

Diurnal Changes of the Titratable Acidity in A New CAM Plant, *Sedum caucasicum* Leaves

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Determination of the photosynthetic pathways of the plants in the studied areas would be very useful for evaluation of the modern vegetation and prediction of changes. Crassulacean acid metabolism (CAM) is a specialized mode of photosynthesis that features nocturnal CO₂ uptake, facilitates increased water-use efficiency. A large variation in CAM has been found within the genus *Sedum*. C₃ species, CAM constitutive species and CAM inducible species under water stress or salinity have been recognized. Diurnal acidity cycle in *Sedum caucasicum* species which is considered endemic in Caucasus occurs similar to that of in CAM plants regardless if they are grown in a greenhouse or naturally. The obtained results confirm that *Sedum caucasicum* is an obligate CAM plant.

Keywords: CAM photosynthesis, *Sedum caucasicum*, titratable acidity, photochemical efficiency

INTRODUCTION

Crassulacean acid metabolism (CAM) is a water-conserving mode of photosynthesis and one of three photosynthetic pathways which occurs in 7% of vascular plant species (Winter and Smith, 1996), many of which dominate the plant biomass of arid, marginal regions of the world. CAM is a modification of the basic C₃ pathway and represents CO₂-concentrating mechanisms that elevate CO₂ around Rubisco and suppress photorespiration. This is achieved in two principal phases separated in time. At night, atmospheric CO₂ is incorporated by phosphoenolpyruvate carboxylase (PEPC) via oxaloacetate into malic acid, which accumulates in the large vacuoles of chloroplast-containing mesophyll cells. During the following light period, malic acid is released from the vacuoles and decarboxylated, and the CO₂ thus liberated is refixed by Rubisco and reduced in the Calvin cycle (Osmond, 1978; Winter & Smith, 1996). Decarboxylation of malate generates high intercellular CO₂ and is associated with stomatal closure, minimizing water loss in the middle of the day when evaporative demand is highest. The net result of CAM is an improvement in water use efficiency generally 6-fold higher than for C₃ plants and 3-fold higher than for C₄ plants under comparable conditions (Nobel 1996).

CAM may operate in different modes: (1) obligate CAM, with high nocturnal acid accumulation (ΔH^+) and CO₂ fixation; (2) facultative or inducible CAM, also known as C₃-CAM, with a C₃ form of CO₂ fixation and nil (ΔH^+) in the non-induced state, and small nocturnal CO₂ fixation and ΔH^+ in the induced state; (c) CAM-cycling, with daytime CO₂

fixation and ΔH^+ but no nocturnal stomatal aperture; and (d) idling, with small ΔH^+ and stomatal closure during the entire day and night in severely stressed plants (Ana Herrera, 2009). On the basis of the magnitude of ΔH^+ obligate CAM, can be strong and weak CAM form (Winter and Holtum, 2002; Silvera et al., 2005; Holtum et al., 2007). In facultative species, CAM may be induced by factors such as drought (Borland and Griffiths, 1990; Herrera et al., 1991; Olivares et al., 1984), salinity (Winter and von Willert, 1972), photoperiod (Brulfert et al., 1988), high photosynthetic photon flux (PPF) (Maxwell, 2002), nitrogen deficiency (Ota, 1988) and phosphorus deficiency (Paul and Cockburn, 1990), among others.

Sedum is a large genus of flowering plants in the family *Crassulaceae*, members of which are commonly known as stonecrops. The genus has been described as containing up to 600 species updated to 470. There are 22 species of the genus *Sedum* in the flora of Azerbaijan. *Sedum caucasicum* (Grossh.) *Hylotelephium caucasicum*, grows on dry stony and limestone slopes in mixed deciduous forests of the Caucasus (Figure 1). The plant is herbaceous, perennial, with the aerial part completely dying for the winter. Roots are thickened, fusiform. Stems are simple, among several, raised, strong, straight, green or dark purple. Due to the arrangement of leaves plants can use solar energy with maximum efficiency.

Work presented in this paper aimed at investigating the presence of CAM in *S. caucasicum*. Investigations involved field and greenhouse studies of diurnal changes in titratable acidity.



Figure 1. *S. caucasicum* plants at anthesis under natural conditions.

MATERIALS AND METHODS

The study site was located at the Lesser Caucasus Mountain (Tovuz region) in the north-west of Azerbaijan (40°59'31.92"N, 45°37'44.04"E) and at the Main Caucasus Mountain (Ismayilli region) in the north-eastern part of Azerbaijan (40°51'11.16" N, 48°23'35.16"E).

Plant material. Mature *Sedum caucasicum* plants were used as plant material.

Titrateable acidity was determined in leaves of *Sedum caucasicum* at different times of. Extracts were prepared by grinding 0.5 gr frozen tissue in distilled water and titrating them to a pH 7.0 end point with 0.01 N NaOH (FJ.Castillo,1996)

Determination of RWC. To obtain the leaf RWC, fresh mass, mass after rehydration (equilibrated at 100% RH, at 4°C in distilled water for 24 h in the dark) and dry mass were determined (Castillo,1996).

RWC was calculated as follows:

$$RWC = \frac{(FW - DW)}{(SW - DW)} \times 100$$

where FW is the fresh weight, DW is the dry weight, and SW is the mass after rehydration.

Fluorescence induction was measured with intact leaves, at ambient temperature with a portable fluorimeter (Mini-PAM fluorometer, WALZ, Germany). Fluorescence parameters were as follows: F_0 , initial fluorescence; F_m , maximum fluorescence, F_v , variable fluorescence ($F_m - F_0$; Bolhar-Norden-kampf et al., 1989). Measured chlorophyll fluorescence parameters included F_v/F_m , and Φ PSII, reflecting the efficiency of PSII antenna and the quantum yield of PSII, respectively.

RESULTS AND DISCUSSION

CAM in *Sedum caucasicum* L. (*Crassulaceae*) was analysed by studying diurnal acidity changes depending on environmental factors both in natural habitat of the plant and in phytotron. The extent of

CAM at these 4 various stages of drought was assessed by measuring the overnight accumulation of titrateable acidity. Diurnal acidity changes were observed under natural as well as controlled condition. The quantity of titrateable acid which was very high in the early morning, decreased during the day and reached a minimum value at 17⁰⁰-18⁰⁰. At the early hours of night (at 22⁰⁰) average acidity was 1/3 of the acidity observed in the morning hours. Total titrateable acidity in flowers and green stems under natural conditions was less than in leaves and it did not change pronouncedly during the day. Despite the fact that, the quantity of titrateable acidity was less in juvenile leaves compared with young and middle-aged leaves of the plants studied under both natural conditions, cyclic changes of acidity occurred in juvenile leaves contrary to flowers and green stems of young and middle-aged ones (Table 1).

Acidity of old leaves, completed their vegetation, was relatively less than that of young and middle-aged leaves. It suggests that the rate of photosynthesis and the intensity of metabolism are positively correlated with the titrateable acidity. The quantity of titrateable acidity in *S. caucasicum* is much less in initial leaves, flowers (mature and immature) and stems compared with leaves and this amount remains unchanged during the day. This shows that CAM photosynthesis does not occur in flowers, stem and initial leaves of *S. caucasicum*. However, quantity of acidity and its diurnal changes have similar patterns in young and middle-aged leaves. This confirms that there is no significant difference in the rate of CAM photosynthesis between these leaves.

The titrateable acidity in different aged leaves of the *S. caucasicum* plant cultivated in controlled conditions was lower in older leaves than in young and middle aged plants (Table 2). Thus, in young and middle aged leaves the quantity of titrateable were $\Delta H^+ = 120.0 \pm 0.2 \mu\text{mol g}^{-1}$ and $\Delta H^+ = 124.0 \pm 0.2 \mu\text{mol g}^{-1}$ respectively, and $\Delta H^+ = 106.0 \pm 0.15 \mu\text{mol g}^{-1}$ in older leaves.

Table 1. Diurnal changes in the titratable acidity in above-ground organs and leaf tiers of *S.caucasicum* under natural conditions.

Plant organs	8 ⁰⁰	12 ⁰⁰	16 ⁰⁰	20 ⁰⁰	24 ⁰⁰
Initial leaf	44.0±0.2	42.0±0.1	38.0±0.2	40.0±0.1	44.0±0.1
1 leaf	114.0±0.1	46.0±0.02	26.0±0.01	20.0±0.2	60.0±0.2
2 leaves	122.0±0.2	76.0±0.02	26.0±0.01	22.0±0.2	70.0±0.3
3 leaves	126.0±0.25	88.0±0.03	24.0±0.01	20.0±0.1	70.0±0.3
4 leaves	130.0±0.3	96.0±0.03	24.0±0.01	20.0±0.2	65.0±0.3
5 leaves	124.0±0.3	88.0±0.03	24.0±0.01	20.0±0.2	60.0±0.3
6 leaves	124.0±0.3	60.0±0.02	22.0±0.01	18.0±0.3	60.0±0.3
Flower	40.0±0.01	38.0±0.01	38.0±0.01	40.0±0.1	40.0±0.1
Stem	36.0±0.01	36.0±0.01	36.0±0.01	40.0±0.1	40.0±0.1

Note: Quantity of titratable acidity is expressed as $\mu\text{mol g}^{-1}$ fresh weight

Table 2. Changes in the titratable acidity in various aged leaves of *S.caucasicum*

	8:00	12:00	16:00	20:00	24:00	04:00
Young aged leaves	134.0±0.3	50.0±0.1	14.0±0.05	14.0±0.05	60.0±0.1	110.0±0.2
Middle aged leaves	140.0±0.4	50.0±0.1	16.0±0.05	16.0±0.05	60.0±0.1	110.0±0.2
Juvenile leaves	126.0±0.3	40.0±0.1	20.0±0.05	20.0±0.05	50.0±0.1	100.0±0.2

Note: Quantity of titratable acidity is expressed as $\mu\text{mol g}^{-1}$ fresh weight.

Table 3. Changes in the titratable acidity in various parts of *S.caucasicum* leaves.

Part of leaf	8 ⁰⁰	12 ⁰⁰	16 ⁰⁰	20 ⁰⁰	24 ⁰⁰	04 ⁰⁰
Top part of the leaf	134.0±2.5	55.0±1.4	14.0±0.9	14.0±0.9	60.0±1.4	110.0±2.2
Middle part of the leaf	134.0±2.4	65.0±1.4	16.0±0.9	16.0±0.9	50.0±1.5	110.0±2.2
Bottom part of the leaf	130.0±2.6	50.0±1.4	20.0±0.9	20.0±0.9	50.0±1.5	110.0±2.2

Note: Quantity of titratable acidity is expressed as $\mu\text{mol g}^{-1}$ fresh weight.

Table 4. Changes in the titratable acidity in different conditions in *S.caucasicum* leaves.

Plant	07 ⁰⁰	11 ⁰⁰	15 ⁰⁰	19 ⁰⁰	23 ⁰⁰	03 ⁰⁰	07 ⁰⁰
20-24°C (Natural conditions)	124.0±2.6	90.0±1.6	20.0±1.2	18.0±1.2	60.0±0.1	116.0±2.2	120.0±2.2
30-32°C (Absheron peninsula)	112.0±1.8	50.0±1.6	16.0±1.2	16.0±1.6	65.0±0.1	128.0±2.6	110.0±1.8

Note: Quantity of titratable acidity is expressed as $\mu\text{mol g}^{-1}$ fresh weight.

The changes in the quantity of titratable acidity were studied in various parts of *S.caucasicum* leaves (Table 3). The total acidity in the different parts of the leaves were approximately the same and dawn was $\Delta\text{H}^+=120.0\pm 0.2 \mu\text{mol g}^{-1}$ and their changes have similar patterns.

The quantity of titratable acidity changed differently in plants grown under natural as well as controlled conditions depending on the environmental parameters. Decline in acidity started at 11:00-12:00 in plants grown under natural conditions (20-24°C). As the temperature of the Absheron peninsula is higher than 30°C in June-July, the decline in the titratable acidity of these plants begins earlier (Table 4).

Dependence of the quantity of titratable acidity on temperature, humidity of the environment and time in the *S.caucasicum* plant under outdoor conditions in June is presented in Table 5.

As seen in the table high environmental temperature has caused a sharp decline in the quantity

of titratable acidity since the early morning hours. Changes in the quantity of the titratable acidity and RWC were also measured in the *S.caucasicum* plants under normal and drought conditions (Figure 2).

Table 5. Changes in the titratable acidity dependence on temperature, humidity of the environment in *S.caucasicum* leaves.

Time, hour	T, °C	RH	Titratable acidity, $\mu\text{mol H}^+\text{g}^{-1}$ fresh weight
07 ⁰⁰	30	45	150.0±2.7
09 ⁰⁰	31	54	128.0±2.6
11 ⁰⁰	33	41	100.0±2.4
13 ⁰⁰	36	44	36.0±0.9
15 ⁰⁰	32	48	24.0±0.8
17 ⁰⁰	30	56	20.0±0.8
19 ⁰⁰	27	63	36.0±0.8
21 ⁰⁰	27	65	56.0±0.9
23 ⁰⁰	26	70	64.0±0.9
01 ⁰⁰	25	73	84.0±0.9
03 ⁰⁰	24	72	98.0±0.9
05 ⁰⁰	24	61	170.0±2.9

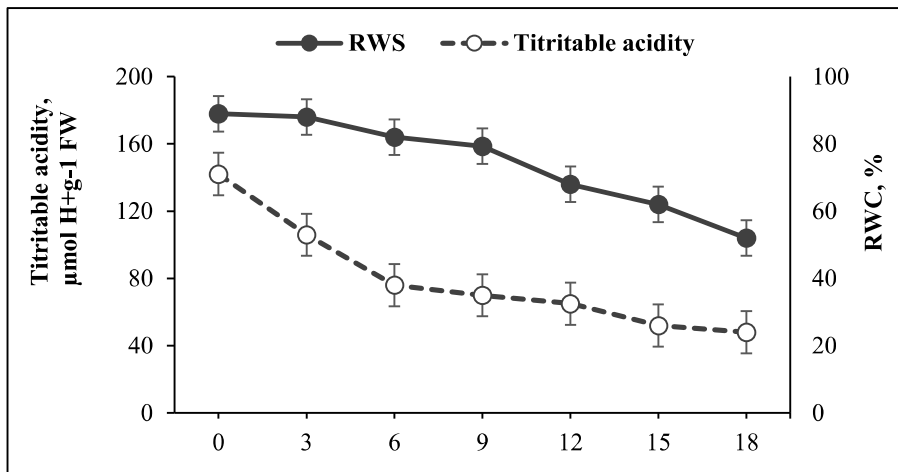


Figure 2. Changes in RWC and titratable acidity depending on the duration of the drought stress, in mature leaves of the *S. caucasicum* plants during early morning hours.

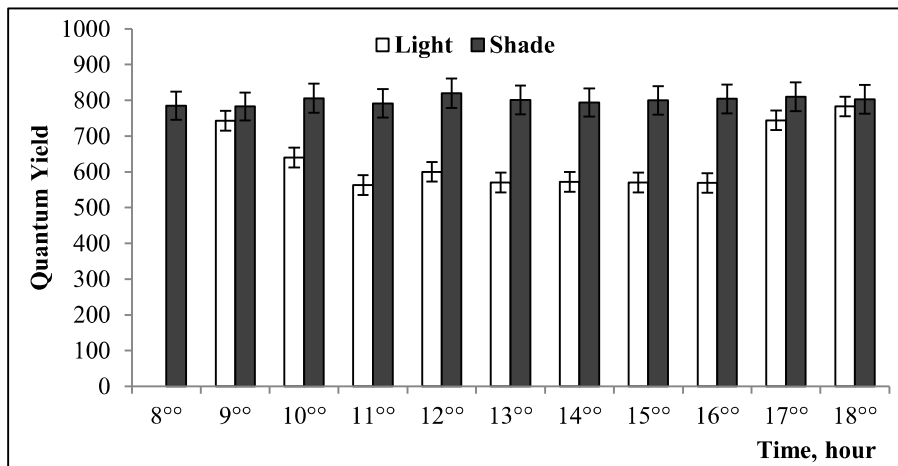


Figure 3. Changes in maximum photochemical efficiency of photosystem II in the leaves of *Sedum caucasicum* under shaded and lighted conditions.

As seen in the figure the quantity of the titratable acidity in *S. caucasicum* leaves decreased with increasing duration of the drought stress. Negative correlation is observed between the quantity of titratable acidity in the plants subjected to drought and relative water content in leaves. The value of lowering ΔH^+ is characteristic of CAM plants under severe water stress (Lee and Griffiths, 1987). CAM plant *S. dasyphyllum* shows, under controlled conditions, a decrease of malate accumulation as relative water content decreases. Recovery from water stress is fairly slow. Water potential quickly increases during rewatering and exceeds the original value after few days, suggesting a consumption of osmotic compounds during the water stress period (A. Fioretto et al., 1990).

PSII is known to be sensitive to the harmful environmental effects. The F_v/F_m ratio is a parameter that allows the detection of any damage to the photosystem II (PS II) and the possible existence of photoinhibition (Long et al. 1994). Diurnal changes in the photochemical activity of PS II depending on the environmental temperature and the intensity of solar radiation were measured in leaves of *S. caucasicum* under outdoor conditions (under

shaded and lighted conditions) in the Absheron peninsula in July (Figure 3).

According to the obtained results the values of the photochemical efficiency of photosystem II (F_v/F_m) in the leaves of *Sedum caucasicum* under shade condition were very close and almost unchanged. However, in plants grown under natural light these values decreased till afternoon with increasing light intensity and then remained stable. Previous values recovered with the decreasing light intensity by the evening. It suggests that the increasing light intensity causes damage to PSII, which leads to photoinhibition. But in accordance with the decreased light intensity and environmental temperature by the evening, this value became equal to the values obtained in the morning. This confirms that recovery process is faster in CAM plants and their plasticity is at the high level.

Phase III of CAM takes place under closed stomata and high irradiance, and PSII can become over-energized (Niewiadomska & Borland, 2008; Lüttge, 2010). Under such conditions, plants evoke photoprotection by non-radiative excess energy dissipation via xanthophyll cycle (Horton & Ruban, 2005; Murchie & Niyogi, 2011). Uptake, and diurnal

acidity changes sustained by nocturnal re-fixation of respiratory CO₂ (Sayed 2001b; Lüttge, 2010). Comparison of chlorophyll fluorescence parameters measured in *E. triaculeata* during wet and dry seasons indicated reduction of Fv/Fm and ΦPSII denoting reduced efficiency of PSII antenna and PSII quantum yield, respectively (Baker, 2008). Similar reduction of PSII activity manifested by reduction of Fv/Fm and PSII was reported for other CAM plants under stress conditions (Mattos et al., 1999).

Determination of titratable acidity both natural and controlled conditions revealed diurnal oscillation of acidification-deacidification cycles reflecting operation of obligate CAM. Nocturnal CO₂ uptake and daytime stomatal closure of CAM implies avoidance of gas exchange when environmental conditions favour transpirational water loss and improved plant water economy (Lüttge, 2008). The obtained results confirm that *Sedum caucasicum* is an obligate CAM plant.

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Yeni CAM Növü *Sedum caucasicum* Bitkisinin Yarpaqlarında Sutkalıq Titirlənən Turşuluğun Dəyişməsi

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Bitkilərdə fotosintetik yolların öyrənilməsi müasir bitki örtüsünün qiymətləndirilməsi və baş verə biləcək dəyişikliklərin proqnozlaşdırılması üçün çox əhəmiyyətlidir. Krassuliyasiya turşuları metabolizmi (CAM), gecə ərzində CO₂ qazının fiksasiyasına əsaslanan və sudan effektiv istifadənin artması ilə səciyyələnən karbonun fotosintetik assimilyasiya yollarından biridir. *Sedum* cinsinin növləri arasında CAM fotosintezin müxtəlif formalarına təsadüf olunur. Buraya C₃, CAM və fakültativ CAM növlər daxildir. Qafqaz üçün endemik olan *Sedum caucasicum* bitkisinin sutkalıq titirlənən turşuluğun dəyişməsi həm təbii şəraitdə, həm də süni iqlim şəraitində bitən bitkilərdə oxşar olmuşdur. Alınan nəticələr belə guman etməyə əsas verir ki, *S.caucasicum* bitkisinin CO₂ qazının fiksasiyası fotosintezin CAM yolu ilə baş verir.

Açar sözlər: CAM fotosintez, *Sedum caucasicum*, titirlənən turşuluq, fotokimyəvi effektivlik

Суточные Изменения Титруемой Кислотности В Листьях Нового САМ Вида *Sedum caucasicum*

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Изучение путей фотосинтеза в растениях очень важно для оценки современной растительности и для прогнозирования климатических изменений. Метаболизм по типу толстянковых (CAM) - один из способов поглощения углерода, который основан на фотосинтетической ассимиляции углерода в течение ночи и характеризуется увеличением эффективного использования воды. В роде *Sedum* обнаружена большая вариация форм САМ фотосинтеза, таких как: C₃, САМ и САМ-индуцируемые виды при водном или солевом стрессе. У вида *Sedum caucasicum*, который считается эндемичным для Кавказа, суточное изменение кислотности было аналогичным как для растений, выращенных в естественных условиях, так и для растений, выращенных в условиях искусственного климата. Результаты показали, что *S.caucasicum* является облигатным САМ растением.

Ключевые слова: САМ фотосинтез, *Sedum caucasicum*, титруемая кислотность, фотохимическая эффективность