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Adaptive CO_2 Fixation and Nitrate Assimilation of *Portulaca oleracea* in *Zoysia japonica* Community

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잔디군락에 출현하는 쇠비름의 CO_2 고정과 질소동화

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요 약

CO_2와 질소동화작용에 있어서의 잎과 줄기간의 세포간 관계가 잔디군락의 자연 환경 상태 하에서 자라는 *Portulaca oleracea* 에서 조사되었다.

기공이 줄기에서는 관찰되지 않는 반면 잎에서는 1 cm^2당 3,275개가 나타났다. 어린 쇠비름 잎의 기공의 저항은 밤에는 높고 낮에는 낮다. 최고 활성과 최저 활성사이의 차이는 원래 식물의 8번째 마디에서 최대가 된다. 최고 활성을 갖는 마디의 순서는 잎이 위에서 아래로 떨어짐에 따라 변화한다. *P. oleracea* 의 줄기조직은 CAM의 최고 활성을 나타내었으나 잎에서는 CAM의 활성이 나타나지 않았다. 빠른 산성화는 새벽에 원줄기에서 보여지나 줄기는 서서히 증가하는 것 밖에 관찰되지 않는다. RuBP carboxylase의 활성은 잎과 줄기 모두에서 오후 2시에 최고를 보이나 오전 1시와 5시 30분의 활성은 거의 관찰되지 않는다. 특히, 잎에서의 PEP carboxylase의 활성은 이른 아침에 줄기에서는 낮고 잎에서는 매우 높음을 보여준다.

이런 결과는 새벽에 열려진 기공을 통과할 CO_2가 PEP carboxylase에 의해 잎에서 동화되고 C_4생산물이 줄기로 이동함을 나타낸다.

Nitrate 축적과 nitrate reductase, nitrite reductase, glutamine symithetase, glutamate symthase, glutamade dehydrogenase의 농도는 잎에서 보다 줄기에서 더 높았고 밤보다 낮에 더 높다. 이것은 뿌리를 통해 흡수된 nitrate의 상당한 양이 줄기에서 동화되고 줄기 조직을 통해 잎으로 이동되어 거기에서 감소된다.

INTRODUCTION

The possible occurrence of CAM or facultative CAM in a succulent C_4 plant species was examined by Koch and Kennedy(1980, 1982). Many aspects of C_4 physiology in *P. oleracea*

have been established well, such as C_4 acid metabolism(Kennedy, 1977), enzyme activities(Kennedy, 1976), compensation point(Kennedy, 1977), anatomy and cytology(Kennedy, 1973), photorespiration(Kennedy, 1976). Photosynthetic rate(Kennedy, 1977), and response to salt and heat stress(Kennedy, 1977). Under 8- hour per days well watered plants showed a CAM-like pattern of acid fluctuation but under 16-hour per days well-watered plants showed no CAM activity(Koch and Kennedy, 1980). Karadge and Joshi(1980), and Koch and Kennedy(1982) showed that malate plays an important role in this CAM-like activity of *P. oleracea*.

The purposes of this study are to elucidate the mechanisms by which CAM activity and nitrate assimilation were occurred markedly in *P. oleracea* stems while its leaves had little CAM activity under the natural condition. The additional systems, C_4 and CAM pathways, become profitable through beta-carboxylation under interrelationship between leaves and stems.

MATERIALS AND METHODS

Naturally grown *P. oleracea* plants were sampled from a field in May. They were potted, and transferred to an open air place near the laboratory and grown for 4 weeks before experimental use. Diurnal change of titratable acidity was measured by the modified method of Chang *et al.* (1981). Stomatal resistance was measured with an Autoporometer(LI-65, LI-COR) in the *P. oleracea* leaves. RuBP and PEP carboxylases, nitrate reductase, nitrite reductase, NAD(P)H-dependent glutamate dehydrogenase, glutamate synthase and glutamine synthetase activities were determined according to the modified procedures of Chang *et al.* (1981).

RESULTS AND DISCUSSION

Titratable acidity was measured in leaves and stems of *P. oleracea* (Fig. 1) and the stomatal resistance in leaves only (Fig. 2) under natural sunlight on a longday condition (June 11, 1984). Acidity level in stems increased gradually during the night, at dawn it dropped a temporary measure and became higher remarkably till 9:00 A.M. In contrast, the acidity of deliated stems increased gradually through the night and even after dawn, and did not show a rapid increase in the morning. This diurnal acid fluctuation in leaves and stems of *P. oleracea* were equal to the results by Koch and Kennedy(1982) in well-watered *P. oleracea* grown under natural conditions in September.

The *P. oleracea* leaves showed a high stomatal resistance during the night which decreased rapidly at dawn. The plateau of stomatal resistance measured in detached leaves during the daytime was higher than that in the leaves of intact plants during the nighttime. These results indicate that stomatal opening occurred during the daytime. It suggests that the dual pattern of titratable acidity(Fig. 1) in intact stems without stomata

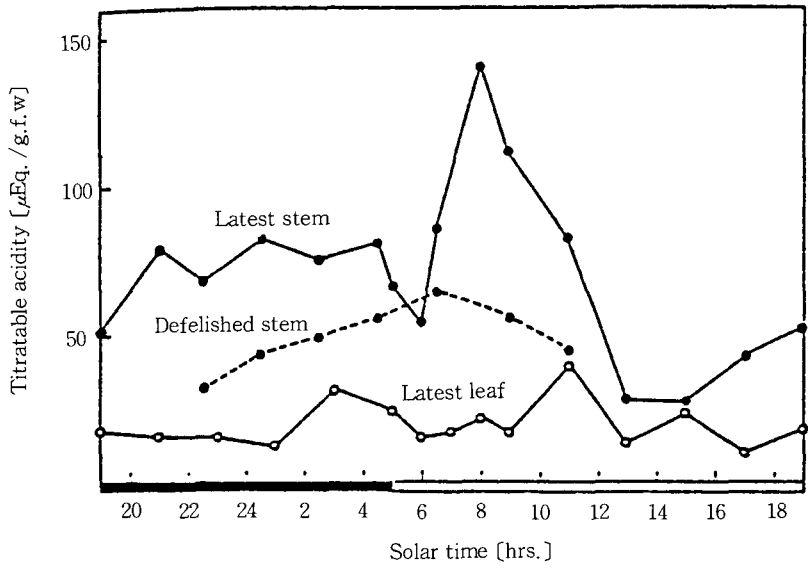

Fig. 1. Diurnal fluctuations of titratable acidity in leaves and stems of *P. oleracea* under natural conditions.

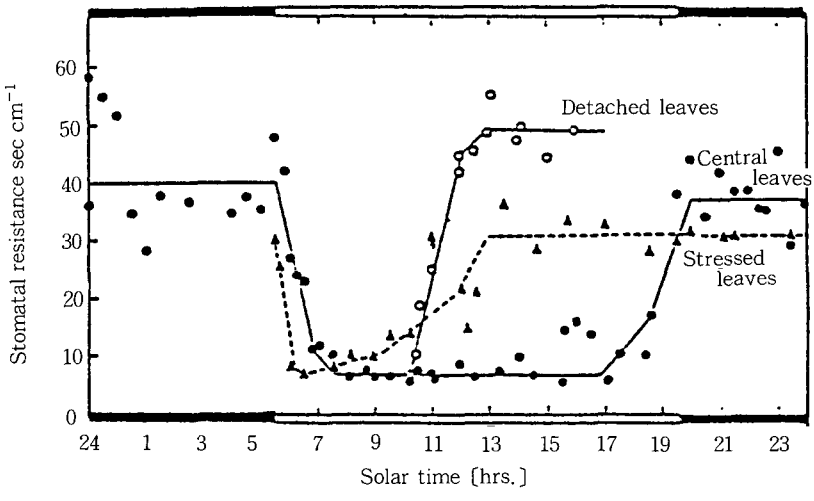

Fig. 2. Diurnal patterns of stomatal resistance for *P. oleracea* growing under natural conditions.

depends on an additional rapid rise at the time of initial stomatal opening of leaves.

Kranz-type arrangement was observed in leaves but not in stems. Stem cells of *P. oleracea* have large vacuoles and a few chloroplasts. The number of stomata was 3,275 cm^{-2} in leaves, but the stomata could not observed in stems.

Results of the assays for various enzymes of nitrate assimilation in *P. oleracea* leaves and stems are compiled in Fig. 1 and Table 2. Both nitrate reductase activity and nitrate ion concentration were higher in stems than in leaves. A peak of nitrate contents in stems

Table 1. The carboxylase activities from leaf and stem extracts of *P. oleracea* (μ mole /g.fw.hr.)

Time	RuBP Leaf	Carboxyalse Stem	PEP Leaf	Carboxylase Stem
14:00	461	339	1239	138
1:00	4	N.D.*	193	13
5:30	4	N.D.*	1484	20

* N.D.; Not detectable

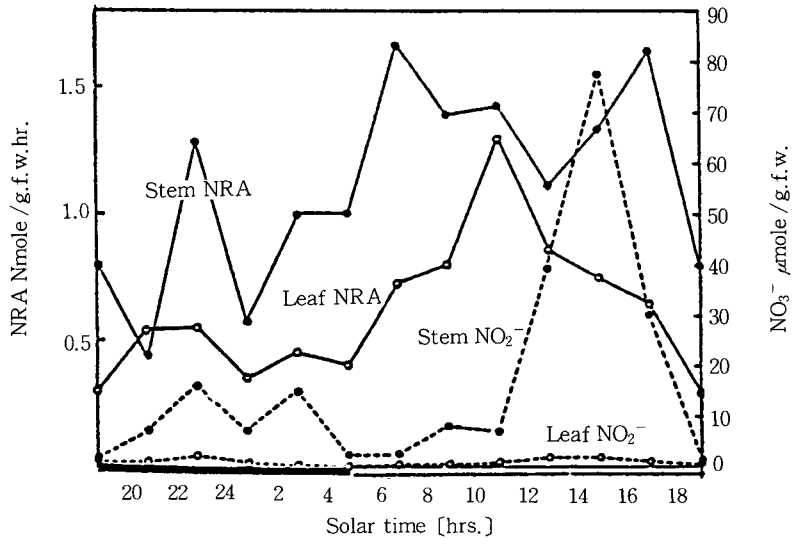

Fig. 3. Diurnal fluctuations of NRA and NO_3^- contents in leaves and stems of *P. oleracea* under natural conditions. NRA, nitrate reductase activity.

would be due to a transpiration through the open stomata and to a circadian rhythm(Pate, 1971). During the daytime, high levels of NRA may result from much amount substrate, NO_3^-, and light energy. The levels of the nitrite reductase, glutamine synthetase, glutamate synthase, and glutamate dehydrogenase activities in the leaves were lower than in the stems.

As shown in Fig. 4, the difference between the highest and the lowest acidities was the greatest at the 2nd node of the intact plants. When 1st pair of leaves on the top of the plant was removed, the level of acidity become lower but the 2nd node also showed the highest level of acidity. *P. oleracea* nodes showed gradual decrease of acidity and the order of the node having the highest activity was changed as the leaves were detached from the top to the bottom. These results also give a possibility of transport of an acid material from leaves to stems.

Both leaf and stem tissues showed very high RuBP carboxylase activity at 2:00 P.M. (Table 1). At 1:00 A.M. and 5:30 A.M., activity of this enzyme was hardly detected. PEP carboxylase extracted from leaves always showed higher activity than from stems. It is thought that absence of stomata in stems may result in producing a little amount of

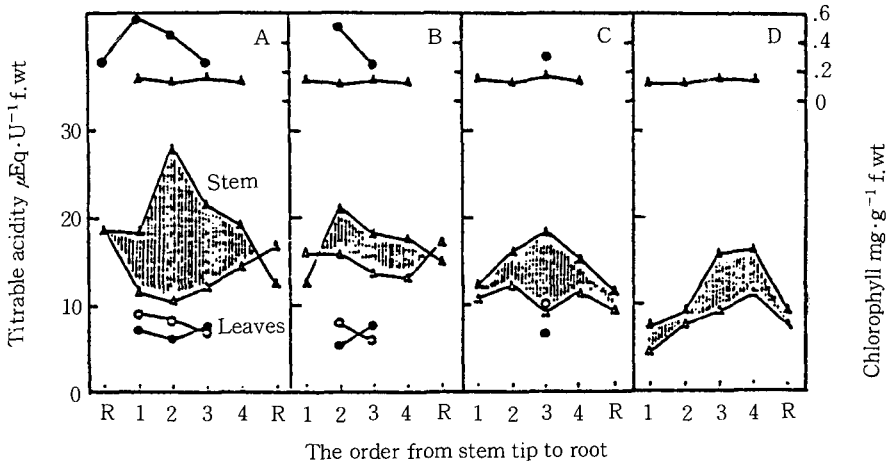

Fig. 4. Titratable acidity and chlorophyll contents in leaves and stems of the different order of the young purslane, *P. oleracea* under different treatments: A, control plants; B, plants whose buds and 1st pair of leaves were detached; C, whose buds and 1st, and 2nd pairs of leaves were detached; D,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

Table 2. The enzyme activities for nitrate assimilation in *P. oleracea* (μ mole /g.fw.hr.)

Enzyme	Leaf	Stem
Nitrate reductase	0.9	1.1
Nitrite reductase	3.9	5.4
Glutamine synthetase	6.4	8.6
Glutamate synthase	1.5	2.0
Glutamate dehydrogenase		
NADH-dependent	2.6	3.1
NADPH-dependent	1.0	1.9

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 night. At 5:30 A.M., PEP carboxylase activity was high in leaves but showed a very low level in stems. These results indicate that carboxylase activities show diurnal fluctuations under the natural conditions.

SUMMARY

The intercellular relationships between leaves and stems for pathways of CO_2 and nitrate assimilation were investigated in *Portulaca oleracea* growing under the natural environmental conditions in *Zoysia japonica* communmity.

The number of stomata was 3,275 cm^{-2} in leaves, while the stomata could not be observed in stems. Stomatal resistance in leaves of the young pursane was high at night and low in daytime. The difference between the highest and the lowest acidity was the greatest at the 2nd node of the intact plant. The order of the node having the highest acidity was changed as the leaves were detached from the top to the bottom. The stem tissues of *P. oleracea* showed high activity of Crassulacean acid metabolism(CAM) but no CAM activity was seen in the leaves. The rapid acidification was seen in the intact stems at dawn but defoliated stems showed only a gradual increase. RuBP carboxylase acitivity 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. Those 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, nitrite reductase, glutamine synthetase, glutamate synthase and glutamate dehydrogenase were higher in stems than in leaves. The levels were also higher in the light than in the dark. It would be suggested that cosiderable amount of nitrate absorbed from roots be assimilated in stems and nitrate transferred to leaves via stem tissues be reduced there.

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