

## Limitation of Light Energy Utilization in the Fallen Stems of *Opuntia bigelovii* without CO<sub>2</sub> and Water Absorption

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地上에 떨어진 *Opuntia bigelovii* 仙人掌의 줄기에 있어서 光에너지의 利用限界

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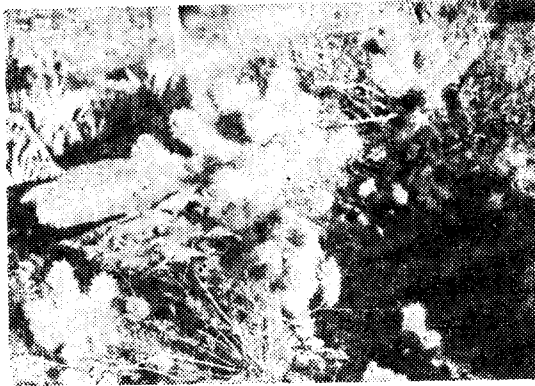
### ABSTRACT

Light energy utilization was investigated in the fallen stems of *Opuntia bigelovii*. Threshold time for the decreasing steady state of acid accumulation in the palisade tissue of *Opuntia* stems was 4 hours under  $1,000 \mu\text{Em}^{-2}\text{sec}^{-1}$  of PAR at 27°C, while stomatal closing throughout the stem stage was illustrated by  $256.0-310.4 \text{ sec cm}^{-1}$  of stem diffusive resistance and  $0.20\text{g day}^{-1}$  of the water loss rate as cuticular resistance. The acid loss rate in the stems per 4 hours was related to tissue water contents and a few acid loss rate could be recognized at the water content range of 56.4~46.8%. Endogenous oscillation of tissue acidity due to the diurnal rhythmic phenomena depended on the tissue water content was found in the *Opuntia* stems with stomatal closing during the normal day/night cycle. The survival rate of 1 segment stems to survive 2 years old cactus was 22.7% in desert environments. Such a compensation photosynthesis which utilizes light energy and maintains the reassimilation of endogenous gases was interpreted as conceptual model.

### Introduction

Under normal photosynthetic conditions, the stems attached to cacti need the CO<sub>2</sub> uptake and water supply in addition to light irradiation. However, when the stems fell on the soil surface, their stomata must be closed and water supply must be cut off. The adaptive significance of CAM plants for growth and survival in desert environments has a 2-fold interpretation

(Szarek and Ting 1974). First, strict stomatal regulation enhances water use efficiency, while reducing loss of endogenous gases. Second, the continuous day/night reassimilation of endogenous gases maintains a moderate level metabolic acidity during periods without atmospheric gas exchanges. Here, annually, the stems produced by *Opuntia bigelovii* had been tolerated for survival in desert environments, rooted in the soils, and grow up as new individuals. In the case of that stem stage, the Crassulacean acid metabolism for light energy utilization contributes to the very strong interest.



**Fig. 1.** Photograph of a part of an *Opuntia bigelovii* stand in the University of California's Philip L. Boyd Deep Canyon Desert Research Center near Palm Desert, California, U.S.A. One segment stems on the soil surface and *Opuntia bigelovii* of 2 segment stems were shown.

The following study was initiated to investigate that light energy utilization and respiration in the stems without CO<sub>2</sub> uptake and water supply contribute to the maintenance of the tolerance level of metabolic activity. Therefore, *Opuntia bigelovii* was selected for this study since it is a cactus which produces the stems for the reproduction except seeds (Benson 1969).

### Materials and Methods

Stems of *Opuntia bigelovii* Engelm were removed from natural stands of plants and were picked up from the soil surface (Fig. 1) at the University of California's Philip L. Boyd Deep Canyon Desert, California, U.S.A. (33°38' N, 116°24' W). The detached stems were put into the vinyl bags, returned to the laboratory, and stored on the roof garden of the laboratory building. Temperature and the light/dark cycle depend on the natural conditions. In each experiment, the stems of uniform storage periods were utilized. Most experiment stems were young without fruiting scars, less than 1 years old, and free from tissue deterioration.

The determination of stem tissue acidity was



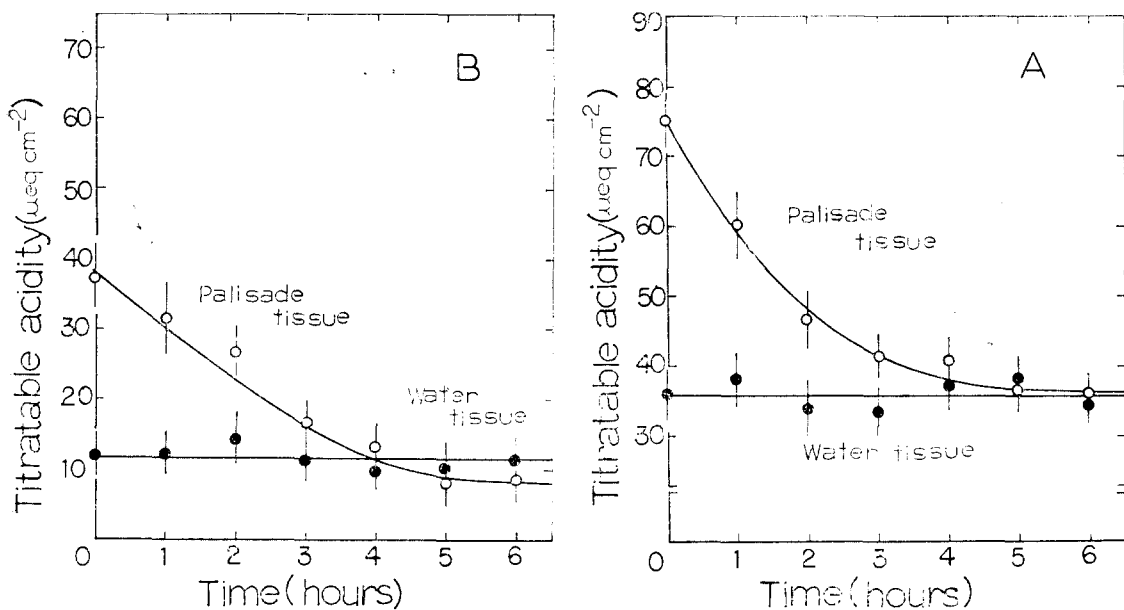
**Fig. 2.** Photograph of the *Opuntia* stems for normal surface with spines (1) and with spines removed (2). The stem sampling was divided into (a) preparing stems removed from natural stands of plants and (b) preparing stems fallen on the soil surface.

conducted with a technique modified from Sideris et al. (1948), and Hartsock and Nobel (1976). Approximately 4g of stem tissue were removed with a cork borer, ground in 100ml of distilled water, boiled for 5 min. and then filtered. Two 40ml aliquots were titrated to an endpoint of pH 6.4 using 0.011 N NaOH.

Stem diffusive resistances for the stems with spines removed were determined with a Lambda Instruments LI-60 diffusive resistance porometer with an LI-20S sensor and self-conducted calibration plates. Cuticular resistances to water vapor loss at day-and night-time intervals were determined using chemical balance with the stems which had the normal surface with spines (Fig. 2).

Light was provided by solar irradiation, and warm-white fluorescent lights supplemented with tungsten-filament lamps. PAR (400~700nm) was measured with a Lambda Instruments LI-190S quantum sensor. The water contents of the *Opuntia* stems were evaluated as the loss rates between their fresh and dry weights.

The survival rate of the *Opuntia* stems was



**Fig. 3.** Relationship between tissue acidity and time for the *Opuntia* stems involved 86.7% water content (A) and 81.3% water content (B). Tissue acidity data of the stems pretreated 3 days in darkness were obtained in the growth chamber at 27°C under 1,000  $\mu\text{E m}^{-2} \text{sec}^{-1}$  of PAR. According to the tissue gradient of palisade parenchymatous tissue to chlorophyllous cortical tissue and nonchlorophyllous tissue in the center (water tissue), data were expressed on the basis of total area (both sides) for these stems.

calculated as the rate of the number of 2 segment stems, which rooted in the soils and grew up during 1 year, to 1 segment stems which were fallen on the soil surface (Fig. 1). Vegetation sampling was also undertaken to determine ages of each *Opuntia bigelovii* in the *Opuntia bigelovii* stand. At each *Opuntia bigelovii* site, ages of all the individuals involved 1 segment stems were estimated by the number of stem segments. According to this method, ages which were less than 7 years old could be determined exactly.

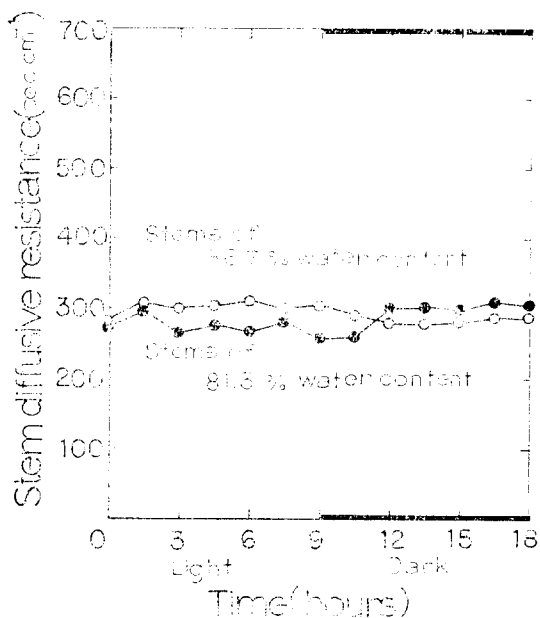
## Results and Discussion

### 1. Tissue acidity in the *Opuntia* stems

Crassulacean acid metabolism to utilize light energy in the *Opuntia* stems were made as shown in Fig. 3. When periods of light irradiation increased from 1 hour to 6 hours, acidity levels in the palisade tissue of the stems of which the

water contents were 86.7% and 81.3% decreased gradually from 74.9  $\mu\text{eq cm}^{-2}$  and 37.5  $\mu\text{eq cm}^{-2}$  to 36.7  $\mu\text{eq cm}^{-2}$  and 8.5  $\mu\text{eq cm}^{-2}$ , respectively. The thresholds of the decreasing steady states in the both water content stems were 38.2  $\mu\text{eq cm}^{-2}$  and 9.3  $\mu\text{eq cm}^{-2}$  of tissue acidity during 4 hours (Fig. 3, A,B). It suggests that transformation level of acid accumulation in the palisade tissue of the stems reaches the steady state under the light conditions over 1,000  $\mu\text{Em}^{-2} \text{sec}^{-1}$  during 4 hours.

However, water tissue (Klung and Ting 1978) in the both water content stems had no changes of tissue acidity throughout 6 hours. The acidity level in the water tissue was higher in 86.7% water content stems as compared with 81.3% stems. When the drought rate between 86.7% and 81.3% water content stems was 6.6%, the changing rate of acidity in the water tissue of the both stems was 66.1%. This result indicates that storage acids in the water tissue removed

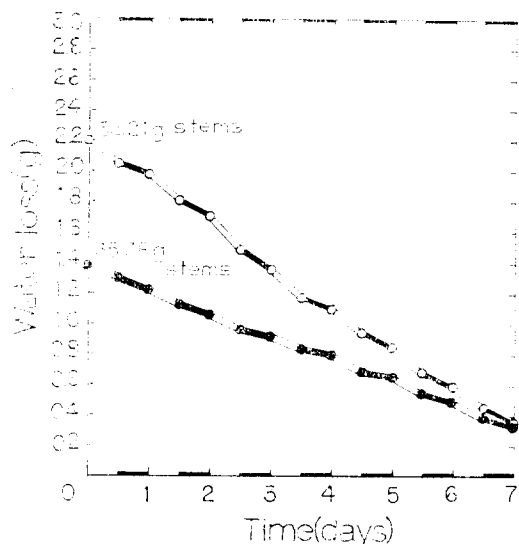


**Fig. 4.** Stem diffusive resistances in the *Opuntia* stems with 86.7% and 81.3% water contents were uniformly maintained at the range from 256.0 sec cm<sup>-1</sup> to 310.4 sec cm<sup>-1</sup>. Illumination was provided by warm-white fluorescent lights supplemented with tungsten filament lamps with values of 1,000 μE m<sup>-2</sup> sec<sup>-1</sup> of PAR. Air in the growth chamber was maintained at 27.0 ± 0.4°C and 5.0 ± 0.1 μg H<sub>2</sub>O cm<sup>-2</sup>.

to the palisade tissue.

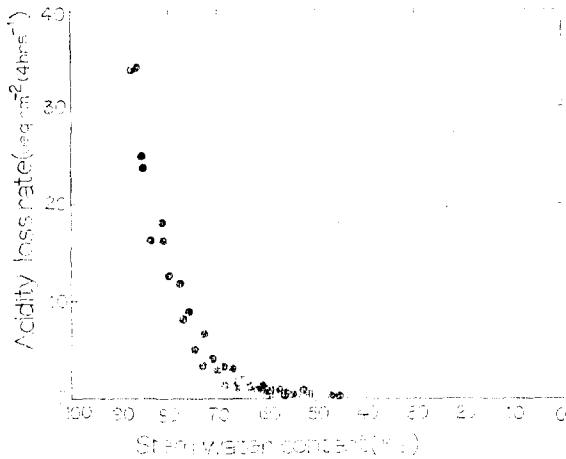
## 2. Stem diffusive and cuticular resistances

Crassulacean acid metabolism in the *Opuntia* stems fallen on the soil surface under light energy was elucidated by the result of Fig. 3. Here, it is important whether the stems uptake CO<sub>2</sub> gas with night-time stomatal opening or do not with all day stomatal closing. Stem diffusive resistances throughout the light/dark conditions maintained the steady level of the range of 256.0 sec cm<sup>-1</sup> to 310.4 sec cm<sup>-1</sup> (Fig. 4). According to Nobel (1976), maximum stomatal opening of *Agave deserti* Engelm did not occur until leaf diffusive resistance was lower than 10 sec cm<sup>-1</sup> for some leaves. In the cactus *Opuntia basilaris* Engelm, minimum stomatal resistances during nocturnal stomatal opening period were 10~15 sec cm<sup>-1</sup> and total gas diffusive resistances were



**Fig. 5.** To determine cuticular resistance, the amount of water loss in the *Opuntia* stems was measured at the day/night intervals. The water loss in the stems with 86.7% water contents were lower than with 81.3% water contents. The water loss rates during the day-time were higher than during the night-time. These data were obtained in the natural conditions.

in excess of 300 sec cm<sup>-1</sup> (Szarek and Ting 1975). The range from 256.0 sec cm<sup>-1</sup> to 310.4 sec cm<sup>-1</sup> are similar to the diffusive resistance level for stomatal closing as compared with data (200 sec cm<sup>-2</sup>) for *Opuntia basilaris* reported by Szarek et al. (1973). The water loss rates of the stems during the night-time throughout the 7 day period were less than the day-time, while the loss rate gradient of 86.7% water content stems was greater than that of 81.3% water content stems (Fig 5). This result with the data of stem diffusive resistances illustrates that stomata of the *Opuntia* stems must be closed throughout the day/night cycle and for all stem life until the stems will root. Therefore, in the stem stage, photosynthesis in the stems depends on CO<sub>2</sub> produced endogenously and so is expressed as compensation photosynthesis i.e.



**Fig. 6.** Change curve of acidity loss rates in the palisade tissue depending on the water contents in the *Opuntia* stems. After the stems were removed from the day/night cycle condition and put into the dark chamber during the 3 day periods, tissue acidity decreased during the 4 hour period was influenced by the water contents in the stems. Illumination and temperature in the growth chamber were similar to the conditions in Fig. 3.

this paper. When the average water loss rate throughout the stem stage is about  $0.20\text{g day}^{-1}$  (Fig. 5), the maximum survival time for the fallen stems is about 262 days during the stem stage and the drought season in desert environments (Szarek and Ting 1974, Nobel 1977), the cacti carry out compensation photosynthesis and survive in deserts.

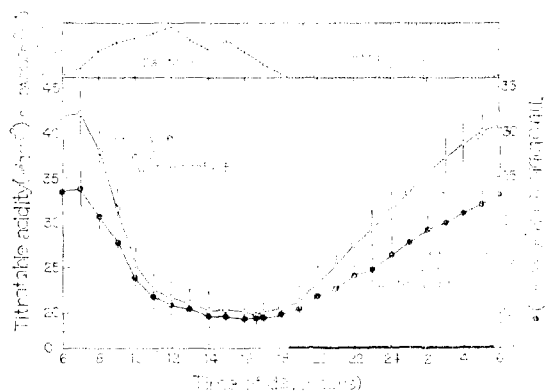
### 3. Compensation photosynthesis and tissue water contents

When the stem water contents were decreased from 86.7% to 66.0%, the loss rate of acidity in the stem palisade tissue per 4 hours was rapidly decreased from  $30.0\ \mu\text{eq cm}^{-2}$  to  $1.5\ \mu\text{eq cm}^{-2}$ . The stem water contents ranging from 66.0% to 52.7%, the loss rate gradually went down from  $1.5\ \mu\text{eq cm}^{-2}$  to  $0.3\ \mu\text{eq cm}^{-2}$  (Fig. 6). However, in a few stems of this water content range, no significant differences between the levels of acid accumulation during 3 days and of tissue acidity decreased during 4 hour periods. Moreo-

ver, in the stem water content range of 52.7~42.8%, the tissue acidity changes in most of the stems was not detectable and under the 42.8% water content, these changes in all the stems could not be recognized exactly (Fig. 6). This result indicates that the *Opuntia* stems maintain compensation photosynthesis until the stem water contents were reduced to the range of 52.7~42.8%. Therefore, the cacti can be survived due to this characteristic during the stem stage and drought season. Szarek et al. (1973) reported that during periods of drought, atmospheric  $\text{CO}_2$  exchange and transpiration were greatly reduced throughout the day/night cycle by stomatal closure and a highly impervious cuticle.

### 4. Diurnal changes and oscillation of tissue acidity

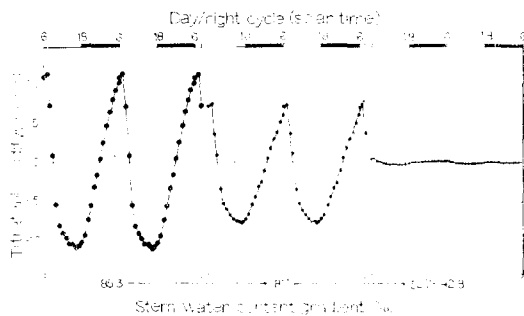
When the *Opuntia* stems were exhibited during a day/night time, diurnal changes of the tissue acidity levels were clearly evident (Fig. 7). The tissue acidity level was the maximum at 7 o'clock in the morning, decreased rapidly to 11 o'clock, gradually went down from 11 o'clock to 16 o'clock 30 minutes, reached the minimum, and increased to 7 o'clock in the next morning. This result accords with tendency to the diurnal change in tissue acidity for *Agave deserti* (Hartsock and Nobel 1976). However, in the *Opuntia*



**Fig. 7.** Diurnal changes in tissue acidity measured in the roof garden of the laboratory building on the spring day (March 18, 1979). At 1 hour intervals from sunrise to sunset, PAR was also measured horizontally.

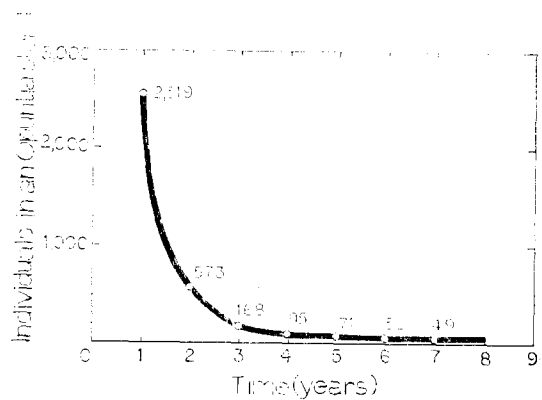
stem, the acid metabolism under the stomatal closing condition was occurred during the day and night-time. As shown in Fig. 7, the minimum loss rate of tissue acidity in the stems during the day-time period was 53.6% of total accumulation acidity for the 85.3% water content stems and 65.7% for 81.1% water content stems. Storage acids seem to transport from the water tissue in the stems into the palisade tissue, too. The time course of the increase in tissue acidity closely followed the net accumulation of CO<sub>2</sub> in the stems during the night-time period under stomatal closing condition. After sunset the increase rates of tissue acidity were greater for the 85.3% water content stems than for the 81.1% water content stems. According to the result of Fig. 7, the respiratory CO<sub>2</sub> in the *Opuntia* stems with closed stomata would be retained in the stems, and activity of stem respiration must be depended on the stem water content. The amount of acid accumulated in the stems with the 85.3% and 81.1% water contents following the night-time period under the stomatal closing condition were 42.3  $\mu\text{eq cm}^{-2}$  and 24.7  $\mu\text{eq cm}^{-2}$ , respectively. The diurnal fluctuation of stem tissue acidity was re-established at the levels similar to the stem water content and the environmental conditions.

In the results of Fig. 6 and 7, activity of



**Fig. 8.** Oscillation of tissue acidity in the *Opuntia* stems based on the dat. in Fig. 6 and 7. This oscillation depends on the stem water content gradient and the day/night cycle periods. This is maintained until the range of 52.7~46.8% of the stem water content.

compensation photosynthesis in the stems depends on the stem water content and rhythmic phenomena of the diurnal changes of tissue acidity throughout a stem stage and a drought season appears to have endogenous oscillation (Fig. 8). Schmitz (1951) first reported rhythmic CO<sub>2</sub> output patterns from excised leaves of *Kalanchoe blossfeldiana* in continuous darkness. In *Bryophyllum fedtschenkai*, Wilkins (1959, 1962 a,b, 1967) studied a CO<sub>2</sub> rhythmic output of CO<sub>2</sub> into CO<sub>2</sub> free air. Nuerbergk (1961) reported a circadian oscillation of net CO<sub>2</sub> uptake by *Bryophyllum daigremontianum* and other CAM species under constant illumination and normal air. The circadian rhythm of CO<sub>2</sub> exchange was explained by Queiroz (1974) in terms of endogenous rhythms of enzyme activity. Oscillation of tissue acidity in the stems with the closed stomata under the day/night cycle has a more importantly different feature depended on the stem water content as compared with rhythmic CO<sub>2</sub> exchange patterns mentioned above. However, the changes of tissue acidity in the stems may be concerned with the decreasing enzyme acti-



**Fig. 9.** Age distribution curve involved in 1 segment stems in an *Opuntia bigelovii* stand in the University of California's Philip L. Boyd Deep Canyon Desert Research Center, California, U.S.A. Ages of the cacti were determined by the number of the stem segments. According to this method, *Opuntia* ages, which are less than 7 years old, could be elucidated exactly.

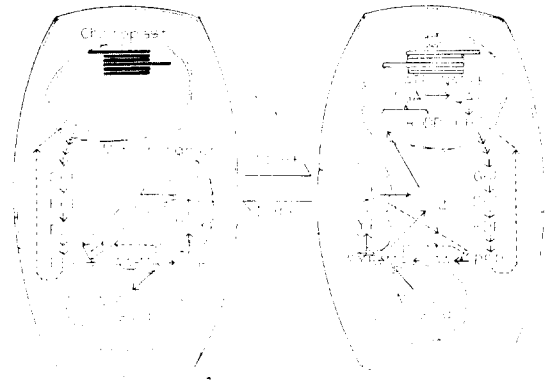
vity due to the stem water content decreased throughout the stem stage. Such speculation presumes that the occurrence of endogenous rhythms is a general feature of CAM plant to possess growth and survival in desert environments.

#### 5. Survival rate of the *Opuntia* stems

The survival rate of 1 segment stems to survive *Opuntia bigelovii* with 2 segment stems was 22.7% in the *Opuntia* stand located in the University of California's Philip L. Boyd Deep Canyon Desert Research Center near Palm Desert, California, U.S.A. Since 1 segment stems produced by the old *Opuntia bigelovii* fell on the soil surface, these stems carried out compensation photosynthesis to utilize light energy for maintenance of stem life, rooted in the soils to uptake water, and grow up as the new individuals due to normal photosynthesis (Fig. 1). As shown in Fig. 9, the survival rates of 2, 3, 4, 5 and 6 years old cacti to grow up as 3, 4, 5, 6 and 7 years old cacti were 29.3%, 56.5%, 74.7%, 73.2% and 94.2%, respectively. The most of the *Opuntia* stems (77.3%) withered in desert environments and died of water deficiency, while some stems were eaten by desert animals such as rats. The survival rates for 1 segment and 2 years old stems were about 2-fold greater than for 3 years old. This result showed that the most of *Opuntia bigelovii* died in the stem stage and 2 years old stage.

#### 6. Conceptual model for compensation photosynthesis

When CAM plants opened the stomata at night, absorbed  $\text{CO}_2$  from the atmosphere and utilized light energy under the stomatal closing condition at daytime, Black (1973) proposed a scheme for net  $\text{CO}_2$  fixation in these plants in dark and light. It is the metabolic pathway, from storage carbohydrate to malic acid in the dark, and then to storage carbohydrate once again during the subsequent light period. Malic acid acts as a night storage molecule for  $\text{CO}_2$  which is donated to the reductive pentose phosphate cycle during the subsequent day (Kluge



**Fig. 10.** A conceptual model of compensation photosynthesis in CAM plants with the stomatal closing during the stem stage and drought season. Abbreviations: ATP, adenosine tri-phosphate; NADPH, nicotinamide adenine dinucleotide phosphate (reduced form); PGA, 3-phosphoglycerate; GAP, glyceraldehyde-3-phosphate; RuDP, ribulose-1, 5-diphosphate; CHO, carbohydrates; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FDP, fructose-1, 6-diphosphate; PEP, phosphoenolpyruvate; OAA, oxaloacetate; MAL, malate; PYR, pyruvate. Main reactions(—); feed-back controls(.....).

and Ting 1978).

The results in this study demonstrated that the decrease of tissue acidity in the *Opuntia* stems during the day-time period depends on light energy as well as normal cacti with day-time stomatal closing but the increase of tissue acidity in the stems with the night-time stomatal closing during the night-time period is related to endogenously produced  $\text{CO}_2$  without  $\text{CO}_2$  absorbed from the atmosphere. This compensation photosynthesis to tolerate water shortage in the stem stage and drought season is characteristic of light energy utilization and continuous day/night reassimilation of endogenous gases. Therefore, a conceptual model of compensation photosynthesis for cacti during the stem stage and drought season modified by this study was presented in Fig. 10.

This model indicates that there is only the light energy utilization in the stems and CAM

plants during the stem stage and drought season in desert environments but there is no growth. This interpretes also that during the periods of the stomatal closing, CO<sub>2</sub> available through the endogenous supply is recycled and metabolic energy continually supplied from the flow of carbon through dark CO<sub>2</sub> fixation, organic acid transformations, photosynthesis, and respiration (Szarek et al. 1973).

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## 摘 要

地上에 떨어진 *Opuntia bigelovii* 仙人掌의 줄기에 있어서 光 energy의 利用限界와 含水量과의 關係를 研究하였다. 이들 떨어진 仙人掌의 줄기는 주·야로 줄기 확산 저항이  $256.0 \sim 310.4 \text{ sec cm}^{-1}$ 인 것으로 보아 氣孔은 주·야를 통하여 閉孔하고 있음을 알 수 있었다. 또한 蓄積된 酸의 減少率은 PAR이  $1000 \mu\text{Em}^{-2}\text{sec}^{-1}$ 인 조건 하에서 4時間이면 steady state로 감소하였다. 組織內的 酸減少率은 組織의 含水量에 따라 감소하며 그 함수량이 56.4~46.8% 일 때에는 4時間

동안의 光照射와 本實驗方法으로는 거의 酸減少率을 확인할 수 없었다. 日週期 rhythm에 의하여 나타나는 酸度の oscillation도 組織의 含水量의 감소에 따라 감소하였다. 한마디의 仙人掌줄기가 沙漠의 땅에 떨어져 두마디의 仙人掌이 되는 生存率은 22.7%였으며 그 동안은 外部로 부터  $\text{CO}_2$ 와 물의 吸收없이 光energy를 利用하여 生存하고 비가 내리지 않는 긴 乾燥期에도 仙人掌은 똑같은 방법으로 생활한다는 것은 알 수 있었다. 이와 같이 外部로 부터 들어오는 光과 呼吸에 依하여 內生되는  $\text{CO}_2$ 만을 利用하는 보상광합성의 代謝過程을 model로 제시하였다.

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