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Effect of Carbon Dioxide Concentration on Malate and Titratable Acidity in *Pereskia aculeata and Kalanchoe rosea*

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The induction of crassulacean acid metabolism(CAM) characterized by day/night acid fluctuation was investigated in leaves from 10 days exposure to elevated CO_2 concentration(1,000 μL L^{-1} CO_2). For Pereskia aculeata, have C_3-like gas exchange pattern in well watered condition and shift into CAM-like in water stress, showed a more typical CAM-like diurnal acid fluctuation. Whereas the massive diurnal fluctuation of acidity in typical CAM of Kalanchoe rosea was declined. The effect of short-term exposure to various concentrations of CO_2 on titratable acidity in *P. aculeata* and *K. rosea* was also investigated. To investigate the response of various CO_2 concentrations, four different CO_2 levels(350, 700, 1,000 and 1,500 μL L^{-1}) were imposed for 24hr and measured the titratable acidity at 06:00, when the acidity was maximum, and 14:00, when the acidity was minimum. The accumulation of acid in *P. aculeata* was enhanced markedly by higher concentration of CO_2, while the level of acidity in *K. rosea* did not highly respond to CO_2. A notable difference between *P. aculeata* and *K. rosea* was the response of de-acidification to a higher concentration of CO_2. Increasing with CO_2, the degree of de-acidification of *P. aculeata* was increased while that of *K. rosea* was depressed.

Key words : Crassulacean acid metabolism, Titratable acidity

1. Introduction

Atmospheric CO_2 concentration could double in the next century if it continues to increase at the present rate(Gates, 1983). Elevated levels of atmospheric CO_2 can have many effects on plants. Doubling ambient CO_2 concentration can increase CO_2 uptake and productivity by 50% or more for C_3 crops, but only small effects occur for C_4 crops (Carson, 1982; Lawlor, 1991). Relatively little is known about the effects of elevated CO_2 on the CAM(crassulacean acid metabolism) plants. CAM is an important physiological and biochemical adaptation of photosynthetic carbon metabolism to water stress evolved to minimize water loss from terrestrial plants in arid environments(Osmond, 1978; Piepenbrock, 1994). This is a consequence of the most notable feature of CAM plants, i.e. nighttime stomatal opening and daytime stomatal closure. Thus while atmospheric CO_2 is assimilated, little water is lost since air temperatures are lower and humidities are higher at night.

CAM species are divided into three groups from the aspect of the responses to environmental conditions(Ting, 1985). In some CAM plants, photosynthetic CO_2 fixation is shifted from C_3-type into CAM-type by drought(weak CAM). In other species, even in the well-watered

condition, they fix CO_2 through CAM-type fixation pathway(full CAM). There are many reports that high concentration of CO_2 causes the stomatal closure. We want to know whether the high concentration of CO_2 induces CAM shift like water stress or not. Thus in the present study, we examined the effect of CO_2 enrichment on acid metabolism in two species of CAM. The species include that follow a typical CAM pattern (*Kalanchoe rosea*) and a species that shows C_3-like gas exchange pattern under the well-watered condition and shift into CAM-type by water stress(*Pereskia aculeata*).

2. Materials and Methods

P. aculeata and *K. rosea* were propagated from cuttings and were grown in a greenhouse. During the growth period, plants were well watered to prevent water stress. Before starting the experiment, plants were transferred into the growth room under day and night, 500 μmol photon $m^{-2}s^{-1}$ at the level of plants, and 70% of relative humidity.

To investigate the short-term effects of various CO_2 concentrations, plants were treated for 24hrs with 350, 700, 1,000 and 1,500 μL L^{-1} CO_2 and then were measured for titratable acidity and malate concentration and then the rate of acidification was estimated. To determine the long-term effect of elevated CO_2, 1,000 μL L^{-1} CO_2 was treated for 10 days and was measured for diurnal fluctuations of acidity in *P. aculeata* and *K. rosea*.

For the determination of acidity and malate contents, leaves were collected and fresh weights were weighed. These collected samples were frozen until use. Samples of leaf material (about 1g in fresh weight) were boiled with distilled water (80℃, 20min) and homogenized. Extracts were titrated to an end point of pH 7 with 0.02 N KOH. The results are expressed as μeq g^{-1} FW. For the determination of malate content, the extract was centrifuged at 15,000 g at 4 ℃ for 15 min, the supernatant fluid was adjusted to a final volume of 50 ml with distilled water and fluorometric enzyme analysis of malate was performed(Schmitt, 1992a). The reaction mixture contained buffer solution (0.4 M glycine, pH 9), 0.12 M NAD^+, 720 U mg^{-1} MDH(malate dehydrogenase) and 100 μL extract. Fluometric measurement was performed in Hitachi MPF-4 fluorescence spectrophotometer.

3. Results

Titratable acidity of *K. rosea* grown under 350 μL $L^{-1}CO_2$ showed a large(233 μeq g^{-1}FW) fluctuation from morning to evening. Fluctuations in titratable acidity of *P. aculeata* leaves were markedly different from those seen in leaves of *K. rosea*(Fig. 1). It show little or no diurnal fluctuation of acidity when compared to *K. rosea* and acid levels remained low throughout the day and night. In both plants, the maximum and minimum levels of acidity were detected at the end of the dark period(06:00) and the light period (14:00), respectively.

After the treatment of 1,000 μL L^{-1} CO_2 for 10 days in *P. aculeata*, moderate diurnal acid fluctuation was induced due to both a greater night accumulation and day depletion of acid relative to 350 μL L^{-1} CO_2. While in *K. rosea* the overall acidity levels were reduced by 10 days treatment of 1,000 μL L^{-1} CO_2. The maximum decline in acidity was observed at the end of the dark period (06:00) from 255 to 170 μeq g^{-1}FW.

P. aculeata and *K. rosea* were treated with 350, 700, 1,000 and 1,500 μL L^{-1} CO_2 for 24hrs and then were measured for diurnal changes of acidity(Fig. 2). The results shown in a figure are

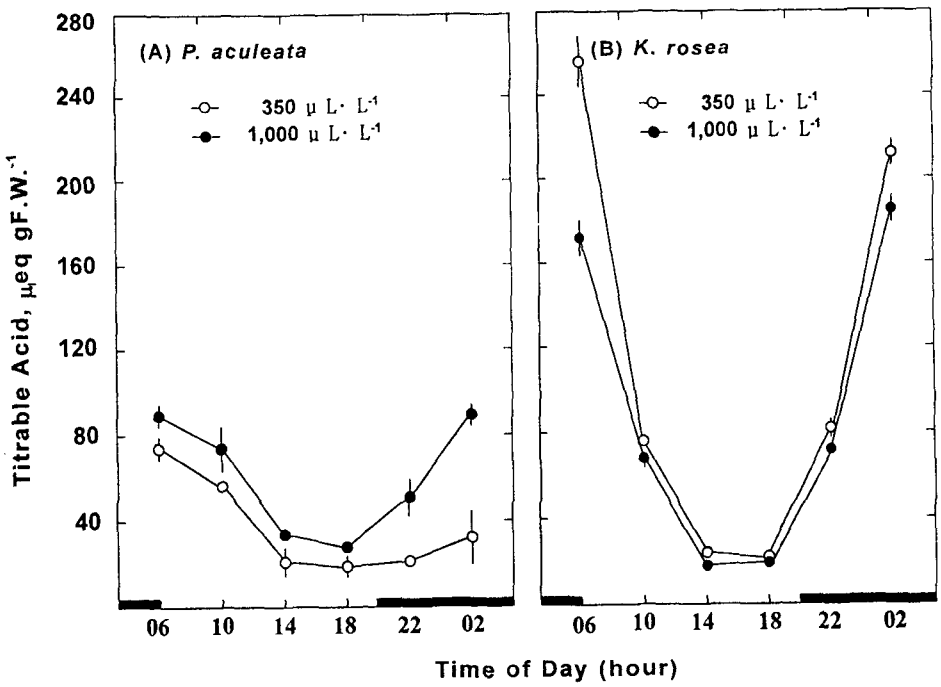

Fig. 1. Diurnal patterns of change in titratable acidity in *P. aculeata*(A) and *K. rosea*(B) under ambient(350 μL L^{-1} CO_2) and elevated CO_2. 1000 μL L^{-1} CO_2 was treated for 10days. Dark bars along the abscissa indicates the dark period. Each point represents the mean of three replicates with S.D.

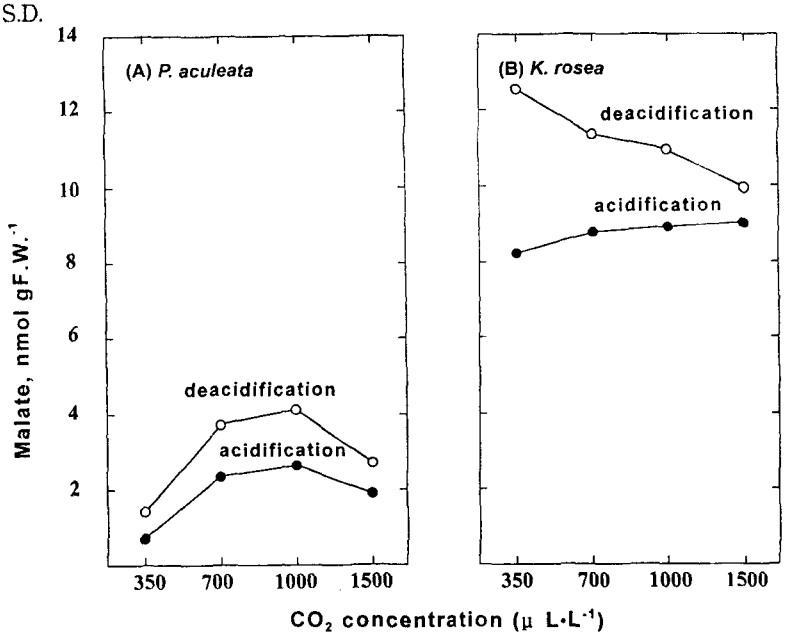

Fig. 2. Effects of CO_2 concentrations on titratable acidity in *P. aculeata*(A) and *K. rosea*(B) at 06:00 and 14:00. Four different CO_2 concentrations was treated for 24days. Each point represents the mean of three replicates with S.D.

those data obtained at dawn(06:00) when the acidity level was the highest and at noon(14:00) when the acidity level was the lowest. At dawn, titratable acidity in *P. aculeata* increased with increasing CO_2 up to 1,000 $\mu L\ L^{-1}$, but the acidity at noon increased to 1,500 $\mu L\ L^{-1}$ and became double. At dawn, acidity in *K. rosea* increased up to 700 $\mu L\ L^{-1}$ CO_2.

There was a linear relationship between the titratable acidity and malate in both plant species, though the slope of the line was different between the two(Fig. 3). The acidity is accounted for by malic acid. From these results we estimated the rates of de-acidification and acidification.

The effect of various concentrations of CO_2 on the rates of acidification and de-acidification are shown in Fig.4. The content of malate was estimated using the relationship between the acidity and the malate content shown in Fig.3. For the estimation of the rates of acidification and de-acidification, the titratable acidity was measured at 02:00, 06:00, 10:00 and 22:00. Then the rates of de-acidification and acidification were calculated by subtracting the acidity at 10:00 from that at 06:00 and by subtracting the acidity at 02:00 from that at 22:00, respectively. There was a significant difference between the two plants in responses of acidification and de-acidification to the CO_2 concentration. The acidification and de-acidification in *P. aculeata* increased with increasing CO_2 to 1,000 $\mu L\ L^{-1}$ and then decreased. The acidification in *K. rosea* was increased with increasing CO_2 concentration, but the rate of de-acidification was significantly decreased.

4. Discussion

The present results indicate that there are significant differences in diurnal changes of titratable acidity and the responses of acidification or de-acidification to various CO_2 concentrations between the two succulent species, i.e., *P. aculeata* and *K. rosea*.

Environmental factors play a role in regulating

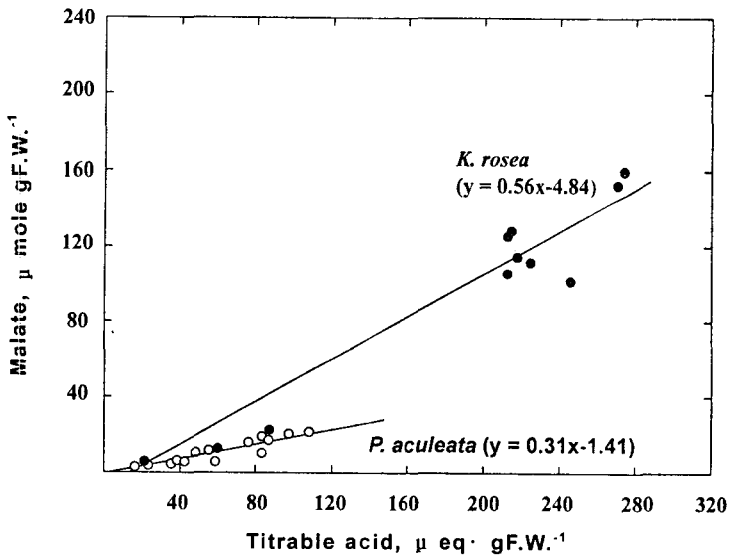

Fig. 3. The relationship between total acid and malate content in *P.* aculeata and *K. rosea*

photosynthetic carbon metabolism in certain succulent species from C_3 metabolism of photosynthetic CO_2 fixation in well-watered plants to CAM in water-stressed plants. *P. aculeata* is one of these succulent species(Ting, 1985). Because water-stress induce the daytime stomatal closure and increase the CO_2 concentration of intercellular air spaces(Cockburn, 1977; Cockburn, 1979), it may also cause the stomatal closure and induce the shift of *P. aculeata* from C_3 metabolism to CAM. On the other hand, *K. rosea* is so called "full CAM" with no shift from C_3 to CAM or vice versa by water status of plants.

Although a higher concentration of CO_2 (1,000 $\mu L\ L^{-1}$) increased overall acidity in *P. aculeata*, diurnal fluctuations of acidity was much smaller than those found in *K. rosea*. In contrast to *P. aculeata*, no significant changes in diurnal fluctuations induced by high CO_2 concentration of acidity could be detected in *K. rosea*. These results indicate that a typical CAM, *K. rosea*, show relatively insensitive response to CO_2 enrichment.

It is now well established that there was a close relationship between total organic acids and malic acid. Our results also showed that malate was linearly related(Fig. 3) the titratable acidity irrespective to CO_2 enrichment. The higher rate in de-acidification compared to acidification in both plants suggest that the rate of degradation in malate is faster than the rate of production in malate. These results might be supported by the hypothesis of Winter(1980) that the rhythm of malic enzyme capacity shifts much faster than

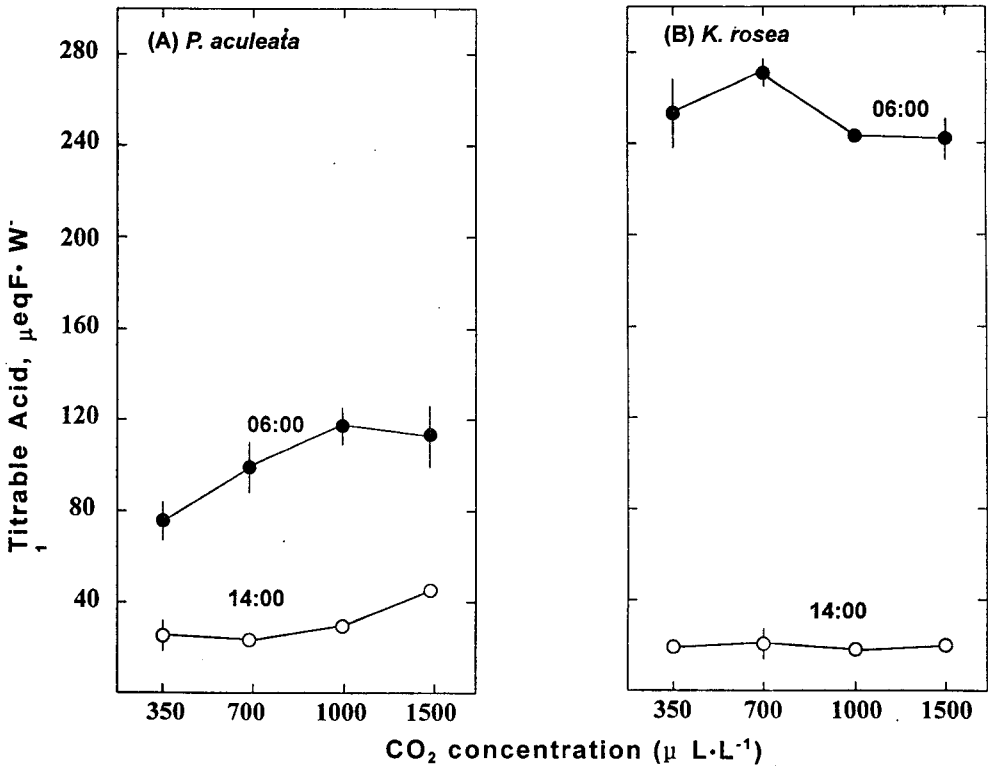

Fig. 4. The rate of change in de-acidification and acidification in *P. aculeata*(A) and *K. rosea*(B) at four different CO_2 concentrations. The values of malate were calculated from the data of titratable acidity in Fig. 2.

that of PEP carboxylase capacity. One notable difference between two species is the response on de-acidification by elevated CO_2 concentrations.

In contrast to that found in *P. aculeata*, the degree of de-acidification in *K. rosea* was greatly depressed by higher CO_2 concentration. The data of decreased de-acidification rate by elevated CO_2 in *K. rosea* supported the results of Nishida (1977), who described that CO_2 plays a regulatory role in de-acidification from the observation of decrease in de-acidification by higher CO_2 concentration. Walker(1960) reported that de-acidification of malate by NADP malate enzyme is inhibited by CO_2 enrichment in *Kalanchoe crenata*. Because there was 10 times higher malate concentration in *K. rosea* relative to *P. aculeata* even under the normal CO_2 condition, it appears that CO_2-induced suppression of de-acidification is probably due to the inhibition of malate enzyme by elevated CO_2 concentration. These different responses to high CO_2 treatment in two species suggest that CAM induction in *P. aculeata* is not caused by 10 days CO_2 enrichment. The enhancement of photosynthesis by higher CO_2 concentration is generally detectable in C_3 plants, however, such enhancement effect could not be detected in a full CAM species, *K. rosea*.

Some scientists(Woodwell, 1978) assume that the desert zones will be enlarged by the increase in CO_2 concentration. Under that condition, species possessing CAM may increase, however, photosynthetic CO_2 fixation in CAM species would not be increased and the growth of CAM species could not be enhanced(Osmond, 1978). After all, our results indicate that all CAM species, especially the weak CAM will play a role for the sink of increased CO_2 and endure long periods of aridity in arid environments.

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