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Sodium Stimulates Growth of *Panicum coloratum* through Enhanced Photosynthesis

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ABSTRACT

A sodium-requiring C_4 plant, *Panicum coloratum* Walt. cv Kabulabula, was grown with and without sodium. Rate of nitrogen uptake and photosynthesis were measured during the recovery from sodium deficiency. The beneficial effect of sodium on growth was apparent irrespective of nitrogen source, ammonium- or nitrate-nitrogen. The leaf photosynthetic rate ($^{14}CO_2$ fixation) doubled by sodium within 1 hour of the application.

Brownell and Wood (4) first reported that a C_4 plant, *Atriplex vesicaria*, required sodium for its normal growth and attempts to elucidate the sodium function in higher plants having C_4 photosynthetic pathway have been made (3). Nable and Brownell (8) suggested that in sodium-deficient *Amaranthus tricolor* plants, their capacity for phospho*enol*pyruvate regeneration was impaired. Ohnishi and Kanai (10) proposed that pyruvate uptake into mesophyll chloroplasts of *Panicum miliaceum* leaves was mediated by pyruvate/sodium symport. *Grof et al.* (5) reported that organization of mesophyll chloroplasts of *Kochia childsii* and *A. tricolor* plants was a sodium-dependent process. In previous papers (11–13), we have reported that sodium affects growth of *A. tricolor* plants through its stimulation of nitrate uptake and reduction. As *A. tricolor* plants had poor growth in the presence of ammonium-nitrogen (N), in this report, *Panicum coloratum* plants were used to examine the effect of sodium on growth in the presence of ammonium-N, then the effect on photosynthesis.

MATERIALS AND METHODS

Plant Culture

Seeds of *Panicum coloratum* Walt. cv Kabulabula were germinated on a sheet of cheesecloth which covered acid-washed polyethylene beads. On d 4, the seedlings were supplied with one-fifth strength nutrient solution (see below) and on d 7 with full strength solution. The seedlings were transferred to water culture in a 1-L plastic container (maximum 18 seedlings each) on d 10, then grown for 14 d. Sodium as 1 mM NaCl was supplied to the plants at the beginning of the dark period on d 24. The reference plants received 1 mM KCl, unless otherwise described.

The basal culture solution prepared in deionized and distilled water contained 1 mM KCl, 1 mM KNO_3, 0.5 mM $(NH_4)_2SO_4$, 0.25 mM KH_2PO_4, 1.0 mM $CaCl_2$, 0.5 mM $MgSO_4$, and micronutrients according to Arnon's formula as cited in Hewitt (6) except that 2 ppm iron was supplied as ferric citrate. The pH of the culture solution was adjusted to 5.0 with solid $Ca(OH)_2$. Nutrient solutions were renewed every 2 d and continuously aerated. Potassium chloride, KNO_3, KH_2PO_4, $MgSO_4$, and H_3BO_3 were purified by recrystallization. Calcium chloride was prepared by dissolving distilled water-washed $CaCO_3$ in conc. HCl of analytical grade. The other salts used were extra-pure grade purchased from Wako Chemicals Co. Ltd. (Osaka, Japan).

For plants raised under ammonium-N, 1 mM KNO_3 was replaced by 1 mM KCl and 0.5 mM $(NH_4)_2SO_4$ on d 10, and when nitrate-N, 0.5 mM $(NH_4)_2SO_4$ was replaced by 1 mM KNO_3, respectively, then grown until d 24 and the sodium treatment was started.

Throughout this study, an environment chamber (NS 280 FHW, Takayama Seisakusho, Kyoto 610, Japan) was used under the following conditions: photoperiod 12 h and PPFD 350 μmol m^{-2} s^{-1}, and temperature 30° C and RH 80% for a whole day.

^{15}N Tracer Study

When the sodium-deficient *P. coloratum* plants were supplied with sodium on d 24, nitrogen was simultaneously labeled with ^{15}N-NO_3 (99.8 atom % excess, Shoko Co. Ltd., Tokyo, Japan) or ^{15}N-NH_4 (99.8 atom % excess), and the plants were harvested at 24 and 48 h after the labeling. The culture solution was renewed each 24 h. At harvest, the roots were dipped into 10 mM $CaCl_2$ for 2 min, then washed with distilled water. The plants were dissected into root and shoot and the fresh weight was determined. The plant parts were then dried in an oven (70° C) and digested by the method of Novozamsky (9). Ammonium in the digestate was recovered by steam distillation, caught in 2 mL of 0.2 N H_2SO_4, and determined by the Indophenol method (16). The atom % ^{15}N were determined by mass spectrometry.

Analyses

Relative growth rate was calculated from the increase in the fresh weights and nitrate reductase was extracted and assayed as described previously (11), except that 0.01 mM DTT was replaced by 1 mM cysteine. Chl concentrations were determined by the method of Arnon (1). Sodium content was determined in the hot-water extracts (11) by atomic absorption spectrometry. The concentration was expressed on the basis of tissue water content.

Photosynthesis

Photosynthetic rate was determined in terms of oxygen evolution and $^{14}CO_2$ fixation. Leaf discs 2 mm in diameter were vacuum infiltrated with 1 mM $CaCl_2$ under reduced pressure. A batch of 20 discs which sank into the medium were transferred to the sample cuvette of a Rank Brothers O_2 electrode containing 2 mL of 50 mM Mes-Tris buffer (pH 6.0) and 1 mM $CaCl_2$ maintained at 30° C. After 3 min of preillumination (an incandescent projection lamp, 2000 μmol m^{-2} s^{-1}), the reaction was started by adding 30 μL of 0.75 M $KHCO_3$. A preliminary experiment showed that the leaf disc oxygen evolution had an optimum pH range of 6.0 to 6.5 (data not presented).

$^{14}CO_2$ fixation experiments were carried out during 6 to 9 h from the beginning of the day using plants with different periods of sodium loading. Twelve plants were transplanted to other containers (1.25 L), four plants each, and sodium was added. The time of sodium addition was counted backward from the start of the experiment. The three containers with plants were placed in an air-tight box made of Plexiglas (36 L) and the box was set in the environment chamber. The air was labeled with 8 μCi $^{14}CO_2$, which was prepared from 20 mg $Ba^{14}CO_3$ and 10 mL 60% $HClO_4$. The initial CO_2 concentration was about 400 ppm. Five min later, $^{14}CO_2$ was aspirated by an air pump and 30 min later the plants were harvested. After determining fresh weight of the leaves, the whole plant was immediately subjected to wet-digestion with Van Slyke reagents (15) and the evolved CO_2 was caught in 1 mL of 2.5 N KOH, then the radioactivity was determined. Radioactivity in a whole plant was divided by the fresh weight of the leaf blade and the $^{14}CO_2$ fixation rate was expressed as radioactivity incorporated by the unit fresh weight of leaf blade.

RESULTS

Sodium-deficient *Panicum coloratum* plants showed poor growth and had yellow-green leaves irrespective of the form of nitrogen source, however, by the application of 1 mM NaCl, relative growth rate (RGR) was enhanced and at 48 h after the application the rates reached to 195 and 182% of the initial rates of the ammonium- and nitrate-grown plants, respectively (Fig. 1). RGR of the reference plants, which received 1 mM KCl in place of 1 mM NaCl, remained constant.

Nitrogen uptake by *P. coloratum* plants during the recovery from sodium-deficiency was examined using $^{15}NO_3$ and $^{15}NH_4$. In the ammonium-grown plants, nitrogen concentrations both in the root and shoot increased by sodium, and the increment was entirely ascribed to the increase in ^{15}N (Table I). Ratio of nitrogen concentrations in the shoot to the root was similar irrespective of the application of sodium, suggesting that sodium may stimulate ammonium uptake in the root and not upward (root to shoot) transport. Ammonium and nitrate taken up per unit weight of root in the initial 24 h of the application of sodium was significantly greater (43 and 36%, respectively) than that of the corresponding control plants (Tables I and II). Sodium stimulation on nitrogen uptake was evident for both forms of nitrogen; however, amounts of nitrogen taken up in the initial 24 h and the degree of stimulation by sodium were less conspicuous in the nitrate-grown plants than those in the ammonium-grown plants.

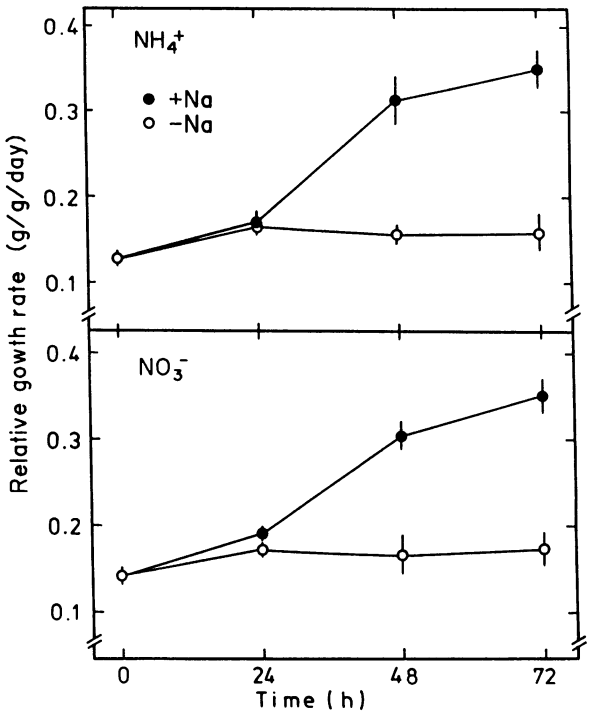

Figure 1. Changes in the relative growth rate of *P. coloratum* plants during recovery from sodium deficiency when the plants were raised under ammonium nitrogen (top) and nitrate nitrogen (bottom). On d 24 after germination, *P. coloratum* plants were supplied with either 1 mM NaCl (●) or 1 mM KCl (○) at the beginning of the dark period (at time 0) and the relative growth rate was calculated from the daily changes in the fresh weight. Both experiments were repeated three times each. Data are the means and SD of four plants.

Table I. *Accumulation of ^{15}N in the Shoot and Root of Ammonium-Nitrogen-fed P. coloratum Plants during 24 and 48 h of the Sodium Application*

The plants grown under ammonium-nitrogen and sodium-deficient condition were supplied with 1 mM NaCl and ^{15}N-ammonium simultaneously on d 24 after germination. Data are the means and SD of three replicates.

Exposure Period	Na	^{15}N content		Total ^{15}N uptake
		Shoot	Root	
h		*μmol/g fresh wt*		*mmol ^{15}N/g fresh wt root*
24	−	50.3 ± 2.0 (302.7 ± 9.8)[a]	49.9 ± 1.2 (194.7 ± 8.0)	0.554 ± 0.054
	+	73.0 ± 1.5 (324.8 ± 13.1)	73.7 ± 1.9 (228.9 ± 2.4)	0.793 ± 0.037
48	−	93.5 ± 11.4 (285.7 ± 17.3)	97.0 ± 6.8 (205.1 ± 8.1)	0.869 ± 0.115
	+	142.5 ± 4.0 (326.5 ± 10.3)	140.5 ± 11.7 (222.1 ± 14.0)	1.057 ± 0.101

[a] Values in parentheses represent the contents of nitrogen (μmol N/g fresh wt).

Table II. *Accumulation of ^{15}N in the Shoot and Root of Nitrate-Nitrogen-fed P. coloratum Plants during 24 and 48 h of the Sodium Application*

The plants grown under nitrate-nitrogen and sodium-deficient condition were supplied with 1 mM NaCl and ^{15}N-nitrate simultaneously on d 24 after germination. Data are the means and SD of three replicates.

Exposure Period	Na	^{15}N content		Total ^{15}N uptake
		Shoot	Root	
h		*μmol/g fresh wt*		*mmol ^{15}N/g fresh wt root*
24	−	41.9 ± 2.2 (302.8 ± 12.1)[a]	60.7 ± 2.8 (214.8 ± 8.8)	0.377 ± 0.024
	+	59.6 ± 4.1 (320.8 ± 9.4)	78.2 ± 3.8 (236.1 ± 12.2)	0.514 ± 0.051
48	−	80.5 ± 4.3 (296.4 ± 5.9)	83.3 ± 1.1 (196.9 ± 2.9)	0.582 ± 0.037
	+	128.4 ± 4.5 (321.7 ± 8.5)	137.3 ± 3.9 (226.6 ± 6.4)	0.789 ± 0.016

[a] Values in parentheses represent the contents of nitrogen (μmol N/g fresh wt).

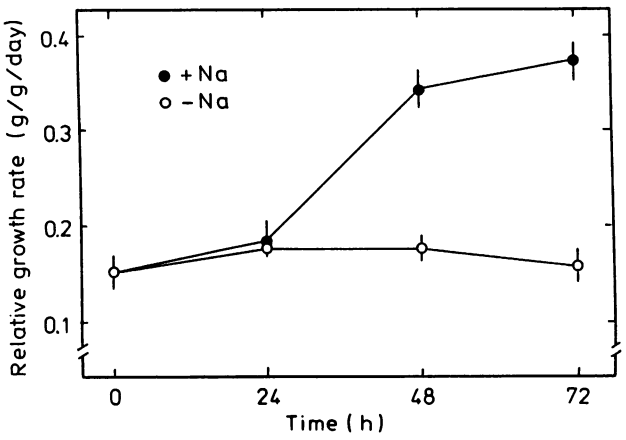

Figure 2. Changes in the relative growth rate of *P. coloratum* plants during recovery from sodium deficiency when the plants were raised under culture solution containing both ammonium- and nitrate-nitrogen. The sodium treatment was given as was explained in the legend for Figure 1. Experiments were repeated four times, and a representative one is shown. Data are the means and SD of four plants.

Irrespective of the form of nitrogen supplied, the beneficial effect of sodium on growth was evident, plants raised in the basal culture solution containing both ammonium- and nitrate-N for the whole growth period were used consequently. Figure 2 shows the sodium effect on RGR of the plants grown under the basal culture solution. The effect of sodium was substantially the same as was observed for the ammonium- or nitrate-grown plants. Changes in the Chl concentrations of the leaves are presented in Figure 3. Sodium-deficient *P. coloratum* plants were supplemented with sodium at the beginning of the light period. Chl concentration increased about 29% within the initial 12 h and did gradually afterward. It did not increase at night. At the dawn of the third and the fourth day, apparent decrease in the concentrations was observed, may be due to the increase in the fresh weights. The

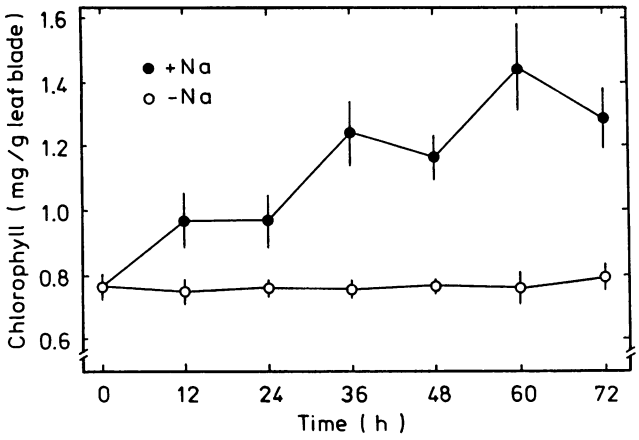

Figure 3. Changes in the Chl concentration of the leaves of *P. coloratum* plants during the recovery from sodium deficiency. On d 24 after germination, the plants were supplied with 1 mM NaCl (●) or 1 mM KCl (○) at the beginning of the light period (at time 0). Experiments were repeated three times and a representative result is presented here. Data are the means and SD of three samples.

concentrations in the control plants remained constant. The recovery from chlorosis was faster in the younger leaves (data not shown), as in the leaves of *Amaranthus tricolor* plants (11).

In previous reports (12), nitrate uptake rate and nitrate reductase activity were stimulated within 6 h after the sodium application to the sodium-deficient *A. tricolor* plants. In *P. coloratum* plants, nitrate reductase activities of the sodium-supplied plants was 185% of the sodium-deprived plants on d 5 after the application of sodium (14). Changes in the nitrate reductase activities during the recovery from sodium deficiency of *P. coloratum* plants are presented in Figure 4. The activity was not stimulated by sodium after 12 h of the addition and it did not increase as promptly as in *A. tricolor* plants (Fig. 4, compared with Fig. 3 of ref. 12). However, it increased at 24 and 48 h after the sodium addition and the increase was little in the control plants (Fig. 4, inset). The different responses of nitrate reductase activities toward sodium between *A. tricolor* and *P. coloratum* may be ascribed to the different preference in the form of nitrogen between the species; as was reported in a previous paper (13), the growth of *A. tricolor* plants was very poor when the plants were raised under ammonium-N as a sole nitrogen source.

Changes in the leaf disc oxygen evolution rate are presented in Figure 5. During the initial 6 h, the stimulation of Na on the rate was statistically insignificant but 6 h after the addition, the increase (23%) was significant and then gradually increased up to 54 h. On the other hand, radioactivity incorporated by the plants increased more than twice within 1 h of the sodium application and the rate did not change up to 30 h (Fig. 6). Within 1 h after the sodium addition, Chl concentration did not increase significantly and the sodium concentration in the shoots was about 2 mM; the initial sodium concentration in the shoot was less than 0.2 mM (data not presented).

DISCUSSION

In sodium-deficient *Amaranthus tricolor* plants, the rates of O_2 evolution of leaf discs did not increase until 24 h after the

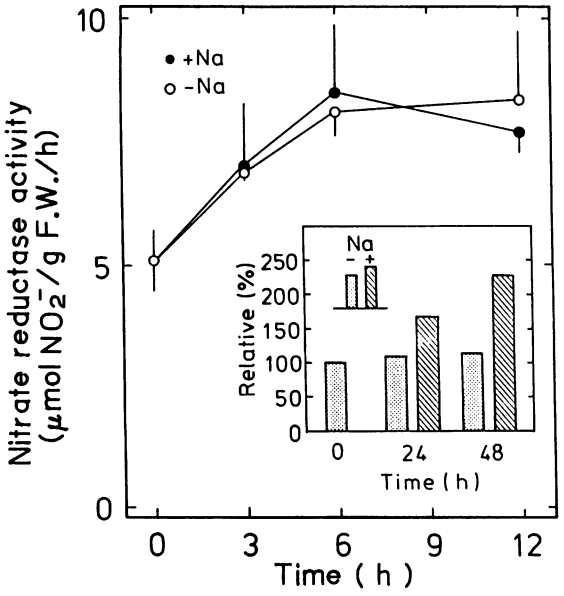

Figure 4. Changes in the nitrate reductase activities of the leaves of *P. coloratum* plants during the recovery from sodium deficiency. The sodium treatment was started at the beginning of the light period on d 24 after germination. The times specified on the X axes designate the period of the sodium treatment. The figure inset is the relative increase of the activities to that of the control plants at time 0. Both short- and long-term experiments were repeated twice and similar results were obtained. Data are the means and SD of three plants.

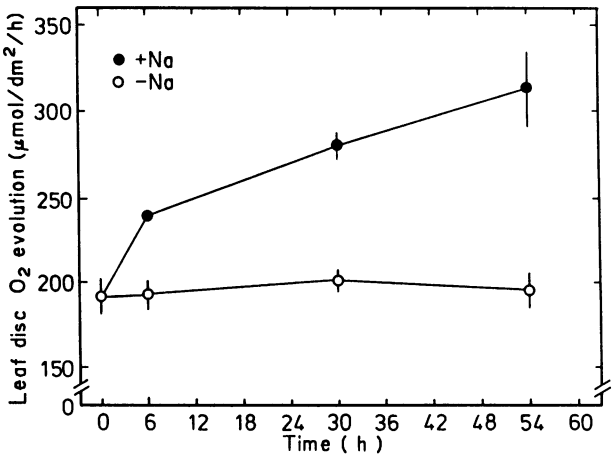

Figure 5. Changes in the light- and bicarbonate-dependent oxygen evolution of the leaf discs of *P. coloratum* plants during the recovery from sodium deficiency. On d 24 after germination, the plants were subjected to the sodium treatment as described in the legend for Figure 3. Experiments were repeated three times and a representative result is presented here. Data are the means and SD of three samples.

sodium application (11), while nitrate uptake and nitrate reductase activity increased within 6 h (12). Consequently, it was concluded that sodium affected growth through nitrate uptake and assimilation rather than directly through photosynthesis. As has been presented here, however, during the recovery from sodium deficiency, $^{14}CO_2$ fixation by the intact leaves of *P. coloratum* plants increased faster than did light-

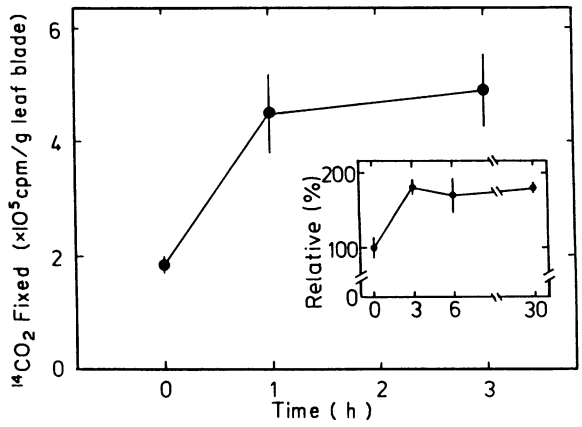

Figure 6. Changes in $^{14}CO_2$ fixation by *P. coloratum* plants during the recovery from sodium deficiency. At 5 h after the beginning of the light period on d 24 after germination, four plants were transferred to the culture solution supplemented with 1 mM NaCl (the plants receive 1 mM NaCl for 3 h) and eight plants were transferred to the culture solution supplemented with 1 mM KCl. At 7 h after the beginning of the light period, four of the eight plants were transferred to the NaCl medium (the plants received 1 mM KCl for 2 h then receive 1 mM NaCl for 1 h) and remaining four plants did not receive sodium (the plants received 1 mM KCl for 3 h; as the control). At 8 h after the beginning of the light period, the $^{14}CO_2$ fixation experiment was started. The times specified on the X axes designate the period of sodium loading. The figure inset is the relative increase of the fixation to that of the control at the longer period. The plants were allowed to incorporate $^{14}CO_2$ for 5 min and the plants were harvested 30 min later, then the leaf blade was weighed. A whole plant was subjected to wet-digestion, and the $^{14}CO_2$ fixation rate was expressed as the radioactivity incorporated by the unit fresh weight of leaf blade. Experiments were repeated seven times with varying periods of sodium loading and a representative result is presented here. Data are the means and SD of four replicates.

dependent O_2 evolution by leaf discs and nitrate reductase activities, and sodium was effective irrespective of the nitrogen source. It is unlikely that both ammonium and nitrate ions share the same uptake mechanism and that sodium affects them simultaneously. Consequently, the sodium effect on nitrogen uptake and assimilation may be secondary and not primary.

The effect of sodium on photosynthesis of the sodium-deficient *P. coloratum* plants was determined using two methods, HCO_3^--dependent O_2 evolution by the leaf discs and $^{14}CO_2$ fixation by the leaves. Based on the former method, the sodium effect did not appear significantly until 6 h of the application. Inclusion of sodium into the incubation medium and/or assay medium did not stimulate the oxygen evolution (data not shown). However, $^{14}CO_2$ fixation more than doubled within 1 h of the sodium application. The latter method has several advantages over the former one, *i.e.* the $^{14}CO_2$ method could avoid artifacts due to cellular damage from vacuum infiltration (7) and reduced gas diffusion in liquid phase (2). It is therefore highly probable that the apparent stimulation of photosynthesis which took place within 1 h of the application of sodium reflects the beneficial effect of this element on cellular metabolism. The different responses of sodium-supplied *P. coloratum* leaves toward oxygen release (23%

increase after 6 h of the sodium application) and $^{14}CO_2$ uptake (147% increase after 1 h of the sodium application) may be important characteristics of sodium function in the sodium-requiring C_4 plants.

These results made us reexamine the sodium effects on the photosynthesis of *A. tricolor* plants. As was detected in *P. coloratum* plants, the tendency was substantially the same; the $^{14}CO_2$ fixation nearly doubled after the addition of sodium within 3 h, while the sodium effect on the rate of oxygen evolution was evident only after 24 h of the application and furthermore the increase was about 20% (data not shown, published elsewhere).

Fractionation of the initial products of the $^{14}CO_2$ fixation and determination of the endogenous pool size of phospho*enol*pyruvate of *P. coloratum* plants during recovery from sodium deficiency and examination of CO_2 uptake mechanisms of the leaves, including responses of the stomata to sodium, are now in progress.

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