Stimulation of CAM Photosynthesis in *Kalanchoë blossfeldiana* by Transferring to Nitrogen-Deficient Conditions

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ABSTRACT

Kalanchoë blossfeldiana Poelln. cv Hikan plants were grown hydroponically with nutrient solution containing 5 millimolar NO_3^- (or NH_4^+) for 1 to 2 months and then transferred to nutrient solution containing no nitrogen. CO_2 uptake at night, nocturnal increase in titratable acidity, and activity of phosphoenolpyruvate carboxylase increased after the transfer. Thus, transfer to nitrogen-deficient conditions stimulates Crassulacean acid metabolism (CAM photosynthesis) in *K. blossfeldiana*. The importance of the plant nitrogen status (nitrogen-withdrawal status) for induction and stimulation of CAM photosynthesis is discussed.

CAM photosynthesis is one of the three major pathways of photosynthetic CO_2 assimilation in green plants. The activity of CAM photosynthesis responds to external and internal factors such as plant water relations, photoperiod, temperature, light intensity, leaf age, genotype, and so forth (7, 12). Induction or stimulation of CAM photosynthesis by water stress, photoperiod, and leaf age has been extensively studied in several succulent plants (7, 12, 14, 19, 23). There are some reports of effects of nutrients on CAM photosynthesis (9–11, 21), and the plants grown with lower N were observed to have low activity of CAM photosynthesis (10, 22). But the effect of N on CAM photosynthesis is insufficiently investigated. So in the present study, I investigate the effects of transfer to N-deficient conditions on CAM photosynthesis in *Kalanchoë blossfeldiana* grown on N-enriched conditions and report the importance of N status for activity of CAM photosynthesis.

MATERIALS AND METHODS

Plant Culture. Cuttings of *Kalanchoë blossfeldiana* Poelln. cv Hikan having 2 or 3 pairs of leaves were rooted in sand without nutrients and then transferred to solution culture. Three of the plants were held by vermiculite in a plastic basket, and the basket was placed on a 1/5000 are (3.8 L) Wagner pot which contained 3 L of nutrient solution. Constituents of the nutrient solution were 1 mM KH_2PO_4, 2 mM K_2SO_4, 2 mM $MgSO_4·7 H_2O$, and 4 mM $CaCl_2·2 H_2O$ for macroelements, and 10 μM $MnSO_4·4 H_2O$, 1 μM $ZnSO_4·7 H_2O$, 1 μM $CuSO_4·5 H_2O$, 50 μM H_3BO_4, 0.5 μM $Na_2MoO_4·2 H_2O$, 0.1 mM NaCl, 0.2 μM $CoSO_4·7 H_2O$, and 50 μM Fe,Na-EDTA·2 H_2O for microelements. When N was supplied, 5 mM $NaNO_3$ or 2.5 mM $(NH_4)_2SO_4$ was added to the solution. The pH of the solution was daily adjusted to 6.8 with 1 N HCl or NaOH, and the solution was renewed once a week. Plants were grown under long day photoperiod (16 h light [6:00–22:00], 25°C; 8 h dark, 20°C; light intensity: 100 W/m²) in an environmental control cabinet.

CO_2 Uptake. CO_2 uptake was measured with instruments in

The System for Biological Fixation of Photoenergy in Nagoya University. The upper portion of an attached shoot (5 or 6 leaf pairs from the top) was enclosed in a chamber, and CO_2 uptake was monitored by an infrared gas analyzer every 5 min for 24 h. In measurements of CO_2 uptake (Fig. 1), although the plants grew in the experimental period and new leaves were formed, the enclosed leaf number was maintained constant as much as possible (5 or 6 leaf pairs from the top) in order to avoid the effects of leaf age. The environmental conditions during the measurement were similar to the growth conditions. The curves drawn in the figures were the typical data in repeated experiments. Similar results were obtained in the repeated experiments.

Titratable Acidity. Leaf discs were excised from the 4th and 5th pairs of leaves from the top at 0600 (morning) or 2200 (evening). They were sliced and boiled in distilled water for 10 min and then homogenized and centrifuged at 2000g for 10 min. The supernatant was decanted, and the residue was again similarly extracted with distilled water. The extracts were combined and titrated with 0.01 N NaOH using phenolphthalein to estimate the end point (pH 8.0).

Enzyme, Protein, and Chlorophyll Assays. Enzyme extraction and assay were performed by the method of Holtum and Winter (5). Leaf discs were excised at 0 time from the same leaves as for the determination of titratable acidity. The discs were ground in a chilled mortar and pestle in cold buffer (0–4°C) containing 0.1 M Hepes-NaOH (pH 8.0), 5 mM DTT, and 0.5% PVP-40. The homogenate was filtered through two layers of Miracloth. Chl was assayed in an aliquot of the filtrate according to Arnon (1). After centrifugation of the filtrate at 10,000g and 0°C for 5 min, the supernatant was passed through a column of Sephadex G-25 (coarse) equilibrated with buffer containing 25 mM Hepes-NaOH (pH 8.0) and 5 mM DTT and the desalted eluate was used for the assay. PEP[1] carboxylase activity was measured at 25°C by following the change in absorbance at 340 nm in a 3 ml reaction mixture containing 25 mM Bicine, 25 mM Hepes-NaOH (pH 8.0), 2 mM $KHCO_3$, 5 mM $MgCl_2$, 0.1 mM NADH, 6 units malate dehydrogenase, 100 μl extract, and 2 mM PEP. Soluble protein content in the extracts was determined by a modified Lowry procedure (2), using BSA as a standard.

RESULTS

Kalanchoë blossfeldiana plants grown on the constant-N solution culture (control plants) had a CO_2 exchange pattern of the weak-CAM type (8), whereas the plants transferred to N-deficient conditions (N-deficient plants) for 4 or 10 weeks had larger net CO_2 uptake during the night and less during the day and had a stronger CAM type pattern (Fig. 1, A–D). Difference of nocturnal CO_2 uptake between N-deficient plants and control

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[1] Abbreviations: PEP, phosphoenolpyruvate; RuBP, ribulose-1,5-bisphosphate.
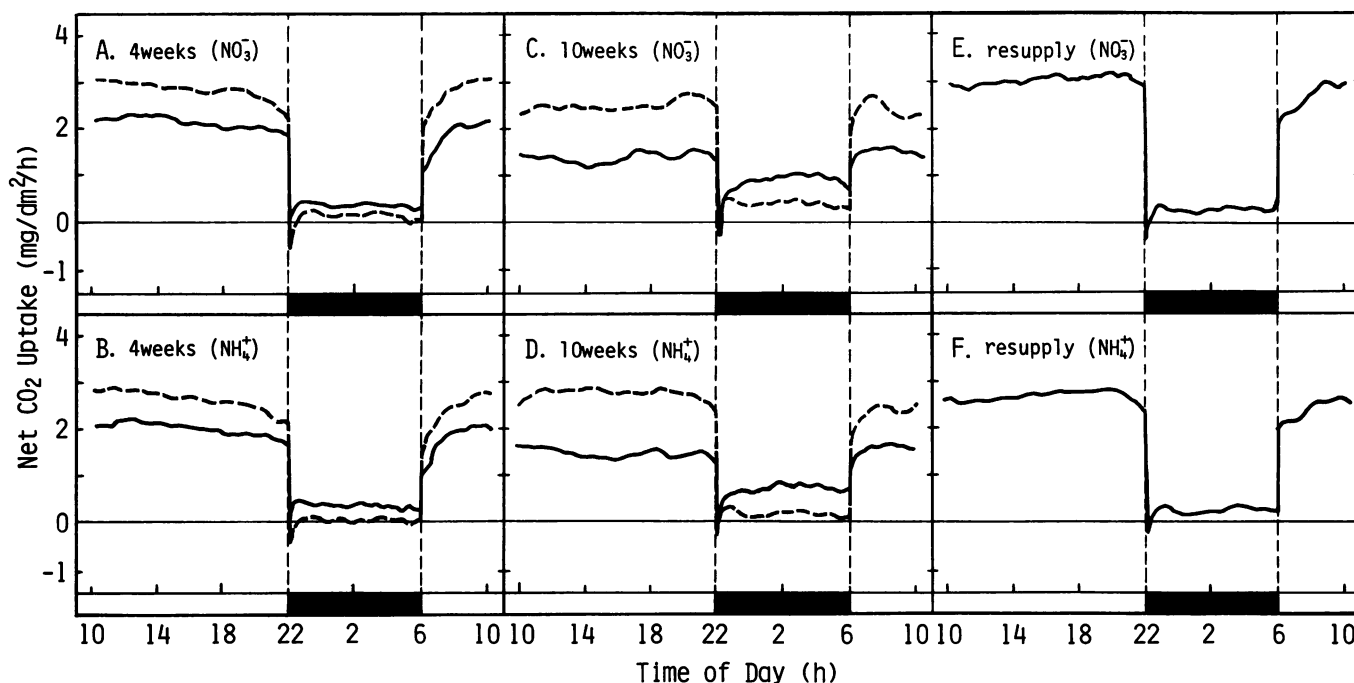
FIG. 1. Diurnal changes in net CO_2 uptake for a shoot of *K. blossfeldiana* cultured under different N conditions. Upper, 5 mM $NaNO_3$ was supplied as an N source; lower, 2.5 mM $(NH_4)_2SO_4$ was supplied as an N source. A–D, solid line, N-deficient plants cultured with N for 4 weeks, then cultured without N for 4 (A, B) or 10 weeks (C, D); dotted line, control plants grown with N during the experimental period of 8 (A, B) or 14 weeks (C, D). E and F, N-deficient plants (8 weeks culture without N after 4 weeks culture with N) followed by the resupply of the N for 1 week. The black bar along the abscissa indicates the dark period.

plants in the 10-week culture was larger than that in the 4-week culture although nocturnal CO_2 uptake rate of control plants grew larger as plants aged (Fig. 1, A–D). Leaves of the plants began to yellow at 4 weeks or more after the transfer, and retardation of growth appeared at 8 weeks or more after the transfer.

When the N supply was resumed at 8 weeks after the transfer to N-deficient conditions, the pattern of diurnal CO_2 uptake exchange recovered to the control level within a week (Fig. 1, E and F). Similar results of the N-deficient culture were observed in the plants supplied with NO_3^- as a N source and in the plants with NH_4^+, although the NH_4^+-grown plants tended to absorb less CO_2 during the night than the NO_3^--grown plants (Fig. 1).

In the experiments depicted in Figure 1, effect of the N-deficient culture appeared slowly (1 month or more after the transfer). So the control plants also had more CAM activity with increasing plant age (Fig. 1, A–D). In this experiment, when the plants were transferred to N-deficient solution from N-enriched solution, the vermiculite which was used for supporting plants was not washed. Thus, remaining N in the vermiculite had to be a reason for the slow appearance of N-deficient effects. Then in the following experiments, the vermiculite was thoroughly washed with water before transfer to N-deficient solution. This procedure brought the similar result of N-deficient culture to the experiment depicted in Figure 1 only in a few weeks of culture. So the plant age effect can be almost excluded. Ten-d culture on N-deficient conditions increased nocturnal CO_2 uptake and decreased daytime CO_2 uptake similarly to the 10-week culture in Figure 1 (Fig. 2).

N-deficient plants showed larger nocturnal increases in titratable acidity and higher activity of PEP carboxylase than control plants (Tables I and II). The ratio of the nocturnal increase in titratable acidity in the N-deficient plants to that in the control plants corresponded with that of the activity of PEP carboxylase in each experiment.

DISCUSSION

These results show that the criteria of CAM photosynthesis correspondingly increased in *Kalanchoë blossfeldiana* when the plants were transferred to N-deficient conditions. This indicates that CAM photosynthesis is stimulated by transfer to N-deficient conditions.

CAM photosynthesis is induced or stimulated under drought conditions (7, 12, 19, 23). Although nutrient and water absorption are independent processes in the root, they are closely related because nutrient transport requires available water in both the plant and soil (20). Under drought conditions, nutrient transport in a plant is decreased by low soil-ion availability and the poor ability of roots to absorb and transport nutrients to the shoot (4, 15). Nitrogen assimilation is also decreased by inhibition of nitrate reductase activity at a low water potential (6, 15, 16). Therefore, plants should lack N under drought conditions. Nevertheless, in past studies, the induction or stimulation of CAM photosynthesis under drought conditions has only been investigated and discussed in relation to low water potentials, and the influence of the N status has not yet been considered. On the basis of the results in the present study and these findings, it is suggested that the lack of N is an important factor for stimulation of CAM photosynthesis under drought conditions.

CAM photosynthesis and flowering are controlled by photoperiodism in a similar manner for almost all plants in which the photoperiodic induction of CAM photosynthesis is reported (14). When flowering is induced, N (a mobile element) and other mineral elements redistribute from the leaves to the inflorescences (21). So the same physiological shift must occur when CAM photosynthesis is photoperiodically induced. On the other hand, it is also known that CAM photosynthesis increases with leaf age. The status of the leaves shifts from that of a source of carbon to a source of N during leaf development (18, 21). Chang *et al.* (3) observed that CAM activity increases while NO_3^- re-
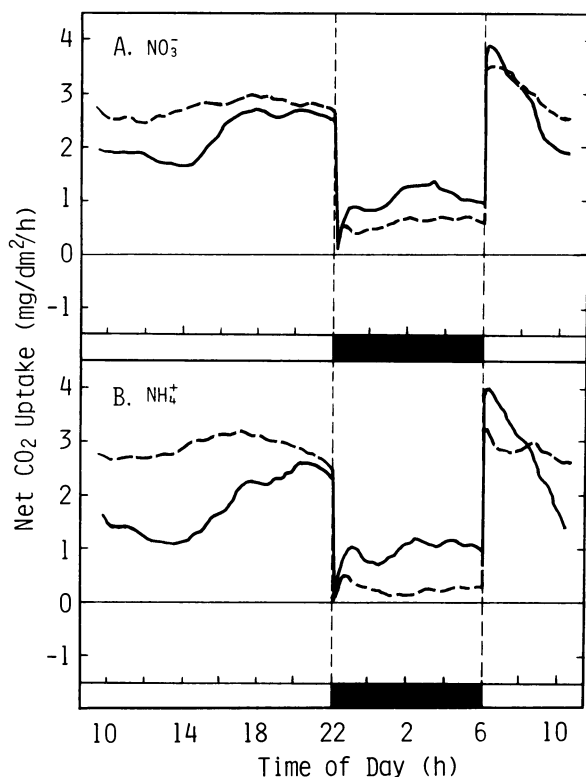
FIG. 2. Diurnal changes in net CO_2 uptake for a shoot of *K. blossfeldiana* before and after transfer to N-deficient conditions. Upper, 5 mM $NaNO_3$ as an N source; lower, 2.5 mM $(NH_4)_2SO_4$ as an N source. Solid line, 4 d before the transfer; dotted line, 10 d after the transfer. The transfer was carried out after 6 weeks of the culture with the N.

Table I. *Effect of Transfer to N-Deficient Conditions on Nocturnal Increase in Titratable Acidity in Leaves of K. blossfeldiana*

Values indicate means ± SE. Each value was calculated from 3 determinations. Each determination was carried out using the excised leaf discs from each different plant. N-deficient plants were cultured for 10 (experiment 1) or 4 (experiment 2) weeks with N (5 mM $NaNO_3$), then for 18 (experiment 1) or 16 (experiment 2) d without N. Control plants were cultured with N constantly during the experimental period.

Treatment	Titratable Acidity		
	Morning	Evening	Nocturnal increase
	μeq/g fresh wt		
Experiment 1			
N-deficient plants	48.7 ± 4.0	12.4 ± 1.2	36.3 ± 3.8
Control plants	23.6 ± 2.6	10.1 ± 0.4	13.5 ± 2.4
Experiment 2			
N-deficient plants	55.8 ± 11.8	13.0 ± 1.4	42.8 ± 13.0
Control plants	14.5 ± 0.3	9.9 ± 0.8	4.6 ± 0.7

ductase activity decreases as the leaves develop (with the exception of the youngest two and the oldest one) in *K. fedtschenkoi*. So lower activity of N assimilation in the leaves is accompanied by higher activity of CAM photosynthesis as leaves age.

The suggestion mentioned above and these findings indicate that the three major factors (drought, photoperiod, and leaf age)

Table II. *Effect of Transfer to N-Deficient Conditions on Activity of PEP Carboxylase, Chl Content, and Soluble Protein Content in Leaves of K. blossfeldiana*

N-deficient plants and control plants were the same as those in Table I. Each value was obtained from one determination. The determination was carried out using the excised discs from the same leaves of the same three plants as the determination in Table I.

Treatment	PEPCase Activity	Chl Content	Soluble Protein Content
	μmol/min/gfw	*mg/g fresh wt*	
Experiment 1			
N-deficient plants	866	0.523	7.27
Control plants	367	0.723	6.66
Experiment 2			
N-deficient plants	1204	0.600	6.68
Control plants	104	0.680	6.87

that stimulate CAM photosynthesis are accompanied by a status of lowering N level in plant or leaf. Therefore, considering the results in the present study additionally, N-withdrawal status should be an important factor in the stimulation of CAM photosynthesis. In the present study, however, plant age also seemed to have an additive effect on the stimulation of CAM photosynthesis to N-deficient effect (Fig. 1). More research is required to clear up the effect of plant age and the correlation among these factors.

Winter *et al.* (22) reported that the plants grown on lower NO_3^- have lower activity of PEP carboxylase in *K. pinnata*, and there is no definite change in the ratio of RuBP carboxylase to PEP carboxylase activity with a varying supply of NO_3^- during growth. Nobel (10) also reported that lower N levels in the hydroponic solutions led to less amounts of nocturnal acid accumulation in some species of cacti. Furthermore, Sugiyama *et al.* (17) reported that the activity and protein content of PEP carboxylase decreases as the N level is lowered in the C_4 plant maize. In contrast to these reports, the criteria of CAM photosynthesis including the activity of PEP carboxylase increased under N-deficient conditions in *K. blossfeldiana*. This discrepancy should be caused by the difference in the experimental conditions to make N lacking. In their experiments, the plants were supplied with different levels of N throughout their growth period. On the contrary, in the present study, *K. blossfeldiana* plants were grown with rather high N concentrations (5 mM NO_3^- or NH_4^+) for 1 month or more, and the plants grew fairly large and were then transferred to the solution containing no N. So N deficiency in the plants was probably not yet severe for the experimental period, although the leaves slightly yellowed. This view is supported by the fact that the soluble protein content was slightly higher in the leaves of the N-deficient plants than the control plants although the Chl content was lower (Table II). This interpretation of the discrepancy confirms the suggestion that N-withdrawal status is an important factor for the stimulation of CAM photosynthesis, although more detailed experiments are required to certify this interpretation.

Additionally, it was also observed that the plants cultured with NO_3^- as a N source performed more CAM photosynthesis than the plants cultured with NH_4^+ in *K. blossfeldiana* (Figs. 1 and 2) (13). Thus, N nutrient condition including both quality and quantity of N should be added to the external factors that affect CAM photosynthesis.

LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24: 1–15
2. BENSADOUN A, D WEINSTEIN 1976 Assay of proteins in the presence of interfering materials. Anal Biochem 70: 241–250
3. CHANG NK, HM VINES, CC BLACK JR 1981 Nitrate assimilation and Crassulacean Acid Metabolism in leaves of *Kalanchoë fedtschenkoi* variety Marginata. Plant Physiol 68: 464–468
4. HANSON AD, WD HITZ 1982 Metabolic responses of mesophytes to plant water deficits. Annu Rev Plant Physiol 33: 163–203
5. HOLTUM, JAM, K WINTER 1982 Activity of enzymes of carbon metabolism during the induction of Crassulacean acid metabolism in *Mesembryanthemum crystallinum* L. Planta 155: 8–16
6. HUFFAKER RC, T RAKIN, GE KLIENKOPF, EL COX 1970 Effects of mild water stress on enzymes of nitrate assimilation and of the carboxylative phase of photosynthesis in barley. Crop Sci 10: 471–473
7. KLUGE M, IP TING 1978 Crassulacean Acid Metabolism. Analysis of an Ecological Adaptation. Springer-Verlag, Berlin
8. MATHUR DD, NJ NATARELLA, HM VINES 1978 Elemental analysis of Crassulacean acid metabolism plant tissue. Commun Soil Sci Plant Anal 9: 128–139
9. NEALES TF 1975 The gas exchange patterns of CAM plants. *In* R Marcelle, ed, Environmental and Biological Control of Photosynthesis. Dr. W Junk Publishers, The Hague, pp 299–310
10. NOBEL PS 1983 Nutrient levels in cacti-relation to nocturnal acid accumulation and growth. Am J Bot 70: 1244–1253
11. NOBEL PS, WL BERRY 1985 Element responses of *Agaves*. Am J Bot 72: 686–694
12. OSMOND CB 1978 Crassulacean acid metabolism: a curiosity in context. Annu Rev Plant Physiol 29: 379–414
13. OTA K, T TEZUKA, Y YAMAMOTO 1988 Changes in Crassulacean acid metabolism of *Kalanchoë blossfeldiana* by different nitrogen sources. Plant Cell Physiol. 29: In press
14. QUEIROZ O, J BRULFERT 1982 Photoperiod-controlled induction and enhancement of seasonal adaptation to drought. *In* IP Ting, M Gibbs, eds, Crassulacean Acid Metabolism. American Society of Plant Physiologists, Rockville, MD, pp 208–230
15. SHANER DL, JS BOYER 1976 Nitrate reductase activity in maize (*Zea mays* L.) leaves. III. Regulation by nitrate flux at low leaf water potential. Plant Physiol 58: 505–509
16. SINHA SK, DJD NICHOLAS 1981 Nitrate reductase. *In* LG Paleg, D Aspinall, eds, Physiology and Biochemistry of Drought Resistance. Academic Press, Sydney, pp 145–169
17. SUGIYAMA T, M MIZUNO, M HAYASHI 1984 Partitioning of nitrogen among ribulose-1,5-bisphosphate carboxylase/oxygenase, phosphoenolpyruvate carboxylase, and pyruvate orthophosphate dikinase as related to biomass productivity in maize seedlings. Plant Physiol 74: 665–669
18. THOMAS H, JL STODDART 1980 Leaf senescence. Annu Rev Plant Physiol 31: 83–111
19. TING IP, L RADER 1982 Regulation of C_3 to CAM shifts. *In* IP Ting, M Gibbs, eds, Crassulacean Acid Metabolism. American Society of Plant Physiologists, Rockville, MD, pp 193–207
20. VIETS FG JR 1972 Water deficits and nutrient availability. *In* TT Kozlowski, ed, Water Deficits and Plant Growth. Academic Press, New York, pp 217–239
21. WILLIAMS RF 1955 Redistribution of mineral elements during development. Annu Rev Plant Physiol 6: 25–42
22. WINTER K, JG FOSTER, MR SCHMITT, GE EDWARDS 1982 Activity and quantity of ribulose bisphosphate carboxylase- and phosphoenolpyruvate carboxylase-protein in two Crassulacean acid metabolism plants in relation to leaf age, nitrogen nutrition, and point in time during a day/night cycle. Planta 154: 309–317
23. WINTER K, U LÜTTGE 1976 Balance between C_3 and CAM pathway of photosynthesis. *In* OL Lange, L Kappen, ED Schulze, eds, Water and Plant Life. Springer-Verlag, Berlin, pp 323–334