throughout their autotrophic lifetime. But another difficult problem in CAM photosynthesis is that, with a few plants, the stage of plant development affects CAM expression in green tissues, in combination with environmental stresses. For example, in nature *Mesembryanthenum crystallinum* will express C_3 photosynthesis when young and slowly shift to

CAM as it matures and is water stressed (Black & Osmond, 2003). *Mesembryanthenum crystallinum* is succulent undershrub and grows in Karru′s subdesert of South Africa (Pukareka, 1982).

Prior to this CAM was recorded in *Sedum* genus, but CAM has not been recorded in *Orostachys* genus of *Crassulaceae* family (http://biodiversity.uno.edu/delta). Thus, a new genus (*Orostachys*) added to CAM by our study. Now, *Sedum aizoon* is not evident whether it is CAM or not and we need to conduct further studies on this species.

An unusual night fixation of CO_2 via CAM has been detected that allows these naturally occurring plants to live in cold, higher altitude from 600 to 2900 m asl) and rocky desert environments. We found that CAM occurred in succulent plant genus *Orostachys* in Mongolia, where it is used as food for pastoral livestock and in herbal medicine. Subsequently we found there are about 30 species of *Orostachys* in the world, they are widely adapted to survival and grow in Asian mountain deserts. The species of this genus are in Russia, Tibet, Japan, Korea, China, Mongolia, Siberia, Sakhalin, Kazakhstan and other areas of Central Asia. Also *Orostachys* was cultivated in gardens and houses in USA (Georgia), Germany (Halle), Belgium and Japan.

Only four species of *Orostachys* have been recorded in the Mongolian flora (Gubanov, 1996). They are represented phytogeographically as the North Central Asian rock steppe element, part of which extends into the Siberian taiga and in East Asia. Occasionally *Orostachys spinosa* and *Chamaerhodos altaica* have been found on bare rock and on cobbled and skeletal soils in the eastern Hangai (Hilbig, 1995).

Orostachys spinosa remains green under snow cover during the Mongolian winter. Mongolia has an extreme continental climate with extremes of cold and heat and very low precipitation. The absolute temperatures vary from -50°C in winter to +40°C in summer (Pyankov *et al*., 2000). Therefore, it seems to be the most cold adapted CAM plant known, its special environmental adaptation mechanisms remain to be elucidated. It is one of the important goals of our future study.

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Хураангуй

Монгол орны ургамлын аймгаас CAM ургамал илрүүлэх зорилгоор Зузаалайн овгийн (*Crassulaceae* D.C.*) Orostachys spinosa* (L.) C. A. Mey., *O.malacophylla* (Pall.) Fisch.*, O.thyrsiflora* Fisch.*,* and *Sedum aizoon* L. зэрэг 4 зүйлийн суккулент ургамлын навчны анатоми, фотосинтез, транспирацийн эрчим, амсрын хөдөлгөөний хоногийн явцыг судлав. Тэдгээрийн фотосинтезийг судлахад изотопи дискриминацийн анализ (d^{13}Co%) болон CAM тодорхойлох тусгай арга зүйгээр, транспирацийн эрчмийг жингийн аргаар, навчны анатоми болон амсрын хөдөлгөөнийг микроскопоор тус тус судалсан болно.

Orostachys spinosa (L.) C. A. Mey., *O.thyrsiflora Fisch*. зүйлийн d^{13}Co%-ийн хэмжээ C_4-тэй төсөөтэй (-13.44o%,-18.10o%), *O.malacophylla* Fisch., *Sedum aizoon* 2 зүйлд C_3-тай төсөөтэй (-25.03o%,-26.32o%) байна.

Бидний судалгаагаар *Orostachys spinosa* (L.) C. A. Mey. *O.malacophylla* (Pall.) Fisch. 2 зүйл нь CAM ургамал болох нь тогтоогдсон бөгөөд *Sedum aizoon* L.-ийн хүчиллэгийн хэмжээ нь шөнө ихэсч, өдөр буурч байгаагаараа CAM ургамалтай адил боловч амсрын хөдөлгөөн ба транспирацийн эрчмийн үзүүлэлт нь өөр байгаа учраас түүнийг CAM эсэхийг тодруулах судалгааг дахин нарийвлан судлах нь зүйтэй юм. Харин *Orostachys thyrsiflora* Fisch. зүйлд CAM ургамлын шинжүүд илрээгүй, d^{13}Co%-ийн хэмжээ нь C_4 ургамалтай төсөөтэй боловч түүний навчны анатомийн судалгаагаар Кранц анатоми илрээгүй.

Дээрх судалгаанд авсан 4 зүйлийн ургамлын алинд нь ч навчны Кранц анатоми илрээгүй бөгөөд *Sedum aizoon* L.-ийн мезофилл эд нь фотосинтез явуулахын зэрэгцээгээр ус нөөцлөх эдийн үүрэгтэй байна.

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