Crassulacean Acid Metabolism Photosynthesis in Pineapple (Ananas comosus (L.) Merr.) Grown under Hydroponic Culture

Crassulacean Acid Metabolism Photosynthesis in Pineapple (*Ananas comosus* (L.) Merr.) Grown under Hydroponic Culture

Miki HORIE, Hiroo TAKARAGAWA, and Yoshinobu KAWAMITSU*

Department of Subtropical Agro-Environmental Sciences, Faculty of Agriculture,University of the Ryukyus,

Keywords: Gas exchange, CO_2 balance, Transpiration, Stomata, Hydroponic

*Correspondng author (E-mail: kawamitu@agr.u-ryukyu.ac.jp)

Abstract

In order to shorten the cultivation period of pineapple, a crassulacean acid metabolism (CAM) plant, it is important to increase the rate of CO_2 exchange rate and improve dry matter production, especially at the early growth stage. When plants are grown under hydroponic conditions, absorption of nutrients and plant water availability are generally increased, and in the case of CAM plants, hydroponics could be expected to improve growth rate through promoting stomatal open during daytime. The objective of this study was to examine the effect of culture method (hydroponic and soil culture) on diurnal changes in gas exchange rate and CO_2 balance in pineapple. The daily CO_2 balance under hydroponics tended to be higher than under soil culture, especially in Phase-4. This was due to a shortened Phase-3 and a prolonged Phase-4 under hydroponics compared to soil culture. In Phase-4, the CO_2 exchange rate was significantly correlated with stomatal conductance, indicating CO_2 balance was affected by stomatal activity. Rubisco consumption activated under hydroponic culture may have shortened the duration of Phase-3 when stomatal opening was suppressed. The CO_2 balance tended to be increased because of the shortening of Phase-3, resulting in higher dry matter productivity. From these results, it is suggested that hydroponic culture could promote growth in pineapple. However, net assimilation rate under soil culture was equivalent to that under hydroponics. Further study is needed to reveal the relationship between hydroponic cultivation and whole-plant photosynthetic ability.

1. Introduction

Pineapple (*Ananas comosus* (L.) Merr.) is a fruit crop of Bromeliaceae originating in tropical South America. It is commonly cultivated in the subtropical Okinawa prefecture in Japan, and the cultivation area is limited to the red acid soil (Kunigami Mahji) areas of the northern part of Okinawa island and Yaeyama islands[1]. The first fruit production requires almost 2 years under natural conditions, and the initial period with a slower growth rate accounts for one third of the cultivation time. It would be desirable to improve the growth rate in the initial stage to shorten the cultivation period and increase fruit yield[2].

Pineapple growth is driven by crassulacean acid metabolism (CAM) [3]. CAM plants have evolved a CO_2 fixation cycle adapted to dry climates alongside high water use efficiency[4]. In particular, CAM plants exhibit a unique diurnal change in CO_2 exchange rate (CER) during which they open their stomata at night to fix CO_2, in contrast to C_3 and C_4 plants which open their stomata during the daytime. According to Osmond[5], the diurnal change in CER is divided into four phases: Phase-1 with CO_2 uptake in the dark period, Phase-2 with CO_2 uptake in the early morning, Phase-3 with stomatal closure during the day, and Phase-4 with CO_2 uptake in evening. The CO_2 taken up in Phase-1 is first changed to oxaloacetic acid (OAA) by phosphoenolpyruvate carboxylase (PEP-C), after which the OAA is decomposed to CO_2 fixed mainly as malic acid stored in vacuoles. In Phase-2, PEP-C and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) work simultaneously to fix and assimilate CO_2. In Phase-3, the stomata are completely closed. Photosynthesis during this phase is driven by CO_2 generated by decarboxylation of malic acid, when CO_2 flows into the C_3 cycle at high concentration while photorespiration is suppressed. In Phase-4, the stomata are open and carbon assimilation by Rubisco begins after malic acid stored in the vacuoles is consumed[4]. In this way, photosynthesis in pineapple is driven during the day (when the stomata are closed) using CO_2 fixed and stored at night. Stomatal closure during daytime results in suppressed transpiration and increased water use efficiency, while the diurnal amount of carbon assimilation is limited by the quantity of CO_2 taken up during the period of darkness. Consequently, dry matter production is normally lower in CAM plants compared with C_3 and C_4 plants[6].

Plant biomass productivity could be improved by increasing the water potential of plants, facilitating stomatal opening and increasing the amount of carbon assimilation via Rubisco[7]. Nose *et al.*[8] investigated the relationship between biomass productivity in pineapple and soil moisture conditions, an environmental factor relating to plant water content, by measuring the leaf gas exchange rate. The study revealed that accumulated CO_2 assimilation, i.e., CO_2 balance, was not maximized under well irrigated conditions (at pF 1.1) because the waterlogged conditions caused physiological disorder; it was considered that the waterlogging led

to a reductive rhizosphere in the soil. Therefore, it is limited to improve water availability by changing soil water conditions; pineapple prefers to be grown in well-drained soil. According to Dhungel *et al*.[9], an increase in pineapple photosynthetic ability and yield has been confirmed with the use of aerated water subsurface drip irrigation. On this basis, we have considered the possibility of growing pineapple in hydroponic culture, which promotes water absorption by the roots[10] while supplying sufficient oxygen for the rhizosphere. Hydroponic culture of pineapple has been utilized as a means of evaluating the absorption characteristics of specific element[11, 12], but there have been no studies investigating the influence of hydroponic culture on CO_2 assimilation and metabolism as compared with soil culture.

The objective of the present research is to compare the effects of hydroponic versus soil culture on CAM photosynthesis with respect to improvements in biomass productivity in pineapple.

2. Materials and Methods

2.1. Plant materials

We examined pineapple *A. comosus* "Smooth Cayenne" cv. N67-10. Seven-month-old seedlings were obtained from Okinawa Prefectural Agricultural Research Center, Nago Branch, on July 25, 2017. After root washing, cutting, sterilization, and drying, eight average seedlings were selected. Four seedlings were planted in hydroponic culture equipment using polyvinyl chloride pipe through which nutrient solution was circulated (storage volume: 45L, Fig. 1), and the other four seedlings were planted in Wagner pots (1/2000 a) containing 10 kg of red soil (Kunigami Mahji) on August 23, 2017, in a greenhouse at the University of the Ryukyus (26°15′N, 127°45′E; altitude 127 m). While the hydroponic solution was changed every 2 weeks, the pH and EC were constantly adjusted to 5.0 ± 0.5 and 1.6 ± 0.4 mS cm^{-1}. On the other hand, the plants in soil were fertilized with 500 ml of liquid fertilizer once a week and irrigated well (soil pF 2.0–3.0). The liquid fertilizer was a modified Hoagland solution containing 3.0 mM $(NH_4)_2SO_4$, 2.5 mM $NaNO_3$, 1.5 mM K_2SO_4, 1.0 mM KH_2PO_4, 1.0 mM $MgSO_4 \cdot 7H_2O$, 25 µM H_3BO_3, 10µM $MnSO_4 \cdot 4H_2O$, 2 µM $ZnSO_4 \cdot 7H_2O$, 0.5 µM $CuSO_4 \cdot 5H_2O$, 0.5 µM $Na_2MoO_4 \cdot 2H_2O$, and 0.1 mM $C_{10}H_{12}FeN_2NaO_8 \cdot 3H_2O$.

2.2. Measurement of gas exchange

Diurnal changes in gas exchange rates were measured in an assimilation chamber according to the open system adopted by Kawamitsu *et al*[13-15]. The following method was employed to maintain the CO_2 concentrations in the assimilation chamber. First, an air compressor (ACP) pumped ambient air through a pipe column filled with soda lime (No.2, Wako) to remove CO_2. We used an arbitrary saturated water vapor pressure to pass this zero gas through a dew point control system connected to a constant water temperature circulator (CTE-82W, Komatsu-Yamato). The zero gas was mixed with 10% CO_2 (N_2 balance) to make 400 µmol mol^{-1} CO_2 and this was introduced into the assimilation chamber at a flow rate of ~5.0 L min^{-1}.

The growth chamber was fitted with a white fluorescent light source (PPFD 270 µmol m^{-2} s^{-1}) providing 12 h light (06:00–18:00) and 12 h dark (18:00–06:00). The air temperature was kept constant by precision cooling circulators (CL100, Yamato). To acclimate the plants under these environmental conditions, the sample plants were introduced into the growth chamber one or two days prior to measurement. After acclimation, gas exchange was measured over 3 days. Results observed only from on the second day to the third day were adopted. The D-leaf, the largest leaf with high physiological activity[11], was used for measurements. Leaf temperature was measured by a T-type thermocouple affixed to the back of the leaf. CO_2 and H_2O at the inlet and the outlet of the assimilation chamber were measured by an IRGA (LI-820A, Li-Cor). These data were D/A-converted every 10 min by a data logger (DA-100, Darwin) and transferred to a personal computer. In the computer, the CO_2 exchange rate (CER), stomatal conductance (g_s) and transpiration rate (E) per unit leaf area were calculated according to the equations of von Caemmerer and Farquhar.[16] The integrated CER in every phase was taken as CO_2 balance, and the CO_2 balance of Phase-1 divided by total CO_2 balance was taken as CAM ability.[17]

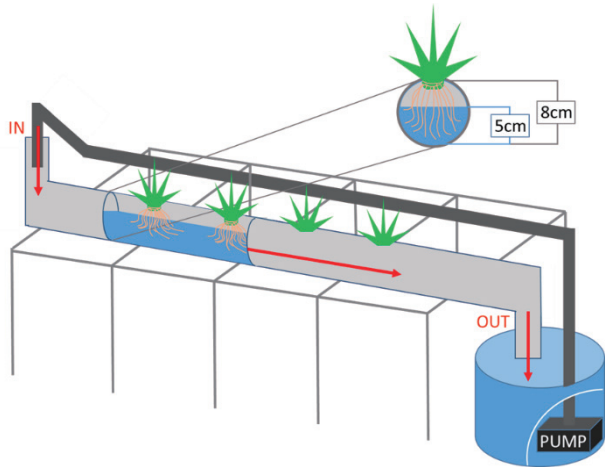

Fig. 1. The outline of hydroponics equipment.
The arrows show the directions of water flow.

2.3. Measurement of organic acid content

Malic acid (malate) content in the leaves was determined by the method of Kawamitsu *et al*[18]. Leaf disks (total 2.25 cm^2) were sampled from the base of the same tip leaves which used for the gas exchange measurement, frozen and fixed with liquid nitrogen, and then stored at −180°C. Samples were taken at six times during each 24-h period: 18:00, 21:00, 24:00, 06:00, 08:00, 12:00, and 15:00. The samples were added to 5 ml of extraction buffer [200 mM Bicine-NaOH pH 8.2, 0.2 mM EDTA, 0.5 mM DTT, 2 mM iodoacetic acid, 50 mg Polyethylene Glycol 2000], a pinch of sea sand (particle diameter 425–850 µm), and 0.03 g PVP, and the mixture was quickly ground in a mortar. The mixture was then

filtered by miracloth (Miracloth, Calbiochem) and 3 ml of the filtrate was added to 1 ml of 0.5% HClO. The extract was centrifuged at 3000 rpm for 10 min, the supernatant was filtered by membrane filter (particle diameter 0.45 µm) and the filtrate was measured by HPLC with automatic sampler (SIL-10A), detector (CDD-6A), pump (LC-10A), and column (SCR-102H, all from Shimadzu Corp.). During measurement, the temperature of column was and the flow rate were fixed at 85°C and 0.8 ml min^{-1}, respectively.

2.4. Plant sampling

Plants were harvested before the start of treatment and on 153 days after treatment, and then divided into component parts. Leaf area was measured by a leaf area meter (LI-3100, Li-Cor) and dry weight was measured after hot air drying at 80°C for 1 week. Growth analysis was assessed using these data. The nitrogen contents of leaves were measured with N-C analyzer (NC-90A, Shimadzu).

2.5. Data analysis

The CO_2 balance and malate content were measured with only one replicate. Therefore, it was not possible to conduct statistical analyses using the data obtained. The relationship between CER and g_s was analyzed by correlation analysis ($p < 0.01$). Statistical differences in leaf area and dry weight between treatments were analyzed by t-test ($p < 0.05$).

3. Results

3.1. Leaf gas exchange, CO_2 balance, and CAM ability

Under both culture conditions, the diurnal change in gas exchange rate exhibited the characteristics of CAM photosynthesis (Fig. 2). The duration of each phase was defined based on the CER under hydroponic culture to avoid the effect of the different end time of CO_2 uptake suppression period on the results of CO_2 balance as follows: Hydroponic Phase-1: 12 hours (18-6 o'clock), Phase-2: 2 hours (6-8), Phase-3: 3 hours (8-11), Phase-4: 7 hours (11-18). Soil Phase-1: 12 hours (18-6 o'clock), Phase-2: 2 hours (6-8), Phase-3: 4 hours (8-12), Phase-4: 6 hours (12-18). Characteristics of each phase were compared between hydroponic and soil cultures (Fig. 2).

In the Phase-1, CER, g_s and E were higher for plants grown hydroponically at the beginning of the phase i.e., night-time (Fig. 2). CER then peaked at 18:30–20:30 and gradually decreased until the end of the phase. The maximum CER was approximately 7.0 µmol m^{-2} s^{-1} under hydroponic culture and 4.0 µmol m^{-2} s^{-1} under soil culture (a difference of ~3.0 µmol m^{-2} s^{-1}). CO_2 balance in hydroponic culture during Phase-1 tended to be higher than in soil culture, the rate of increase being ~40% (Table. 1).

In Phase-2, maxima for CER, g_s and E appeared at same time in both treatments but tended to be higher in hydroponic culture (Fig. 2). The CO_2 balance in Phase-2 was similar between treatments (Table. 1). The maximum value for g_s during Phase-2 tended to be higher than that in Phase-1, but the maximum for CER, observed simultaneously with g_s, tended to be lower than that in Phase-1 (Fig. 2).

Since Phase-3 was the CO_2 uptake suppression period, CO_2 uptake was suppressed by low g_s (Fig. 2 & Table 1).

In Phase-4, values for CER, g_s, and E under hydroponic culture rose higher than under soil culture (Fig. 2). The difference tended to be shown in CO_2 balance where plants grown hydroponically increased their CO_2 balance by ~142% compared to plants grown in soil. This rise was greater than in Phase-1 (Table. 1). The rise in CER in Phase-4 under soil culture was delayed about 2 h (Fig. 2). There were large differences between treatments in CER, g_s and E at the end of Phase-4, with values for plants grown under hydroponic culture tending to be higher (Fig. 2).

CAM ability, the ratio of nocturnal CO_2 balance in Phase-1 to total CO_2 balance, tended to be higher for plants grown hydroponically than in soil.

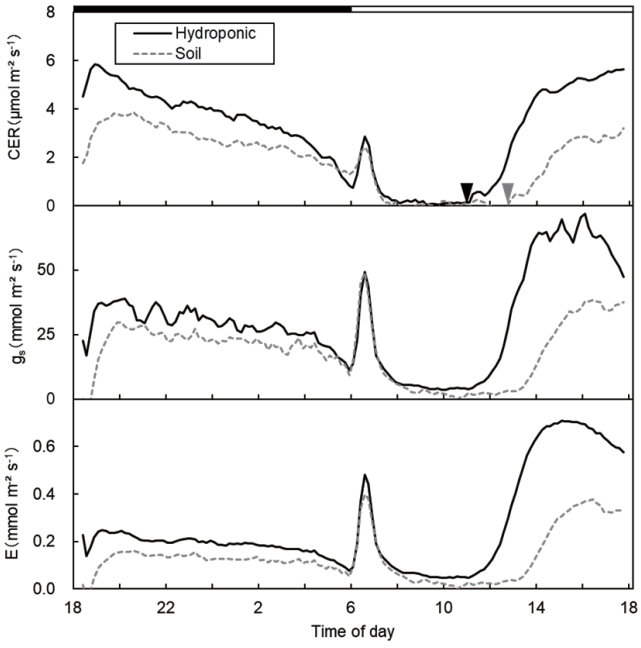

Fig. 2. Effects of culture methods on diurnal changes in CO_2 exchange rate (CER), stomatal conductance (g_s) and transpiration rate (E) in pineapple. Note: The starts of Phase-3 were shown under hydroponics (▼) and soils (▼).

Table 1. Effect of culture method on CO_2 balance and CAM ability.

Culture	CO_2 balance (mmol m^{-2} $period^{-1}$)					CAM ability (%)
	Phase-1	Phase-2	Phase-3	Phase-4	Total 24h	
Hydroponic	160.9	8	0.8	93.4	263.2	61
Soil	115.4	6.1	0.8	38.1	160.3	72
Hydr / Soil[a]	1.4	1.3	1.0	2.5	1.6	0.8

Note: [a] the ratio of Hydroponic to Soil culture.

3.2. Diurnal cycle of malate content

Fig. 3 illustrates the diurnal change in pineapple leaf malate content grown under hydroponic and soil culture. Both treatments showed similar time-course trends in malate content. Malate started to accumulate at 18:00 at the beginning of Phase-1 and reached a maximum at 06:00 at the end of this phase. Plants grown hydroponically tended to show a higher value than those grown in soil during Phase-1. Subsequently, malate content in both treatments decreased to zero at 12:00 to 15:00 when the difference between treatments became small.

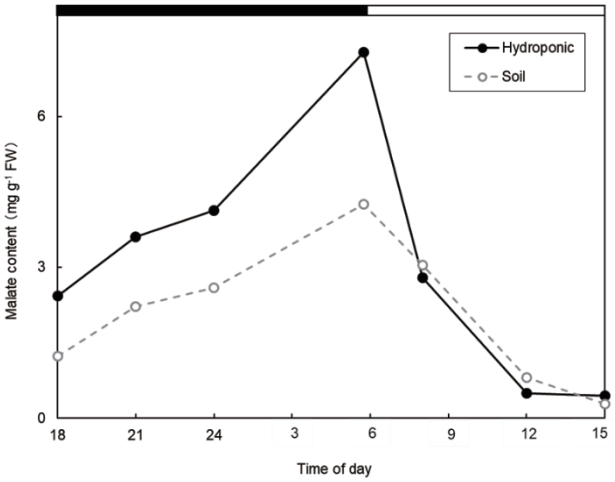

Fig. 3. Effect of culture method on time courses of leaf malate content in pineapple.

3.3. Dry weight of shoot, root, whole plant, and leaf area

Shoot and total dry weight under hydroponic culture tended to be higher than under soil culture (Table 2), with leaf area, in particular, significantly increased under hydroponics. The increment in total dry weight was due to an increase in shoot dry weight because root dry weight did not differ between treatments. With respect to growth analysis, relative growth rate, leaf area ratio (LAR), and specific leaf area (SLA) of plants grown hydroponically were higher than those grown in soil only, significantly so for LAR and SLA (Table 2). In contrast, the net assimilation rate (NAR) of plants grown hydroponically was lower than those grown in soil.

4. Discussions

The pineapple plants grown both hydroponically and in soil showed similar trends in diurnal changes in CER, g_s, and E (Fig. 2). In CAM photosynthesis, CO_2 uptake is closely related to stomatal opening.[3,19] The correlation between CER and g_s recorded every 10 min is shown in Fig. 4. In Phase-1, 2, and 4, significant positive linear correlations are observed in both hydroponic and soil cultures, indicating that CER was closely related to stomatal opening. Thus, it is suggested that the increase in total CO_2 balance in hydroponic culture was mainly due to the facilitation of stomatal opening. In addition, when comparing the CO_2 balance of each Phase for each cultivation method, the ratio of hydroponic to soil culture was highest in Phase-4 (Table 1). These results suggest that the prolonged duration of stomatal opening in Phase-4 due to the shortening duration of Phase-3 may have influenced the increase in total CO_2 balance for the whole day (Fig. 1 and Table 1). The stomatal closure in Phase-3 occurs as a reaction to high CO_2 produced by decarboxylation of malic acid, while subsequent stomatal opening in Phase-4 occurs as the internal CO_2 concentration decreases owing to carbon fixation by Rubisco.[4] On this physiological basis, the early stomatal opening exhibited in hydroponic culture may have been due to the decrease in susceptibility to high CO_2 concentration or to the increase in the consumption rate of malate by activation of malic enzyme. However, malate content at 12 pm, the turning point from Phase-3 to Phase-4, was close to zero for plants grown both hydroponically and in soil, indicating that decarboxylation of malic acid was almost complete (Fig. 3). Therefore, the main reason for early stomatal opening in Phase-4 may have been not the activity of malic enzyme in Phase-3 under hydroponic culture but the consumption speed of the high concentration of CO_2 generated after decarboxylation of malic acid, i.e., Rubisco activity. Also, it is suggested that Rubisco activity may have been higher in hydroponics considering significantly higher total nitrogen contents of leaves than that in soil culture (Table 2). As the carbon accumulation in Phase-3 was concurrent with stomata closing, Rubisco activity in this phase could not be measured in this study. Further research is required to investigate Rubisco activity in Phase-3 to identify factors responsible for this phenomenon.

Table 2. Effect of culture method on dry weight, leaf area, leaf nitrogen contents, relative growth rate (RGR), leaf area ratio (LAR), net assimilation rate (NAR) and specific leaf area (SLA) in pineapple.

Culture	Dry weight (g plant^{-1})			Leaf area (cm^2 plant^{-1})	Leaf N (g m^{-2})	RGR (g g^{-1} day^{-1})	LAR (m^2 g^{-1})	NAR (g m^{-2} day^{-1})	SLA (cm^2 g^{-1})
	Shoot	Root	Total						
Hydroponic	34.4 ±7.8	6.7 ±1.8	41.8 ±9.7	2027*±454	5.21*±0.26	0.015	0.0058*	2.65	49.57*
Soil	27.0 ±2.8	6.6 ±1.0	35.6 ±7.7	1344 ±259	3.37 ±0.28	0.014	0.0051	2.76	39.72
Hydr / Soil[a]	1.3	1	1.2	1.5	1.5	1.1	1.14	0.96	1.25

Note: * means significantly difference between treatments ($P< 0.05$, n=4).

[a] the ratio of hydroponic to soil culture.

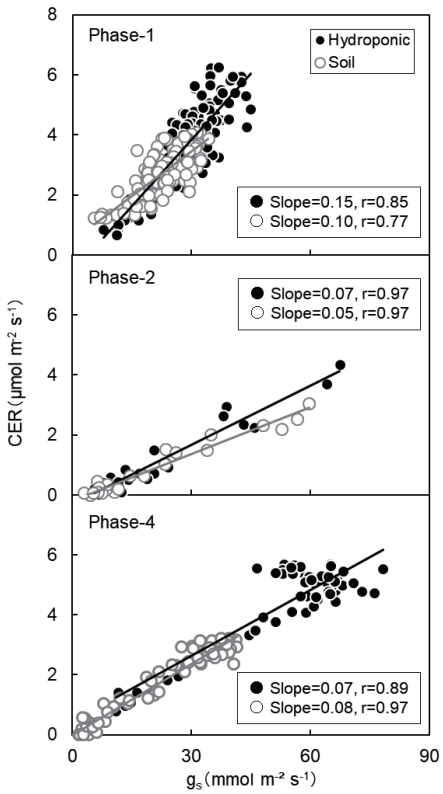

Fig. 4. Effect of culture method on the relationship between CO_2 exchange rate (CER) and stomatal conductance (g_s) during each phase in pineapple.
Note: All correlation coefficient had significance, 0.1% level.

Total CO_2 balance and leaf area per plant grown under hydroponics were higher than those of plants grown in soil (Tables 1 and 2). However, the ratio of total dry weight between hydroponic and soil culture was small, indicating that the effect of CO_2 balance per individual leaf on total dry weight was small. There are two possible reasons for this disagreement. The first may be the difference between environmental conditions in the assimilation chamber during gas exchange measurement and those under natural growth conditions. Several climate factors such as temperature, light intensity, and day length change naturally and temporally which may be more suitable for pineapple photosynthesis than the controlled conditions of the experiment. Pineapple photosynthesis has been shown to be influenced by such environmental conditions[20]. For example, Neales [21] changed the night temperature between 15°C and 35°C and showed a negative correlation between the temperature and pineapple CO_2 balance. In the present study, it is possible that the photosynthetic capacity may have declined in the period from July to October (data not obtained) when the monthly average temperature was higher than the experimentally-fixed night temperature for photosynthesis measurement (25°C). On the other hand, the fact that plants grown under hydroponics were more sensitive to the change in environmental conditions indicates that plant productivity could be improved easily by controlling these factors.

The present study exhibited that photosynthetic ability differed between individual leaves and the whole plant. The influence of leaf position on malic acid content has been reported in *Kalanchoe daigremontiana* and *K. blossfeldiana*[22]. In addition, it is known that the physiological ability of the D-leaf was higher than those of other leaves[11]. Therefore, in this study, it is suggested that the photosynthetic ability of aged leaves was decreased, especially under hydroponics, resulting in the small differences seen in dry weight between treatments. This was also supported by the fact that the leaf area was higher in hydroponic culture than in soil culture (Table 2) and that the NAR was nearly equal for plants grown hydroponically and in soil (Table 2). It has been shown that aging of leaves in paddy rice is promoted when stomata open well, which causes "over-transpiration" [23]. Similarly, in the case of the hydroponics in this study where gs and E were high (Fig. 2), it is possible that aging was promoted due to over-transpiration. Since there was a negative correlation between SLA and leaf longevity (i.e., life span)[24], leaf longevity was suggested to be shorter in hydroponics with significantly higher SLA than that in soil culture (Table 2). In agreement with a high SLA, the leaves of plants grown hydroponically seemed thinner, softer, and more sensitive to scorch than those of soil-grown plants (visual observation, no data obtained). It suggests that the development of the cuticular layer may have been suppressed under the hydroponic conditions. Thus, leaf morphology of pineapples grown under hydroponic culture could lead to an acceleration in transpiration rate. To clarify this, it would be necessary to further investigate the photosynthetic characteristics in leaves at different positions and the relationship between leaf aging and the silica content of the leaf cuticle.

References

1) Shoda, M. 2011. Pineapple. Food preservation science. 37(5): 249-255. (in Japanese with English summary)
2) Onaha, A., Ikemiya H. and Nakasone F. 1986. Studies on the dry matter production of pineapple; relationships between dry matter production and an establishing process of yield. J. Jpn. Soc. Hort. Sci. 54(4): 438-449. (in Japanese with English summary)
3) Neales, T. F. 1975. The gas exchange patterns of CAM plants. Env. Bio. Cont. Photosynthesis. Marcelle, R. (ed.): 299-310.
4) Kluge, M. and Ting I. P. 1978. Crassulacean acid metabolism analysis of an ecological adaptation. Springer-Verlag Berlin Heidelberg. Ecological studies. 30. p.209.
5) Osmond, C. B. 1978. Crassulacean acid metabolism: A curiosity in context. Plant Physiol. 29: 379-414.
6) Shikata, S. 1983. Green energy project (6). Agriculture and horticulture 58(3): 383-388. (in Japanese with English summary)
7) Kramer, P. J., Boyer J. S. 1995. Water relations of plants and soils. Academic Press, Inc. p.495.
8) Nose, A., Miyazato K. and Murayama S. 1981. Studies on

matter production in pineapple plant. Ⅱ. Effects of soil moisture on the gas exchange of pineapple plants. Jpn. J. Crop Sci. 50(4): 525-535.

9) Dhungel, J., Bhattarai S. P., Midmore D. J. 2012. Aerated water irrigation (oxygation) benefits to pineapple yield, water use efficiency and crop health. Adv. Hort. Sci., **26**(1): 3-16

10) Nakano, A., Kawashima H., Watanabe S. and Uehara Y. 2005. Effects of cultivation methods on the yield, sugar content δ15N value of tomato. Bull. Natl. Inst. Vege. Tea Sci. 4: 1-7.

11) Sideris, C. P., Krauss B. H. and Young H. Y. 1938. Assimilation of ammonium and nitrate by pineapple plants grown in nutrient solutions and its effects on nitrogenous and carbohydrate constituents. Plant Physiol. 13(3): 489-527.

12) Lin, Y. H. and Chen J. H. 2011. Effects of aluminum on nutrient uptake in different parts of four pineapple cultivars. Afr. J. Agric. Res. 6(6): 1438-1446

13) Kawamitsu, Y., Naka M., Nakayama H., Sekizuka F. 1995. Effects of elevated CO_2 on Crassulacean Acid Metabolism in Phalaenopsis. The science Bull. Fac. Agr. Univ. Ryukyus. 42: 23-32. (in Japanese with English summary)

14) Kawamitsu, Y., Nakayama H., Takeuchi M. and Murayama S. 1999. Effect of CO_2 concentration on gas exchange rate and CO_2 balance in some CAM plants. Bull. Fac. Agr. Univ. Ryukyus. 46: 15-27. (in Japanese with English summary)

15) Kawamitsu, Y., Oshiro T. and Murayama S. 2001. The effect of continued light period and temperature change on the diurnal changes in CO_2 exchange rate and stomatal conductance in pineapple. Bull. Fac. Agr. Univ. Ryukyus. 48: 39-52. (in Japanese with English summary)

16) von Caemmerer, S. and Farquhar G. D. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta. 153(4): 376-387.

17) Nose, A., Matake S., Miyazato K. and Murayama S. 1985. Studies on matter production in pineapple plants. Ⅲ. Effects of nitrogen nutrition on the gas exchange of shoots. Jpn. J. Crop Sci. 54(3): 195-204.

18) Kawamitsu, Y., Shigehisa R., Fukuzawa Y. and Murayama S. 2002. On the factors controlling stomatal closure during light period in pineapple. Bull. Fac. Agr. Univ. Ryukyus. 49: 1-14. (in Japanese with English summary)

19) Kluge, M. and Fisher K. 1967. Relations between CO2-exchange and transpiration in bryophyllum daigremontianum. Planta (Berl.) 77(3): 212-223. (in German with English summary)

20) Nose, A. 1992. Variations in crassulacean acid metabolism (CAM) and its regulation. Jpn. J. Crop Sci. 61(1): 161-171. (in Japanese with English summary)

21) Neales, T. F. 1973. Effect of night temperature on the assimilation of carbon dioxide by mature pineapple plants, Ananas comosus (L.) Merr. Aust. J. Sci. 26(3): 539-546.

22) Buchanan-Bolling, I. C., Kluge M. and Lüttge U. 1980. PEP-Carboxylase Activities and the Regulation of CAM: Effects of Extraction Procedures and Leaf Age. Zeitschrift für Pflanzenphysiologie. 97: 457-470. (in German with English summary)

23) Agarie, S., Agata W., Kubota F. and Kaufman P. B. 1992. Physiological roles of silicon in photosynthesis and matter production in rice plants. Jpn. J. Crop Sci. 61(2): 200-206. (in Japanese with English summary)

24) Reich, P. B., Walters M. B. and Ellsworth D. S. 1992. Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. Ecological Monographs, 62(3): 365-392.

水耕栽培がパイナップルの CAM 型光合成に与える影響

堀栄美希，寶川拓生，川満芳信*
琉球大学農学部亜熱帯農林環境科学科

キーワード：ガス交換，CO_2 収支量，蒸散，気孔，水耕栽培

要約

CAM 型光合成植物であるパイナップルの栽培期間の短縮には CO_2 交換速度を増大させ乾物生産を向上させることが重要である．植物を水耕栽培すると養水分の吸収が容易に行われるため植物体内の水分条件は改善され，CAM 植物の場合，明期の気孔開孔促進が期待される．そのため，本研究ではパイナップルの物質生産能力の向上を目的に，水耕栽培が CO_2 交換速度の日変化および CO_2 収支量に与える影響を土耕栽培と比較検討した．水耕栽培したパイナップルの CO_2 交換速度の日変化は土耕と同様に CAM 型特有のものであった．1 日の総 CO_2 収支量は水耕区が土耕区に比べ高く，特に，明期後半の CO_2 吸収期（Phase-4）の CO_2 収支量の増加が著しかった．これは CO_2 吸収抑制期（Phase-3）から Phase-4 への転換期が約 2 時間早くなり，Phase-3 が短縮され Phase-4 が長期化したことによる．Phase-4 の CO_2 交換速度と気孔伝導度の間には高い正の相関関係が見られ，CO_2 収支量は気孔の影響を受けていた．Phase-4 への転換期において，リンゴ酸の脱炭酸が土耕・水耕ともに終了していることから，水耕栽培でリブロース 1, 5-ビスリン酸カルボキシラーゼ（Rubisco）が活性化し，気孔開孔の阻害時間が短縮されたと推察される．Phase-3 が短縮されたことで CO_2 収支量が増大し，土耕栽培より高い乾物生産能を示したことから，水耕栽培法はパイナップルの成長を促進させることが示唆された．