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A Study on Photosynthesis and Nitrogen Assimilation in Cactus
—*Portulaca oleracea* L.—

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Cactus의 光合成과 窒素同化作用에 關한 硏究
—한국산 쇠비름(*Portulaca oleracea* L.)—

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ABSTRACT

Crassulacean acid metabolism (CAM) was investigated in leaves and stems of the succulent C_4 dicot *Portulaca oleracea* L. Under 14-hour days, stem tissues showed much greater fluctuation of acidity than leaf tissues. But leaf and stem tissues showed almost same CAM-like pattern of acid fluctuation under 8-hour days. Stem tissues of *P. oleracea* grown under the natural environment showed high CAM activity, but no CAM activity was seen in leaves of those plants. In the naturally growing plants, the rapid acidification was seen in intact stems at dawn, but defoliated stems showed only a gradual increase.

RuBP carboxylase activity was very high at 2:00 P.M. in both leaves and stems. However, its activity at 1:00 A.M. and 5:30 A.M. was hardly detected. Particularly, activity of PEP carboxylase in leaves was very high in the early morning, though that in stem tissues was little. These results indicate that CO_2 passed through open stomata at dawn may be assimilated by PEP carboxylase in leaves, and then C_4 products move to stems.

The levels of nitrate concentration and of nitrate reductase were higher in stems than in leaves. The levels were also higher in the light than in the dark. It would be suggested that considerable amount of nitrate absorbed from roots be assimilated in stems, and nitrate transferred to leaves via stem tissues be reduced there.

Key words: *Portulaca oleracea*, Cactus, Photosynthesis, Nitrogen assimilation, Crassulacean acid metabolism (CAM).

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INTRODUCTION

Crassulacean acid metabolism (CAM) represents a variation on the photosynthetic CO_2 assimilation occurring in succulent plants (Randson & Thomas, 1960; Ting, 1971; Ting, 1972). The CAM plant is characterized by a marked diurnal fluctuation of the malic acid content, with malic acid accumulating during the night and decreasing during the day. The starch content of the leaves shows a diurnal rhythm inverse to that of malic acid, i. e., a decrease during the night and an increase during the day (Kluge, 1975).

Some succulents have the unique capacity to shift their photosynthetic mode between the C_3 type and CAM, depending on environment and age (Osmond, 1978; Osmond *et al.*, 1976; Treichel, 1975). CAM has been shown to be affected by salt treatment (Ting & Hanscom, 1977; Winter & Troughton, 1978), water stress (Allaway *et al.*, 1974; Neals *et al.*, Neals *et al.*, 1968; Osmond *et al.*, 1976; Szarek *et al.*, 1973; Ting & Hanscom, 1977), photoperiod (Queiroz, 1974), temperature fluctuations between hot day and cool night (Nalborczyk *et al.*, 1974), stage of maturing (Jones, 1975; Osmond *et al.*, 1976) and flowering (Randson & Thomas, 1960).

The possible occurrence of CAM or facultative CAM in a succulent C_4 plant species was examined by Koch and Kennedy (1980). Many aspects of C_4 physiology in *Portulaca oleracea* have been established well such as four-carbon acid metabolism (Kennedy, 1977; Kennedy and Laetsch, 1973), enzyme activities (Kennedy, 1976; Kennedy, 1977), compensation point (Kennedy, 1977; Treguna & Downton, 1967), anatomy and cytology (Kennedy, 1973), photorespiration (Kennedy, 1976), photosynthetic rate (Kennedy, 1977), and response to salt and water stress (Kennedy, 1977). Under 8-hour days well-watered plants showed a CAM-like pattern of acid fluctuation, but under 16-hour days well-watered plants showed no CAM activity (Koch & Kennedy, 1980).

There is no question as to the universality of the C_3-carbon fixation pathway for net CO_2 incorporation by all plants. The C_4 and CAM pathways are consequently looked upon as additional systems supporting this basic C_3 pathway (Kelly *et al.*, 1976).

The purposes of this study are to examine the mechanism by which CAM was occurred in *P. oleracea* stems as well as the possible occurrence of CAM in this C_4 plant grown under the natural condition. It would be thought that beta-carboxylation existing in both C_4 and CAM plant (Black, 1973) be performed with variable time schedule according to the specific conditions, such as water-stressed condition. It is important to know how the additional systems, C_4 and CAM pathways, become profitable through beta-carboxylation under a certain condition.

MATERIALS AND METHODS

1. Plants

Portulaca oleracea L. was grown in the green house under natural light condition (about 14 hr day/10 hr dark) for its first 4 weeks. And then some of them were transferred to a growth chamber adjusted to the short day condition (8 hr day/16 hr dark) and grown for 3 weeks before experimental use. High pressure mercuric lamp (HP 6-400 W) provided an intensity of 8,500 Lux at plant level.

Temperature of 30℃ day/15℃ night was maintained. Plants remained in the green house were used to compare the diurnal acidity changes with those of short day plants. The temperature was in the range of 13℃ to 23℃, and maximum light intensity was about 8,000 Lux.

Naturally grown *P. oleracea* plants were sampled from a field in May. They were potted, and transferred to the open air place near the laboratory and grown for 4 weeks before experimental use.

2. Morphology, anatomy and observation of stomata

Cross section through a leaf and a stem of *P. oleracea* was observed with a microscope (VANOX, PM-10-A). The number of stomata and their opening could be investigated by replicating the epidermis with the manicure.

3. Titratable acidity determination

Diurnal change of titratable acidity was measured by the modified method of Moradshahi *et al.* (1977). Both kinds of plants grown under 14-hour day and 8-hour day conditions were removed every 3~4 hours and immediately their leaves and stems were separated. Tissues were then ground with about 20 ml of boiled distilled water in a glass handy homogenizer and acid was extracted by boiling for 5 min. Additional distilled water was added to bring the final volume to 20 ml, and it was centrifuged for 20 min at 1,000 G. A 10 ml of aliquot from the supernatant was removed and 30 ml of distilled water was added to it. This was titrated to an end point of pH 6.4 using 0.01 N NaOH.

4. Stomatal resistance

Stomatal resistance was measured with an Autoporometer (LI-65, LI-COR) in order to investigate the stomatal movements in the *P. oleracea* leaves.

5. Enzyme assays

RuBP and PEP carboxylases activities were determined according to the modified procedure of Dittrich *et al.* (1973). Each of 1~1.5g of leaf and stem tissues was sampled at 2:00 P.M., 1:00 A.M. and 5:30 A.M. respectively, and thoroughly disrupted in a chilled

glass handy homogenizer containing (in a total volume of 4 ml); 50 mM Tris-HCl at pH 20 mM $MgCl_2$, 0.1 mM Na_2-EDTA, 5 mM DTT, 5 mM D-isoascorbate, and 2% PVP-40. The resulting homogenate was then centrifuged for 5 min at 10,000 G and 2℃, and 0.9 ml of supernatant was mixed with 0.1 ml of 100mM $NaHCO_3$ and it was preincubated for 10 min at room temperature in order to activate carboxylases.

RuBP carboxylase was assayed as ^{14}C-incorporated in 1 ml of medium including 0.5 mM RuBP, 20 mM $MgCl_2$, 0.1 mM Na_2-EDTA, 20 mM $NaH^{14}CO_3$, 50 mM DTT, and 50 mM Tris-HCl, pH 8.3. PEP carboxylase was also assayed as ^{14}C-incorporated in 1 ml of reaction mixture containing: 5 mM PEP, 10 mM $NaH^{14}CO_3$, 5 mM DTT, 10 mM $MgCl_2$, and 50 mM Tris-HCl buffer at pH 8.3. The reaction was halted by addition of 0.1 ml of 6 M HCl and 0.1 ml of 6 M HCl saturated with 2,4-dinitrophenyl hydrazine in the each case of RuBP carboxylase and PEP carboxylase. The solutions were dried in an air stream before determining the radioactivity by liquid scintillation counter (Packard, Model 3390).

The extraction media used for nitrate reductase consisted of 25 mM K-phosphate buffer adjusted to pH 8.8 with KOH, 1 mM EDTA, 25 mM cysteine, 3% (w/v) BSA, and 0.1% (w/v) PVP-40. Nitrate reductase was assayed according to Neyra and Hageman (1975), nitrite reductase extracted and assayed according to Dalling *et al.* (1973), NAD(P)H-dependent glutamate dehydrogenase according to Joy (1969), glutamine synthetase (synthetase activity) according to Rhodes *et al.* (1975), and glutamate synthase according to Lea and Miflin (1974). All enzyme activities were linear with time and enzyme concentration. Boiled extracts as well as controls lacking substrates showed no activity.

6. Nitrate determination

The amount of nitrate in tissues was estimated according to Cataldo *et al.* (1975). Leaf and stem tissues were sampled at the same time of extracting nitrate reductase. Each of 0.2~0.3g of leaf and stem tissues was thoroughly homogenized in a chilled glass homogenizer containing 2 ml of chilled distilled water. The resulting homogenate was then centrifuged for 15 min at 30,000 G and 2℃. A 0.2 ml of supernatant was pipetted into 50 ml Erlenmeyer flasks. After mixing with 0.8 ml of 5% (w/v) salicylic acid in concentrated H_2SO_4 thoroughly, the aliquots were kept for 20 min at room temperature. Nineteen ml of 2 N NaOH was added slowly with a pipette to raise the pH above 12. After samples were cooled to room temperature, absorbance at 410 nm was determined with spectrophotometer (Bausch & Lomb).

RESULTS

1. Morphology, anatomy and the number of stomata

The number of stomata in the leaves and the stems of *P. oleracea* was shown in Table 1.

The average number of stomata in the *P. oleracea* leaf was about 2 times more than that in CAM plants reported by Kluge and Ting (1978).

Table 1. Number of stomata per cm^2 on the adaxial and abaxial surfaces of leaves of *P. oleracea*

Young leaves		Stems
Adaxial	Abaxial	
8,500	5,143	0
8,071	5,143	0
8,071	4,500	0
7,285	4,429	0
7,000	4,071	0
6,000	3,786	0
5,786	3,643	0
5,357	2,857	0
5,000	2,857	0
4,929	2,643	0
6,600*	3,950*	0

* The mean estimate for the 10 randomly selected plants

Table 2. Values of mesophyll succulence (Sm) in leaves and stems of *P. oleracea*

Samples	Water contents (g/g. f.wt.)	Chlorophyll contents (mg/g. f.wt.)	Sm*
Leaves	0.898	0.578	1.55
	0.895	0.744	1.20
Stems	0.903	0.132	6.84
	0.897	0.145	6.18

However, no stomata were observed in the epidermis of the *P. oleracea* stem.

Mesophyll succulence (Sm) was suggested as a reasonable index of succulence at cellular or tissue level by Kluge and Ting (1978).

These values were measured in the leaf and in the stem tissues of *P. oleracea* (Table 2). Sm

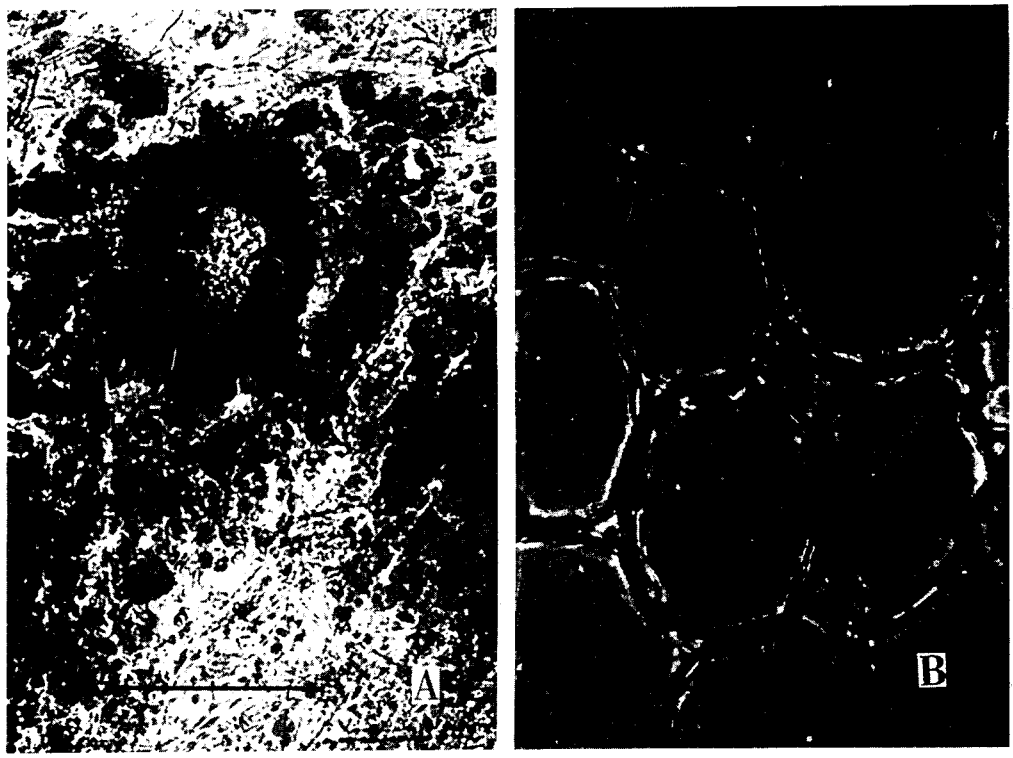

Fig. 1. Cross sections of leaf (A) and stem (B) of *P. oleracea*. 'A' shows Kranz-type anatomy and 'B' shows cells with relatively large vacuoles and a few chloroplasts. The bars designate 50μm length.

value of stem tissues was about 5 times higher than that of leaf tissues.

The cross section of the *P. oleracea* leaf showed an Kranz-type anatomy (Fig. 1) which was one of the characteristics of C_4 plants (Laetsch, 1974). But stem cells of this plant which had the large vacuoles and a few chloroplasts were much alike to the cells of CAM plants.

$$* \text{Sm} = \frac{\text{Tissue water content (g)}}{\text{Chlorophyll content (mg)}}$$

2. Acid fluctuation

Large changes in titratable acidity were mainly exhibited in the stem tissues of *P. oleracea* (Figs. 2 and 3). *P. oleracea* grown under the long day condition showed CAM-like amplitudes of acidity fluctuation of 16.73 and 53.21μ eq. /g. fresh weight in leaves and stems respectively. Under the short day condition, *P. oleracea* also showed acidity fluctuation typically seen in CAM plants (Milburn *et al.*, 1968).

Amplitudes of acidity fluctuation were 15.74 and 19.31μ eq. /g. fresh weight in leaves and stems, respectively, under the short day condition. The ratios of acidity changes in leaves to those in stems were 0.31 and 0.82 under the 14-hour days and the 8-hour days individually.

The ratio of acidity fluctuation in leaves to that in stems became greater under the short day than under the long day. The short day plants were grown for 3 weeks under 8-hour day after being transferred from long day condition, but their diurnal rhythm of acidity change was not broken yet and only the peak of acidity level was moved in stems.

Plants under the natural condition were investigated every 1 hour and every 2 hours during a day in September and in May. In both cases stem tissues showed a CAM characteristic of acid fluctuation, but no CAM was seen in leaf tissues (Fig. 2)

In stems, acidity level became high during the night, and at dawn the level of acidity was increased suddenly (Fig. 3 B). On the contrary, acidity of defoliated stems increased gradually through the night, and their level of acidity was lower than that of intact stems. At dawn, defoliated stems did not show a rapid increase. This result suggests that CAM-like amplitudes of acidity fluctuation exhibited in stems be due to the transport of acid materials from leaves.

The difference between the highest acidity at 9:00 A.M. and the lowest one at 2:00 P. M. was investigated in the individual node of the *P. oleracea* plants (Fig. 4). This difference was the greatest at the second node of the intact plant. When the first pair of leaves on the top of the plant was removed, the level of acidity became lower but the second node also showed the highest level of acidity. *P. oleracea* stems showed gradual decrease of acidity and the order of node having the highest acidity was changed as the leaves were

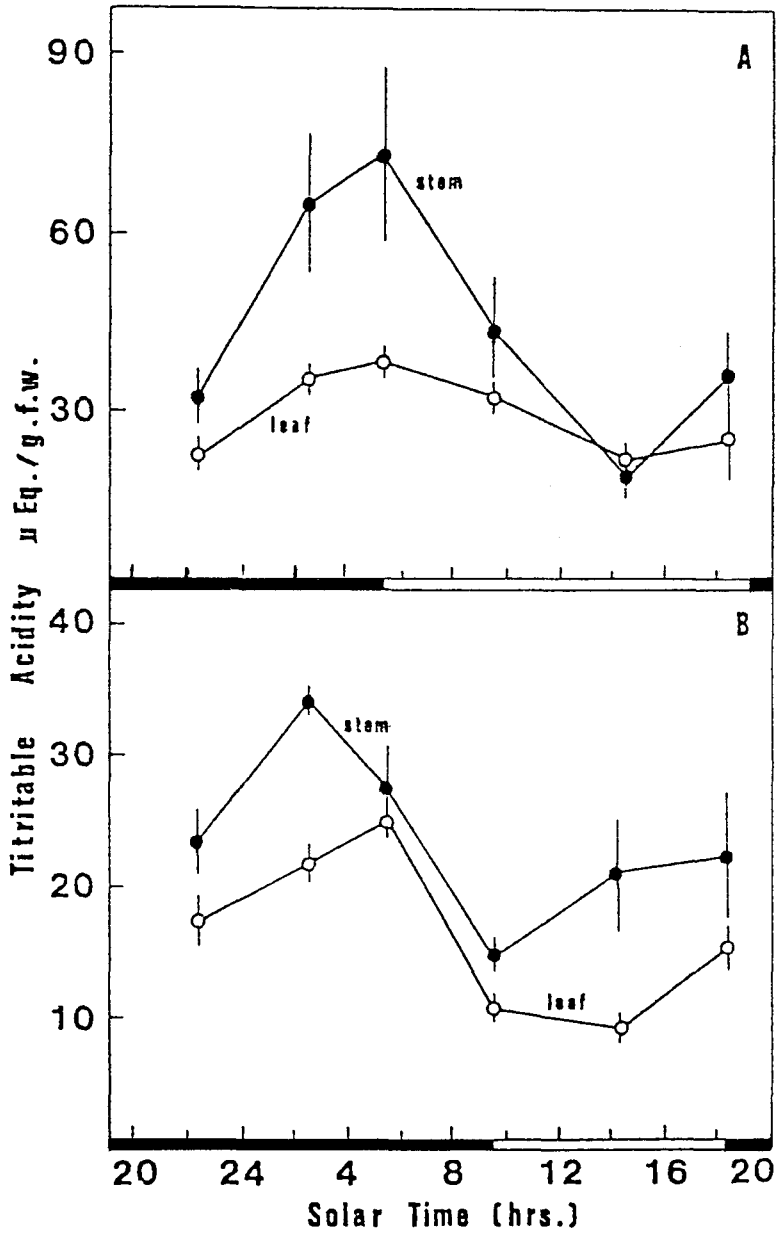

Fig. 2. Diurnal fluctuations of tiratable acidity in leaves and stems of *P. oleracea* L. under 14-hour day condition (A) and under 8-hour day condition(B).

detached from the top to the bottom. These results also give a possibility of transport of an acid material from leaves to stems.

Diurnal rhythms of titratable acidity in excised leaves and stems of *P. oleracea* were

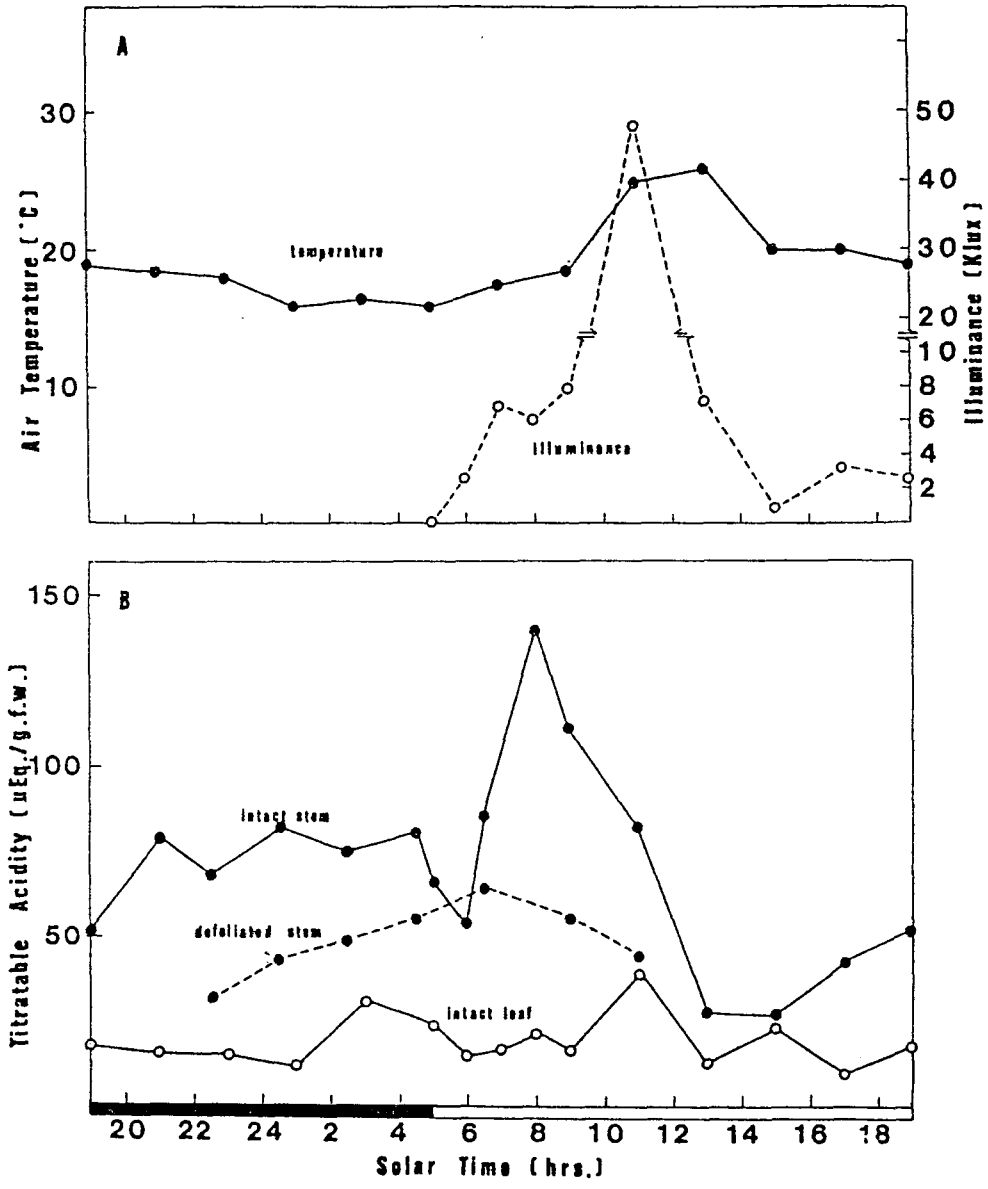

Fig. 3. Diurnal fluctuations of titratable acidity in leaves and stems of *P. oleracea* L. under natural conditions (B). Air temperature and solar illuminance are shown (A).

investigated under the continuous darkness (Fig. 5). The tissue water content in leaves decreased and reached a steady state after only a day, but it took 3 days until the steady state in stems. Diurnal fluctuation of acidity change became reduced according to the reduction of the water content.

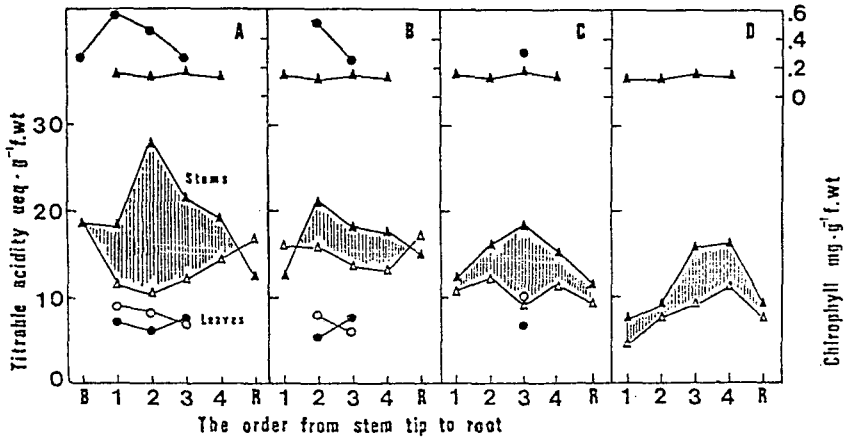

Fig. 4. Titratable acidity and chlorophyll contents in leaves and stems of the different order of the young purslane, *P. oleracea* under different treatments: A, control plants; B, plants whose buds and 1 st pair of leaves were detached; C, whose buds and 1 st and 2 nd pairs of leaves were detached; D, whose buds and all pairs of leaves were detached. 'B' and 'R' on the traverse line designate bud and root, respectively. Filled circles and triangles represent titratable acidity in leaves and stems sampled at 09:00 A.M., and blank symbols represent that sampled at 2:00 P.M., respectively, Each value of titratable acidity is the mean for duplicate samples. Each value of chlorophyll contents is the mean for tetraplicate samples.
The shaded areas indicate acidification.

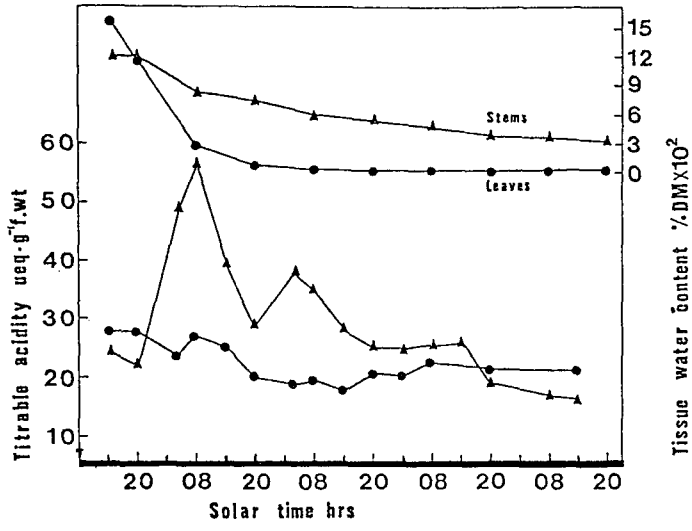

Fig. 5. Diurnal rhythms of titratable acidity in excised leaves and stems of *P. oleracea* under continuous darkness. Tissue water content measurements during these experiments are shown.
Each value of titratable acidity and tissue water contents is the mean for duplicate samples. The shaded lines designate continuous darkness. Air in the darkroom was 65±5% RH and 20±1℃ during these experiments.

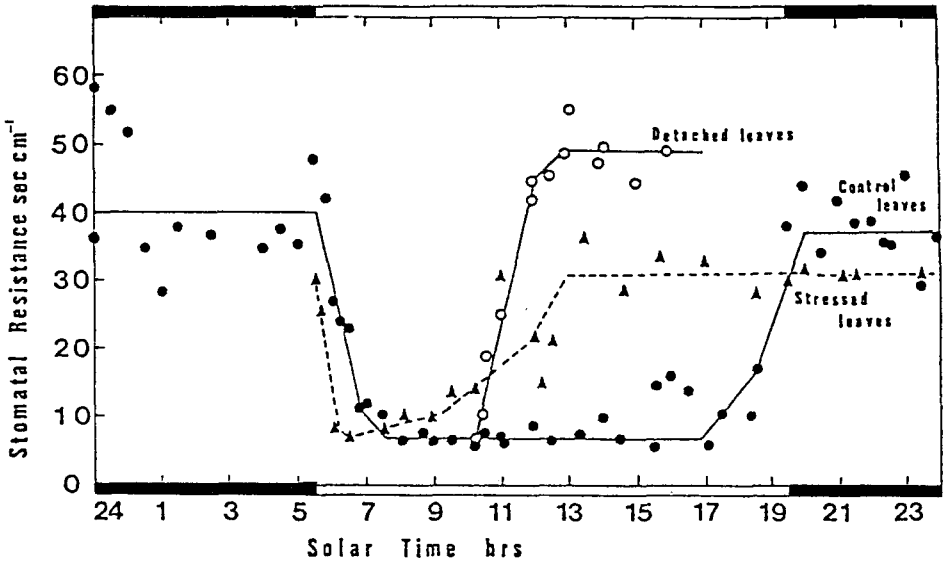

Fig. 6. Diurnal patterns of stomatal resistance for *P. oleracea* growing at a habitat in Bucheonsi, Kyeonggido, Korea under different conditions. The soil water contents for the control plants and those for the stressed plants were 21.2 and 12.1% DM, respectively. The field capacity of that soil was 44.6%. The shaded lines indicate night. Values represent the mean of three plants measured individually.

3. Stomatal resistance

Stomatal resistance was measured in *P. oleracea* growing naturally during a day (Fig. 6). The *P. oleracea* leaf showed a high stomatal resistance during the night and a rapid decrease at dawn. On the other hand stomatal resistance measured in water-stressed *P. oleracea* was lower than that in the control plant during the night, and decreased rapidly at dawn and increased gradually at about 10:00 A.M. These results represent that the interval at which the stomata are open becomes short during the day and stomatal aperture becomes wide during the night under the water-stressed condition.

4. RuBP and PEP carboxylase activity

Both leaf and stem tissues showed very high RuBP carboxylase activity at 2:00 P.M. (Table 3). At the other times, 1:00 A.M. and 5:30 A.M., activity of this enzyme was hardly detected. In the case of PEP carboxylase, the crude enzymes extracted from leaves always showed higher activity than those from stems. It is thought that absence of stomata in stems may result in producing a little amount of substrate for PEP carboxylase and decreasing enzyme activity. The high activity of this enzyme at 2:00 P.M. represents that *P. oleracea* leaves have a C_4 metabolism (Kennedy, 1976) during the light. At 5:30 A.

Table 3. The carboxylase activities from leaf and stem extracts of *P. oleracea*. Activity in μ mole /g. fresh weight.

Time	RuBP carboxylase		PEP carboxylase	
	Leaf	Stem	Leaf	Stem
14 : 00	461.84	339.08	12.39	1.38
1 : 00	4.00	0.19	1.93	0.13
5 : 30	4.27	N.D.	14.84	0.20

M., PEP carboxylase activity was high considerably in leaves, but PEP carboxylase extracted from stem tissues at that time showed a very low activity.

These results have a close relation to the result (Fig. 6) that stomata in leaves become open rapidly as soon as sun rises. From these results, a model for transport of C_4 acid from leaves to stems when light is not sufficient to activate RuBP carboxylase fully was suggested.

5. The enzyme assays of nitrogen assimilation

Results of the assays for various enzymes of nitrogen assimilation in *P. oleracea* leaf and stem are compiled in Fig. 7 and Table 4.

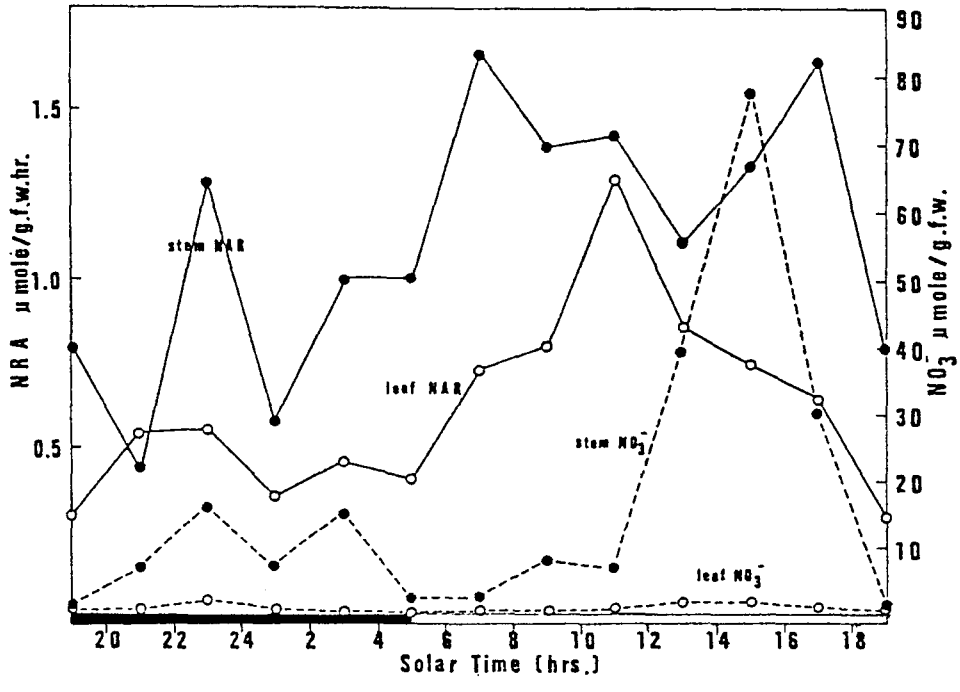

Fig. 7. Diurnal fluctuations of NRA and NO_3^- contents in leaves and stems of *P. oleracea* under the natural conditions. Air temperature and solar illuminance are shown in Fig. 3.

Table 4. Activities (μ mole /g.fw.hr.) of nitrogen assimilation in *P. oleracea*

Enzyme	Leaf	Stem
Nitrate reductase	0.863	1.115
Nitrite reductase	3.916	5.410
Glutamine synthetase	6.432	8.552
Glutamate synthase	1.534	2.004
Glutamate dehydrogenase		
NADH-dependent	2.642	3.056
NADPH-dependent	1.091	1.990

Both nitrate reductase activity (NRA) and nitrate ion concentration were higher in stem tissues than in leaf tissues (Fig. 7). In the light, nitrate contents and NRA increased in comparison to those in the dark. A peak of nitrate contents in stems would be due to a transpiration through the open stomata and to a circadian rhythm (Die, 1959; Pate, 1971). During the daytime, high level of NRA may result from much amount of substrate and light energy. The enzyme activity levels of nitrogen assimilation were higher in the leaf tissue extracts of *P. oleracea* plants which were sampled at 1:00 P.M. than in the stem tissue extracts. The levels of NRA in the leaf and stem tissue extracts were lower than the activity levels of the nitrite reductase, glutamine synthetase, glutamate synthase and glutamate dehydrogenase. The NADH-dependent form of the glutamate dehydrogenase has a greater activity on a fresh weight basis than the NADH-dependent form. Thus the difference in enzyme activities found in leaves and stems of *P. oleracea* in Table 4 is not likely to be the results of an inhibition or inactivation in a leaf or stem tissue extracts.

DISCUSSION

Acidification during the night period is an important characteristic of CAM plants (Randson *et al.*, 1960). CAM characteristics in a *P. oleracea* were investigated by Koch and Kennedy (1980). They reported that plants grown under the short day condition showed CAM-like pattern of acidity fluctuation, while no CAM activity was seen in well watered plants grown under 16-hour days. In this study, possible occurrence of CAM in *P. oleracea* under the natural environment and under the controlled conditions of long day and short day was investigated. Besides plants grown under water-stressed condition were also used to investigate CAM characteristics. In all of these cases stems showed an obvious CAM characteristic of acidity fluctuation. In leaves, CAM became clear under the short day condition. It has found that in short days, there is a progressively rapid increase in the enzyme activity of CAM pathway (PEP carboxylase, malate dehydrogenase, aspartate tranferase) and a net accumulation of malate(Brulfert *et al.*, 1975; Queiroz *et al.*, 1974).

Koch and Kennedy (1980) also tested stems without leaves and detached leaves. Stem tissues were acidified in the dark after leaves were removed, but total acid levels in them

were reduced from 148 to 93μ eq. /g. fresh weight. Both excised and intact leaves were deacidified in the light, but intact leaves lost 100μ eq. /g. fresh weight, compared to only 12μ eq. /g. fresh weight in those which were previously detached.

These results suggest the transport of some acid materials between stems and leaves. In this study, acidification during the night was greater in the intact stems than in the defoliated stems. At daybreak, moreover, rapid increase of acidity occurred in the intact stems, but only gradual increase in acidity was seen in the defoliated stems. It was thought from these results that CO_2 entered through open stomata in leaves may move to stems or that CO_2 be fixed to C_4 acids in leaves and then these C_4 acids move to stems. Stomata have not been found in stems yet, and stomatal resistance in *P. oleracea* leaves decreased suddenly as soon as sun rose(Fig. 6).

PEP carboxylase activity was measured at dawn to find out whether rapid acidification occurred in stems was due to a transport of HCO_3^- or that of C_4 acids. At 5:30 A.M., PEP carboxylase activitiy was very low, but crude enzyme extracted from leaf tissues had higher activities at that time rather than at 2:00 P.M. The fact that leaf tissues showed low level of acidity in spite of having a high PEP carboxylase activity at dawn and that stem tissues exhibited high acidification though their PEP carboxylase activity was very low can be the extensive proofs to our hypothesis that C_4 acids produced from CO_2 by PEP carboxylase in leaves(Hatch, 1975) move to stems while RuBP carboxylase in leaves does not have a sufficient activity for producing photosynthetic final products through Calvin cycle. Thus, an integrated scheme for transport could be suggested (Fig. 8). CO_2 fixed in leaves during the night and dawn may move to stems as C_4 acids, and there C_4 acids be decarboxylated during the day. CO_2 seperated from C_4 acids by the decarboxylating enzymes (Dittrich *et al.*, 1973) may be reassimilated into C_3 products through the Calvin cycle in the light(Osmond, 1978).

These expected procedures would give rise to CAM in stem tissues of *P. oleracea*. In the case of C_4 plants, since they can produce both C_3 and C_4 products of photosynthesis, the rate of one carboxylation reaction relative to the other may change the type of photosynthesis : C_3, C_4 or CAM (Kennedy *et al.*, 1973).

The dark CO_2 incorporation has been studied in C_4 plants (Kennedy, 1977; Kennedy & Laetsch, 1973; Miyachi, 1976). Malate contents of products by dark $^{14}CO_2$ incorporation was 57.8%, compared to 12.0% in the light, and the subsequent transfer of label from C_4 acids to phosphorylated compounds requires light in the *P. oleracea* (Kennedy *et al.*, 1973). But Kluge and Ting (1978) reported that malic acid synthesized in CAM is immobile and carbon previously bound in malic acid is transported in the phloem only after conversion in photosynthesis to carbohydrate. On the contrary, Kluge *et al.* (1979) reported that a transport of the intermediates of beta-carboxylation might be possible.

It is suggested in this paper that C_4 acids move from leaves to stems when RuBP carboxylases are not active yet. It has been known that RuBP carboxylase is activated directly or indirectly by the light and deactivated in the dark(Black, 1973).

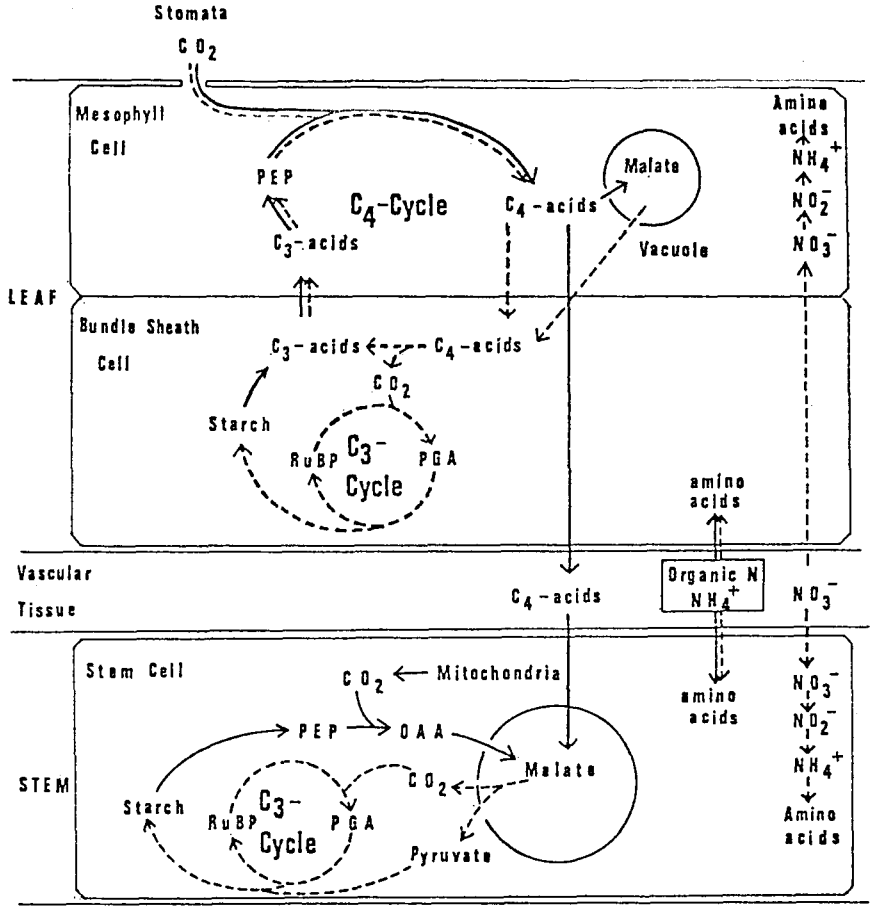

Fig. 8. An integrated scheme for intercellular pathways of CO_2 assimilation and nitrogen assimilation in leaves and stems of *Portulaca oleracea* L. →, reactions dominating at night; ----→, reactions dominating during the day.

The importance of considering events "downstream" from carbon fixation has been reinforced (Moss & Rasmussen, 1969). Such a rapid transport of photosynthate from the point of assimilation results in rapid photosynthesis (Laetsch, 1974). Even during the night and at dawn rapid transport of C_4 products would also increase CO_2 fixation in leaves, thus acidity level would become high in stems. CAM characteristics like this prevent the C_4 plants from losing much amount of water under the seriously acid condition, and when water is sufficient dark CO_2 fixation may be an auxillary process in addition to the active C_4 metabolism during the day. The C_4 plants are adapted to habitats where periods of extreme drought alternate with periods of abundant water. They must be able to survive during periods of drought, and they must be able to compete with rapidly growing mesophytes during periods when water is not limiting (Laetsch, 1974). An integrated

scheme for CO_2 fixation during the day and night suggested in this paper is able to explain these variable characteristics of C_4 plants properly.

Nitrate reduction was investigated together with CO_2 fixation. Both levels of NRA and nitrate uptake were high in the light. Light is directly required for the assimilation of nitrate into amino-N (Canvin & Atkins, 1974), or light has an indirect role via generation of reductant and nitrate movement (Beevers & Hageman, 1972).

In *P. oleracea,* stem tissues could reduce much amount of nitrate, and residual nitrate transferred to leaves could be assimilated there. Moor and Black (1979) proposed that the increased nitrogen use efficiency in crabgrass and other C_4 plants be due partially to a "division of labor" between mesophyll and bundle sheath cells. Nitrate assimilation in the mesophyll cells of corn leaves as related to the C_4 pathway of photosyhthesis was proposed by Neyra and Hageman (1978). C_4 plants utilize their available nitrogen more efficiently than C_3 plants in producing dry matter and in fixing CO_2 at atmospheric concentration (Borwn, 1978). Thus much more studies of the relationship between the photosynthetic C_4 pathway and nitrate assimilation in C_4 plants need to be done as a whole.

적 요

C_4형의 다육질 식물인 쇠비름(*Portulaca oleracea* L.)에서 CAM성격을 조사한 결과, 하루에 14시간씩 빛을 받는 경우에는 산도의 변화가 잎에 비해 줄기에서 현저한 반면에 8시간 동안 빛을 준 상태에서는 잎과 줄기에서 거의 비슷한 CAM성격을 보았다. 하루의 산도 변화가 줄기에서 뚜렷한 자연 상태의 쇠비름의 잎을 제거한 후 밤동안 산도 변화를 측정하였을 때, 새벽까지 점차적인 산도의 증가를 보였다. 그러나, 잎을 가지고 있는 정상적인 줄기는 밤 동안에 이보다 산도가 높았을 뿐 아니라 새벽에는 급격한 증가를 보였다. 그리고, 5:30 A.M에 측정된 PEP carboxylase의 활성도는 잎에서만 매우 높게 나타났고 줄기에서는 거의 활성을 보이지 않았다. 따라서, 잎에서 PEP carboxylase에 의해 합성된 C_4형 광합성의 초기 산물은 빛이 없거나 충분하지 않은 상태에서는 줄기로 이동되어 저장되었다가 빛에 의해 활성화된 RuBP carboxylase에 의해 재고정됨을 추론할 수 있다.

또한 쇠비름은 빛이 있을 때 많은 양의 NO_3^-를 흡수하여 일부는 줄기에서, 또 일부는 잎으로 이동되어 환원됨을 알 수 있었다.

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