

## Photosynthetic Patterns of 3 Crassulacean Plants under Drought Conditions

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**ABSTRACT:** Higher plants can be categorized as C3, C4 or CAM according to their photosynthetic pathways, and some succulent plants are known to shift their patterns of photosynthesis from C3 to CAM in response to environmental stresses such as salt treatment or water deficiency. To investigate fundamental photosynthetic patterns and the induction of pattern shifts (C3, CAM, C3-CAM etc.) as a result of environmental stresses, we measured the water content, diurnal changes in pH, net CO<sub>2</sub> exchange, transpiration rate, total ionic contents, and osmolality of *Kalanchoe daigremontiana*, *Sedum kamschaticum* and *Sedum sarmentosum* which belong to Crassulaceae known as representative CAM plant, after 10 days of drought treatment.

*S. kamschaticum* and *S. sarmentosum* did not show a significant difference in diurnal pH variation in the treatment and control conditions. However, the pH of drought-treated *Kalanchoe* was low at night and high in the daytime, with a pH value between 4 and 5. Typical CAM plants display a net CO<sub>2</sub> exchange that increases at night and decreases in the daytime. *Kalanchoe* displayed the predicted pattern. However, *S. kamschaticum* and *S. sarmentosum* showed a photosynthetic pattern more typical of C3 plants, and did not show changes in photosynthetic pattern under drought stress. *Kalanchoe* also showed a transpiration rate typical for CAM photosynthesis, whereas the transpiration rates of *S. kamschaticum* and *S. sarmentosum* were in the typical range for C3 photosynthesis. *Kalanchoe* had high total ionic contents during the night, which decreased somewhat during the daytime, whereas *S. kamschaticum* and *S. sarmentosum* displayed the opposite pattern. This result is similar to the diurnal patterns of changes in pH in the three plant species, which suggests a relationship between pH and ionic contents. *S. sarmentosum* showed lower osmolality under drought stress than in the control condition, whereas the osmolality of *Kalanchoe* and *S. kamschaticum* did not differ between conditions. *S. sarmentosum* may have maintained internal water content by lowering its osmolality and raising its total ionic contents.

In conclusion, *Kalanchoe* displayed the characteristic responses of a typical CAM plant, whereas *S. kamschaticum* and *S. sarmentosum* displayed aspects of the C3 photosynthetic pattern under drought conditions. These results suggest that *S. kamschaticum* and *S. sarmentosum* (Crassulaceae) in Korea overcome drought stress by increasing solute and ionic contents internally rather than changing their photosynthetic pattern from C3 to CAM under drought stress.

**Key words:** Crassulaceae, Drought stress, *Kalanchoe*, Photosynthetic patterns, *Sedum*

### INTRODUCTION

Most higher plants can be categorized as C3, C4 or CAM (Crassulacean acid metabolism) according to their photosynthetic pathways (Ehleringer and Monson 1993). CAM plants are usually found in arid land such as deserts. In general, CAM plants have thick leaves with a small surface area relative to their volume and have a low transpiration rate, which maximizes the efficiency of water use. In photosynthetic carbon assimilation, incoming CO<sub>2</sub> is usually fixed by the carboxylation of PEP during the nighttime (Ting 1985, Luetge 1987). CAM plants fix CO<sub>2</sub> by the C4 pathway and store malate in large vacuoles as malic acid. During the daytime, malic acid leaves the vacuole and enters the cytoplasm as malate. In the cytoplasm, CO<sub>2</sub> is released from the malate and enters the chloroplasts,

where it is fixed by the Calvin cycle. CAM plants also show diurnal changes in malic acid and dark acidification. Higher plants in 33 families, 328 genus and 10,000 species are known to use the CAM photosynthetic pathway (Winter and Smith 1996). Representative families showing CAM include Crassulaceae, Cactaceae, Orchidaceae, Aizoaceae, Agavaceae, Aspidelaceae, Bromeliaceae and Euphorbiaceae. In Lilliceae and Asclepidaceae, succulent species are also known to employ the CAM pathway (Kluge and Ting 1978).

CAM is generally related to stressful conditions such as water deficit, and the use of this pathway is based on stomata which close during daytime and open during the night to conserve water. However, *Isoetes howellii* inhabiting wet environments also use the CAM pattern (Ting 1985). Therefore, factors other than water deficiency may also induce the CAM mechanism.

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Some succulent plants show a shift in their photosynthetic patterns from C3 to CAM under certain conditions. For example, *Mesembryanthemum crystallinum* (Aizoaceae) is induced to shift from C3 to CAM under salt treatment or water deficiency (Osmond 1978, Winter 1979, Holtum and Winter 1982). A similar shift from C3 to CAM (C3-CAM) is also found in *Portulacaria afra* (Ting and Hanscom 1977).

*Opuntia basilaris* also shows diurnal changes under water deficiency, but the change in levels of malic acid is very small and no gas exchange stage occurs. This alternative pattern, known as CAM-idling, has been found in many kinds of plants (Szarek 1973, Osmond et al. 1975). Plants displaying CAM-idling maintain their physiological and metabolic characteristics under water deficiency, and return to a normal CAM mechanism when re-watered (Rayder and Ting 1985). Through this mechanism, plants are capable of surviving severe temporary water stress.

Some succulent plants display yet another pattern, showing changes in levels of organic acids, but no CO<sub>2</sub> fixation at night. This is known as CAM-cycling (Sipes and Ting 1985). In the family Crassulaceae, there are many plants showing a C3 pattern without the change in organic acids (Teeri 1982). This CAM-cycling is found in many plants, including Bromeliceae and Orchidaceae. Research to date on the photosynthetic patterns of Crassulacean plants suggests that most plants occur as C3-CAM (Dodd et al. 2002).

In Korea, 25 species of Crassulacean plants have been reported to inhabit various habitats such as swamps, mountainous regions, coastal rocks, alpine areas, rice paddies and dry fields. Research on CAM plants in Korea so far has been described seasonal dimorphism and organic metabolism of *Portulaca grandiflora* and *portulaca oleracea* (Portulacaceae) (Chang et al. 1981, Chang and Jin 1982). However, no systematic research about photosynthetic patterns or ecophysiological characteristics of Crassulacean plants has been conducted to date.

The aim of this study is to investigate the fundamental photosynthetic patterns of three Crassulacean plants, *Sedum sarmentosum*, *Sedum kamtschaticum* and *Kalanchoe daigremontiana*, and to determine whether they display changes in their photosynthetic patterns (C3, CAM or C3-CAM) under drought stress.

## MATERIALS AND METHODS

### Plant Materials and Treatments

Our study was conducted on young *Sedum sarmentosum* Bunge and *Sedum kamtschaticum* Fisch (Crassulaceae) plants, and plant of an induced species, *Kalanchoe daigremontiana* Hamet & Perrier. We selected plants showing uniform growth, planted them in a

plastic pot filled with perlite, cultivated them in a greenhouse (25°C/20°C) and supplied them with 60 mL of modified Hoagland solution (1/5 strength) every day. After 20 days of cultivation, we withdrew water from the study plants for 10 days (D10).

### Harvest and Analysis

We harvested leaves from the experimental plants at 4-hour intervals for 24 hours and measured their fresh weight (FW). We then dried the leaves for three days at 70°C and measured their dry weight (DW). Plant water (PW) was the difference between FW and DW.

To measure the pH, we collected about 1.0 g of fresh leaves and ground them into a powder with liquid nitrogen. We then mixed 10 ml of distilled water with the leaf powder, centrifuged the mixture, and measured the pH of the supernatant.

To analyze the net CO<sub>2</sub> exchange in each plant, leaf samples were measured using an infrared gas analyzer (IRGA, LCI photosynthesis System, ADC BioScientific Ltd) at intervals of 4 hours.

To measure the total ionic contents and osmolality, we ground dried leaves to a uniform powder using a UDY cyclone sample mill, and then added a 0.5 g sample to 5 mL of distilled water, and heated the mixture for 1 hour in a water-bath at 95°C. We then adjusted the final volume of the sample to 10 mL with distilled water and filtered the sample with a GFC filter (0.45 μm). The total ionic contents of the extracted samples were then measured using the conductivity method (MX300 X-matepro: Mettler-Toledo), and the total ionic contents were calculated by NaCl equivalent. Osmolality of a 50 μL sample was measured using Osmometer (Micro-osmometer 3MO, Advanced Instrument).

## RESULTS

### Water Contents

*Kalanchoe* and *S. sarmentosum* consisted of approximately 95% and 94% water, but *S. kamtschaticum* had a rather lower water content of only 88%. Under drought stress, the three species displayed similar water contents to control specimens. Even after 10 days of drought stress, the water content for all three species did not differ significantly from the controls (Table 1).

### Diurnal pH Variation

Control of *Kalanchoe* had a pH value of 4.5, which was lower than those of the other two species, and the pH of *Kalanchoe* gradually decreased during the night and increased during the day. The pH of *S. kamtschaticum* and *S. sarmentosum* ranged between 5 and 6, and did not fluctuate between day and night. All three species had slightly lower pH values after 10 days of drought stress

than in the control condition, but the difference was not significant.

### Photosynthetic Characteristics

#### Net CO<sub>2</sub> Exchange

The diurnal changes in net CO<sub>2</sub> exchange of three Crassulacean

Table 1. Changes in the water content of leaves of *Kalanchoe daigremontiana*, *Sedum kamschaticum* and *Sedum sarmentosum* treated with drought. Mean values of 7 replicates with standard errors (g/g f.w. : %)

Plant species	Control	10 days (-H <sub>2</sub> O)
<i>K. daigremontiana</i>	95.60 ± 4.09	94.15 ± 1.75
<i>S. kamschaticum</i>	88.38 ± 0.73	86.91 ± 1.50
<i>S. sarmentosum</i>	95.53 ± 0.29	94.45 ± 0.48

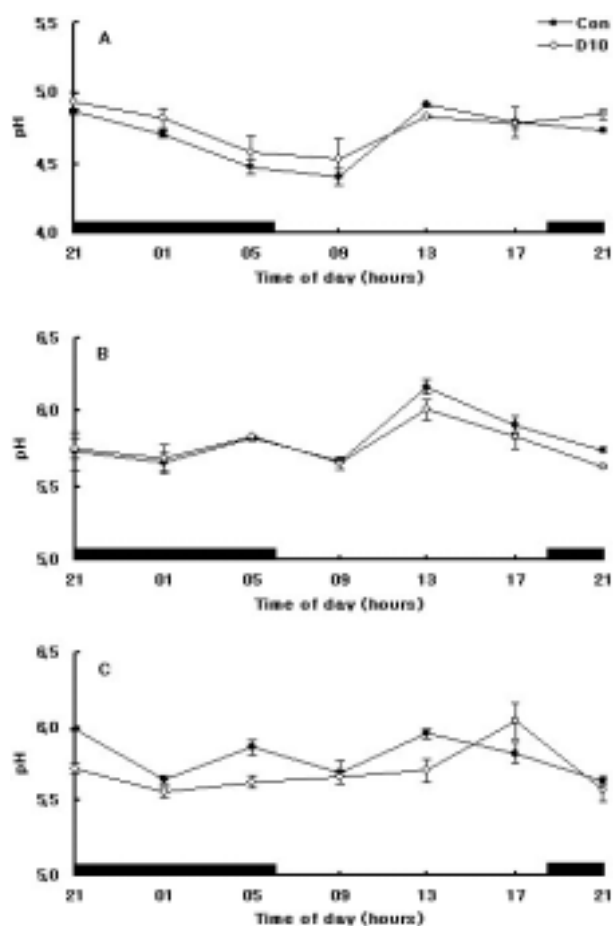


Fig. 1. Diurnal changes in the pH of well-watered (Control; Con) and drought-treated (D10) leaves of *K. daigremontiana* (A), *S. kamschaticum* (B), *S. sarmentosum* (C). Mean values of three replicates. Horizontal bars indicate hours of darkness.

plants exposed to drought conditions for 10 days are shown in Fig. 2. Control of *Kalanchoe* plants had the highest net CO<sub>2</sub> exchange at 5:00, after which the CO<sub>2</sub> exchange gradually decreased, with the lowest net CO<sub>2</sub> exchange value occurring at 13:00. Under drought stress, *Kalanchoe* showed a similar pattern, but the net CO<sub>2</sub> exchange value was somewhat lower than that of the control. In contrast, control of *S. kamschaticum* plants showed the lowest net CO<sub>2</sub> exchange at 21:00 and the highest net CO<sub>2</sub> exchange at 17:00, and control of *S. sarmentosum* plants showed the highest CO<sub>2</sub> exchange at 21:00 and the lowest at 13:00. Both species showed higher net CO<sub>2</sub> exchange rates in the daytime than the night, and even after 10 days of drought treatment showed similar net CO<sub>2</sub> exchange values.

#### Transpiration Rate

Diurnal changes in the transpiration rates of the 3 species after 10 days of drought stress are shown as Fig. 3. The transpiration rate

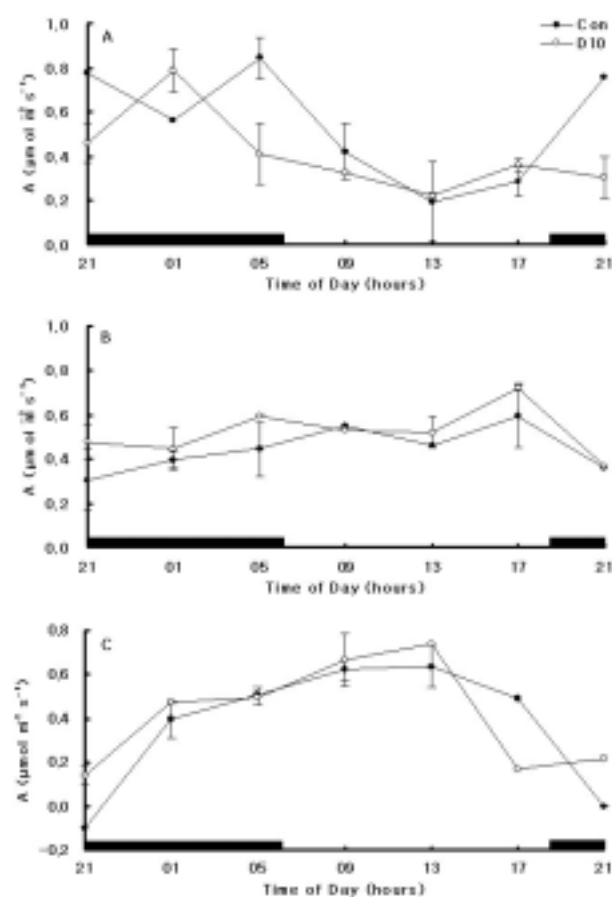


Fig. 2. Diurnal changes in net CO<sub>2</sub> exchange of well-watered (Control; Con), and drought-treated (D10) leaves of *K. daigremontiana* (A), *S. kamschaticum* (B), *S. sarmentosum* (C). Mean values of three replicates. Horizontal bars indicate hours of darkness.

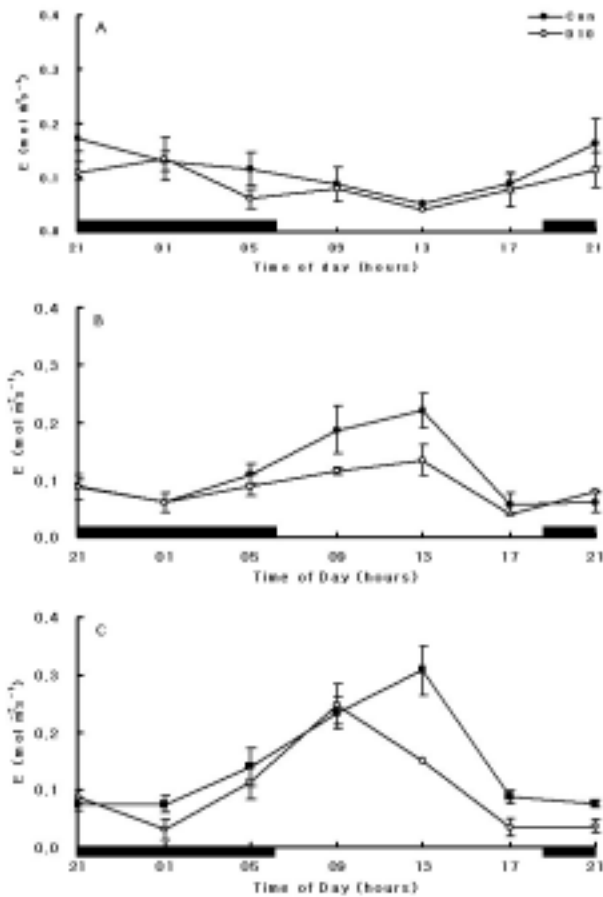


Fig. 3. Diurnal changes in transpiration rates of well-watered (Control; Con), and drought-treated (D10) leaves of *K. daigremontiana* (A), *S. kamschaticum* (B), *S. sarmentosum* (C). Mean values of three replicates. Horizontal bars indicate hours of darkness.

of *Kalanchoe*, like its net CO<sub>2</sub> exchange, gradually decreased during the night until the rate hit its lowest value at 13:00, and then increased slowly. Overall, *Kalanchoe* had higher transpiration rates at night than in the day. Conversely, *S. kamschaticum* and *S. sarmentosum* showed a higher transpiration rates during the daytime than the nighttime. All three species showed a somewhat lower rate of transpiration under drought stress than in the control condition.

#### Total Ionic Contents and Osmolality

Changes in the total ionic contents of the three plant species under drought stress are shown in Fig. 4. Leaves of drought-treated *S. kamschaticum* and *Kalanchoe* plants had higher ionic contents than control leaves, but *S. sarmentosum* did not show any consistent difference between treatments. In general, the ionic contents of the plants were high after 13:00 and decreased after sunset. No significant differences between species in diurnal patterns of osmolality

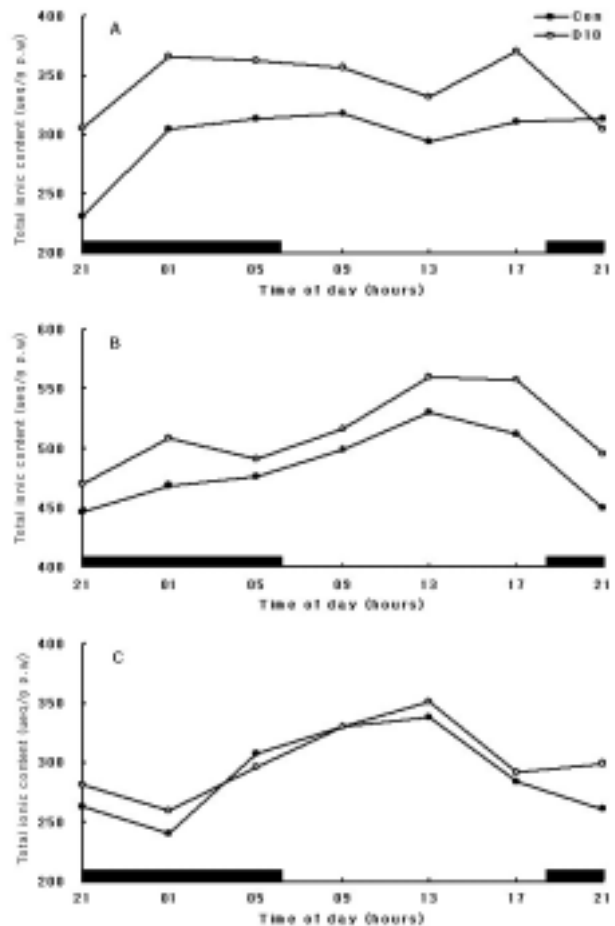


Fig. 4. Diurnal changes in total ionic content of well-watered (Control; Con), and drought-treated (D10) leaves of *K. daigremontiana* (A), *S. kamschaticum* (B), *S. sarmentosum* (C). Horizontal bars indicate hours of darkness.

were observed. However, leaves of the control plant of *S. kamschaticum* had higher osmolality than those of the other two species. Under drought stress, the osmolality of *Kalanchoe* and *S. kamschaticum* plants increased, whereas the osmolality of *S. sarmentosum* plants tended to decrease (Fig. 5).

## DISCUSSION

#### Water Contents

Because CAM species open their stomata during the night, when temperatures are lower and humidity is higher, they are able to maintain a high water content (Klug and Ting 1978, Winter 1985, Luttege 1998). To minimize water loss under drought conditions, they have evolved effective water storage and regulatory mechanisms (Reddy et al. 2003). In this study, three plant species in the family Crassulaceae did not show significant differences in water

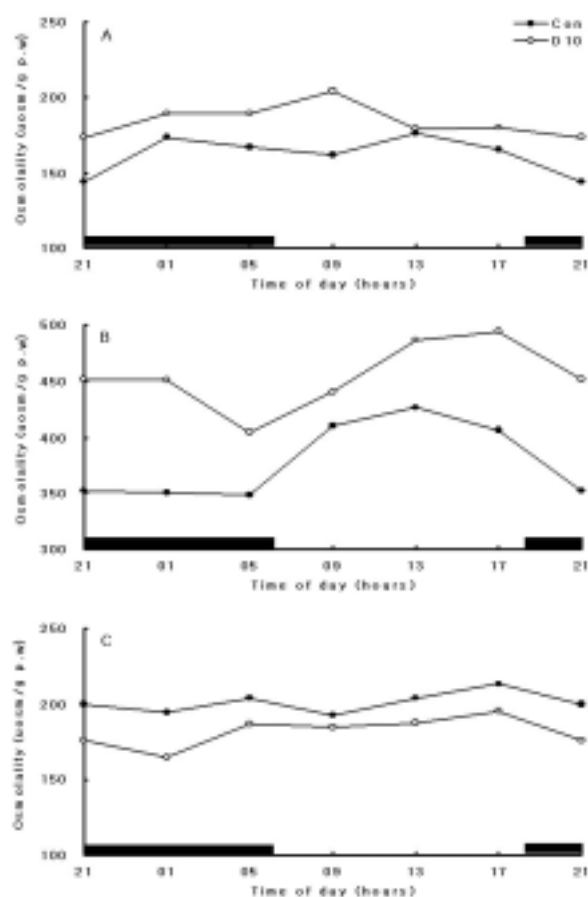


Fig. 5. Diurnal changes in osmolality of well-watered (Control; Con), and drought-treated (D10) leaves of *K. daigremontiana* (A), *S. kamschaticum* (B), *S. sarmentosum* (C). Horizontal bars indicate hours of darkness.

content relative to the control condition after 10 days of drought stress, containing 98% water regardless of treatment. This suggests that succulent Crassulacean plants may have higher tolerance for water stress than many other plant species.

#### pH Changes

CAM species open their stomata at night to fix CO<sub>2</sub> and store malic acid, which leads to acidification during nighttime (Bonner and Bonner 1948). Malic acid fixed at night is stored in the vacuoles of mesophyll cells, and during the daytime malic acid is released from the vacuoles and enters the cytoplasm as malate. In the cytoplasm, CO<sub>2</sub> is released from malate and enters the chloroplasts, where it is fixed in the Calvin cycle. Therefore, the internal pH is relatively high during the day and lower at night (Holtum and Winter 1982). Reddy et al. (2003) found that *Pedilanthus* spp. (Euphorbiaceae) not experiencing drought stress showed almost no diurnal changes in total acid contents, which indicated a C3 pattern.

On the other hand, plants subjected to drought stress for 20 days displayed an increase in total acidic contents at night and a decrease during the daytime, which is characteristic of the CAM photosynthetic pattern. The *Kalanchoe* species used in this study, which is indigenous to Madagascar, had a low pH during the night and a high pH during the day in both the control and drought-treated conditions. Therefore, the *Kalanchoe* displayed the diurnal variation in pH typical of CAM plants, but the other two plant species, *S. kamschaticum* and *S. sarmentosum*, which are indigenous to Korea, revealed no diurnal change in pH under drought stress, which suggests that they maintained a C3 photosynthetic pattern.

#### Photosynthetic Characteristics

##### Net CO<sub>2</sub> Exchange

Under drought stress, plant leaves generally become thin and leaf growth is inhibited by a reduction in photosynthesis. It is well known that CAM species that are resistant against drought stress also display changes in their photosynthetic patterns under drought stress or at high temperatures (Martin et al. 1994). CAM plants experiencing drought stress absorb CO<sub>2</sub> at night (Kluge and Ting 1978). *Kalanchoe* had high CO<sub>2</sub> exchange rates at night and lower CO<sub>2</sub> exchange rates during the daytime, the typical CAM photosynthetic pattern, and CO<sub>2</sub> exchange values were somewhat higher in drought-treated plants compared to controls. *S. kamschaticum* and *S. sarmentosum*, however, showed the typical C3 photosynthetic pattern of high CO<sub>2</sub> exchange in the daytime and lower CO<sub>2</sub> exchange at night in both control and drought-treated plants (Woerner and Martin 1999).

##### Transpiration Rate

Normal C3 plants open their stomata during the day, and rarely at night, but CAM plants open their stomata mostly at night (Chang et al. 1981, Buchanan 2002). *Kalanchoe* showed patterns of transpiration indicative of the typical patterns of stomatal opening and closing for CAM plants, but *S. kamschaticum* and *S. sarmentosum* displayed diurnal variation in transpiration similar to that of C3 plants. Drought stress induces various biochemical changes in plants. In particular, solute transport from roots to stems is reduced, which may be due to inhibition of active transport because of the restriction of the transpiration rate (Cui et al. 2004). In this study, all three Crassulacean plants examined showed a decrease of transpiration rate under drought stress as compared to control. The result of our study of photosynthetic activity suggest that *S. kamschaticum* and *S. sarmentosum* have a C3 photosynthetic pattern, even though *S. kamschaticum* shows the morphological features typical of CAM plants (Chang et al. 1983).

### Total Ionic Contents and Osmolality

It is well known that plants reduce energy consumption and store ions in the vacuole by increasing inorganic solutes and succulency under stress (Cuartero et al. 1992, Alarcon et al. 1993, Gurrier 1996). Accordingly, all three species of Crassulaceae examined showed increasing total ionic contents in the drought-treated condition compared to the control condition. However, *S. kamschaticum* showed significantly higher total ionic contents than the other two species. It seems that *S. kamschaticum* contains lower internal water content than the other two species.

*Kalanchoe* had higher total ionic contents during the night time and lower total ionic contents during the daytime, while the other two species displayed the opposite pattern. This suggests that diurnal variation in pH is closely related to total ionic contents. In general, plant total organic solutes, such as carbohydrates, amino acids, organic acids, etc., are effected by salt and drought stress (Strogonov 1973). The osmolality of *Kalanchoe* and *S. kamschaticum* was higher under drought stress than in the control condition. However, in *S. sarmentosum*, the osmolality was lower under drought stress than in the control condition. This suggests that *S. sarmentosum* raises its total ionic contents to retain water and thereby tolerate drought stress, rather than changing its photosynthetic pattern.

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