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Sodium-Stimulated NO_3^- Uptake in *Amaranthus tricolor* L. Plants

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ABSTRACT

Nitrate uptake of Na^+-deficient *Amaranthus tricolor* L. cv Tricolor seedlings from complete culture solution was stimulated by about 210% within 5 hours by application of 0.5 millimolar NaCl. From a Na^+-preloading experiment, intracellular Na^+ was shown to be responsible for the stimulation of NO_3^- uptake. The results suggest a possible role of Na^+ in NO_3^- uptake in C_4 plants.

Many C_4 plants have been shown to require Na^+ for growth (2). However, the physiological mechanism for the Na^+ requirement has never been elucidated. We have recently (9) presented evidence for the involvement of Na^+ in the enhancement of NR[1] activities in *Amaranthus tricolor* plants.

We now report on the stimulation of NO_3^- uptake in *A. tricolor* seedlings by Na^+.

MATERIALS AND METHODS

Plant Culture. Seedlings of *Amaranthus tricolor* L. cv Tricolor were cultured under Na^+-deficient conditions until 30 d after germination as described previously (7, 9). The standard culture solution (pH 6.0) prepared in distilled and deionized water contained 1 mM KCl, 1 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.25 mM $(NH_4)_2HPO_4$, and 0.5 mM $MgSO_4 \cdot 7H_2O$. The micronutrient composition was that of Arnon's solution cited in Hewitt (6) except that all the iron was supplied as ferric citrate. The concentration of Na^+ as an impurity in the culture solution was estimated to be less than 5 ppb, using atomic absorption spectrophotometry. Throughout this study, an environment chamber (NS280FHW, Takayama Seisakusho, Kyoto 610, Japan) was used under following conditions: temperature 30°C for whole growth period, RH 70%, photoperiod 15 h, and light intensity 350 $\mu E\ m^{-2}\ s^{-1}$. Culture solutions were renewed every 3 d and continuously aerated.

Ion Uptake Studies. Half-strength standard culture solution, adjusted with 0.1 M HCl to pH 6.0, was used as an uptake solution.

Experiment 1. Roots of seedlings grown under the Na^+-deficient condition for 30 d after germination were rinsed with the uptake solution, and then four seedlings each were transferred to 60 ml of the uptake solution supplemented with either 0.5 mM NaCl or 0.5 mM KCl at the end of the dark period. Uptake of ions by the seedlings was determined by following the disappearance of the ions from the uptake solution.

Experiment 2. The following experiment was carried out to evaluate the effects of intracellular Na^+ on the NO_3^- uptake of *A. tricolor* plants. The 30-d-old Na^+-deficient seedlings were treated with 0.5 mM NaCl during the dark period (9 h), and the seedlings were transferred to the uptake solution at the end of the dark period. They were allowed to stand for 10 min, then four seedlings each were placed in 60 ml of the uptake solution supplemented with either 0.5 mM NaCl or 0.5 mM KCl, and ion fluxes were determined at 3 h intervals during 6 h of the light period.

The amount of water lost from the uptake solutions due to evapotranspiration was determined from the decrease in the weight of vessels containing the experimental seedlings, and water was added to return the vessels to their initial weight. The uptake solutions were continuously aerated throughout the experiment and were renewed at every sampling. All the data are the means of at least three replications and are presented as the g FW basis of roots unless otherwise noted.

Analyses. Nitrate, Cl^-, and SO_4^{2-} were determined using ion chromatography (IC6A, Shimazu Co., Ltd., Kyoto 604, Japan). Phosphate (8) and NH_4^+ (14) were determined colorimetrically. Sodium, K^+, Ca^{2+}, and Mg^{2+} were determined using atomic absorption spectrophotometry. Sodium concentrations in the plant materials are presented on a tissue water basis.

Proton release was measured with a pH electrode and calculated on the basis of quantity of hydroxide required to return the solution to the initial pH.

NR assay. For NR assay, the 30-d-old seedlings grown under the Na^+-deficient condition were treated in the same manner as experiment 1. All the leaves except the cotyledons were sampled and the NR activities were determined as described previously (9). The enzyme activity is expressed as μmol of NO_2^- formed$\cdot g^{-1}$ FW$\cdot h^{-1}$.

RESULTS AND DISCUSSION

Within 30 min of application of Na^+ at a concentration of 0.5 mM, the seedlings showed significantly higher NO_3^- uptake capacities (4.51 ± 0.06 μmol/g FW root$\cdot h^{-1}$) than seedlings which received 0.5 mM KCl (1.90 ± 0.56 μmol/g FW root$\cdot h^{-1}$) (Fig. 1A). After 4 h, the difference in the NO_3^- uptake rate further increased. The total amount of NO_3^- taken up by the Na^+-treated seedlings during the experimental period was about twice that by the K^+-treated seedlings (Fig. 1B).

Figure 2 shows changes in Na^+ concentrations in the Na^+-treated seedlings. Following the Na^+ application, Na^+ concentration in the shoots increased linearly with time from an initial value of 0.06 to 0.35 mM after 5 h. On the other hand, that in the roots increased from 0.35 to 0.60 mM within 1 h, and then leveled off.

Table I shows a summary of fluxes of Na^+, K^+, Cl^-, and NO_3^-. The Na^+ treatment stopped the K^+ and Cl^- uptake, and significant amounts of K^+ and Cl^- were released. Taking into account the K^+ and Cl^- contents of the plants in long-term

[1] Abbreviations; NR, nitrade reductase; FW, fresh weight.

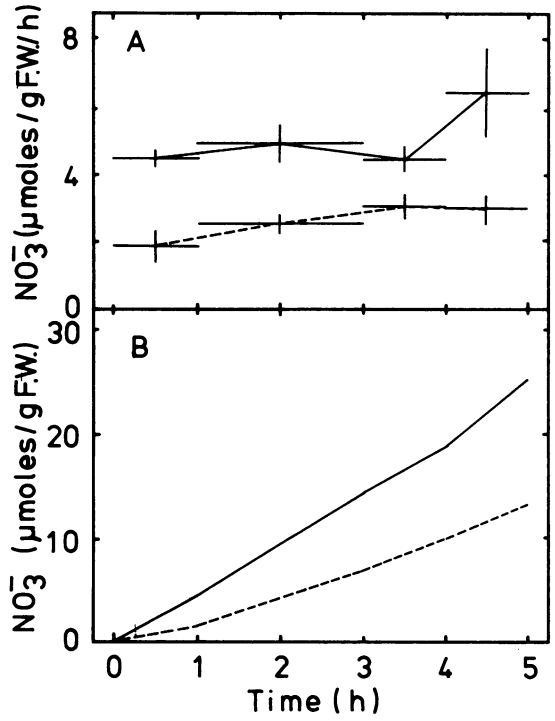

FIG. 1. Effects of Na^+ application on NO_3^- uptake of *A. tricolor* seedlings. The 30-d-old Na^+-deficient *A. tricolor* seedlings were placed in the uptake solution supplemented with either 0.5 mM NaCl or 0.5 mM KCl at the end of the dark period (at time 0 h). A, Changes in the NO_3^- uptake rates with time; B, cumulative NO_3^- uptake. Results are represented by solid lines for the Na^+-treated seedlings and by dotted lines for the K^+-treated seedlings. Data are the means and SD of three replications of each four plants and are expressed as μmol g^{-1} root FW.

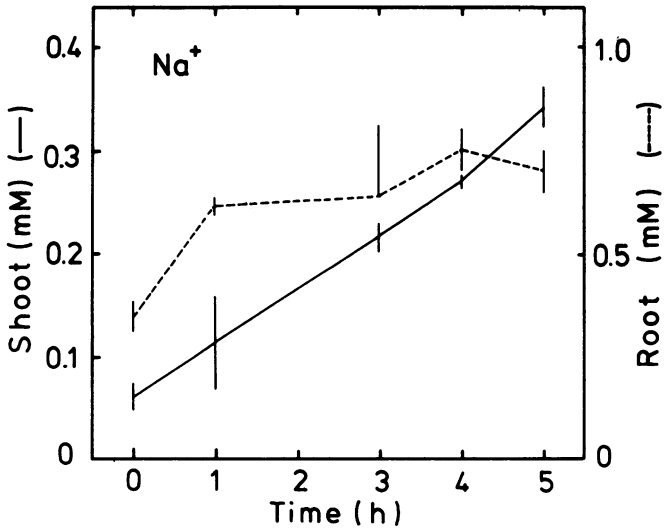

FIG. 2. Changes in Na^+ concentrations in the shoots (——) and the roots (– – –) of the 30-d-old *A. tricolor* seedlings following Na^+ application. The Na^+-deficient *A. tricolor* seedlings were transferred to the uptake solution containing 0.5 mM NaCl at the end of the dark period (at time 0 h) and were sampled at specified intervals during 5 h of the light period. Data are the means and SD of determinations of four individual seedlings. Sodium concentrations (mM) were calculated on the basis of tissue water contents.

Table I. *Fluxes of Na^+, K^+, Cl^-, and NO_3^- Affected by Na^+ Application to A. tricolor Seedlings*

Values represent net influxes (+) and effluxes (−). Sodium influx was calculated from the data of Figure 2. Net fluxes of K^+ and Cl^- during 5 h of the uptake period were taken from the identical experiment presented in Figure 1. Data are the means and SD of three replications.

Element	Na^+-Treated Seedlings	K^+-Treated Seedlings
	μmol/g FW root/5 h	
Na^+	+2.3	
K^+	−1.2 ± 1.7	+10.3 ± 3.9
NO_3^-	+26.6 ± 4.2	+12.6 ± 1.7
Cl^-	−3.5 ± 2.3	+35.2 ± 18.4
H^+[a]	−14.5 ± 0.6	−6.1 ± 1.8

[a] Amount of OH^- required to return the medium pH to the initial value.

Table II. *Effects of Intracellular Na^+ on the NO_3^- Uptake in 30-d-old Na^+-Preloaded A. tricolor Seedlings*

Values are the means and SD of five replications of each four plants. Sodium-preloaded seedlings were placed in the uptake solution supplemented with either 0.5 mM NaCl (+) or 0.5 mM KCl (−) at the beginning of the light period (at time 0 h). The NO_3^- uptake and Na^+ release were determined at 3 h intervals.

		0–3 h	3–6 h
		μmol/g FW root/h	
NO_3^- uptake	+	21.5 ± 3.74	25.3 ± 2.59
	−	22.1 ± 5.69	29.5 ± 3.38
Na^+ release	+	ND[a]	ND
	−	0.16 ± 0.07	0.05 ± 0.01
H^+ release[b]	+	2.64 ± 0.12	3.33 ± 0.40
	−	2.52 ± 0.25	2.91 ± 0.20

[a] Not determined. [b] Amount of OH^- required to return the medium pH to the initial value.

growth experiment (7), these effluxes were transient phenomena. Uptake rates of HPO_4^{2-}, SO_4^{2-}, NH_4^+, Ca^{2+}, and Mg^{2+} did not differ significantly between the treatments during the experimental period (data not presented). The anion uptake exceeded the cation uptake more in the K^+-treated seedlings, and the excess mainly consisted of Cl^-. Acidification of the medium was faster in the Na^+ treatment.

How is Na^+ involved in the enhancement of the NO_3^- uptake? The uptake of NO_3^- was about 11-fold higher than that of Na^+ (Table I), and Na^+ in the uptake solution had no significant effect on the NO_3^- uptake and acidification of the medium when the plants were preloaded with Na^+ (Table II). Moreover, the Na^+-preloaded seedlings released Na^+ into the uptake solution supplemented with 0.5 mM KCl. Accordingly, the Na^+ influx was not essential to the NO_3^- uptake process in the roots, but intracellular Na^+ stimulated the NO_3^- uptake. These results argue against the Na^+/NO_3^- symport which appeared in the report of the Na^+-dependent NO_3^- uptake by a marine diatom, *Phaeodactylum tricornutum* (11).

It is well known that NR activity is induced by its substrate, NO_3^-, and in turn NR activity then influences NO_3^- uptake from the external medium (1, 5). Therefore, NR activities in the leaves were measured at the same time as NO_3^- uptake using the same batch of the plants (Fig. 3). Enhancement of the NR activity became appreciable after 1 to 3 h of the addition of Na^+, *i.e.* the stimulation of NO_3^- uptake by the roots preceded the increase in NR activity in the leaves (Figs. 1 and 3). In addition, NR activities were not detected in the roots. Accordingly, it seemed difficult to correlate the stimulation of NO_3^- uptake by Na^+ with the increased levels of NR activity. The maintenance

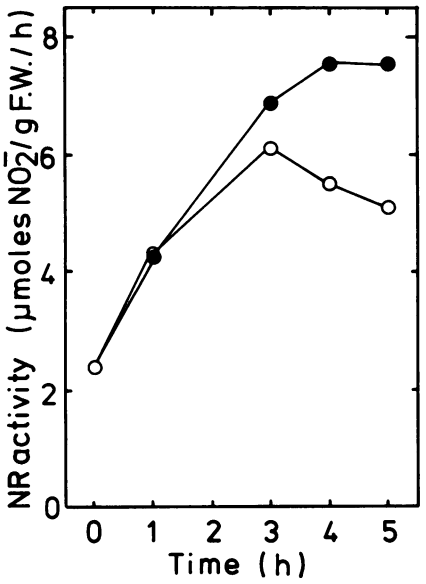

FIG. 3. Effects of Na^+ application on NR induction in the Na^+-deficient *A. tricolor* leaves. The 30-d-old seedlings were placed in the uptake solution supplemented with either 0.5 mM NaCl (●) or 0.5 mM KCl (○) at the beginning of the light (at time 0 h), and the changes in the extractable NR activity in the light period were followed. Curves are the representatives of three independent experiments.

of NR in corn leaves has been shown to be more closely associated with the flux of NO_3^- to the leaves from the roots than to the existing NO_3^- concentrations in the leaves (12, 13). It is considered that electrogenic proton pumps located at the plasma membrane of epidermal and cortical cells provide a means for the ion fluxes from outside to the symplast (10). Recently, it has been reported (3, 4) that electrogenic proton pumps working at the xylem/parenchyma symplast interface mediate ion exchange between xylem and surrounding tissue, then facilitate the upward transport of ions. In *A. tricolor* plants, it is possible that the flux of NO_3^- to the leaves may increase by the addition of Na^+, thereby the levels of NR activities increase. From Figures 2 and 3, the stimulatory threshold of Na^+ concentration in the leaves for the enhancement of the NR activity is presumed to be in a range of 0.1 to 0.2 mM on a basis of whole cell water, although intracellular localization of Na^+ is probable. An effort to locate the newly absorbed Na^+ in the plant body is in progress.

Sodium has been shown to be an essential micronutrient for C_4 plants but not for C_3 plants (2). However, the physiological mechanism for the Na^+ requirement has never been elucidated. This is the first report of a possible role of Na^+ in NO_3^- uptake in C_4 plants. We previously reported that Na^+ stimulated the NR induction in *A. tricolor* seedlings (9). Taken together, it was suggested that Na^+ promoted NO_3^- assimilation resulting in enhancement in *A. tricolor* plants and that Na^+ can be involved in some other functions than C_4 photosynthetic pathway. While it remains to be determined whether Na^+ stimulates the NO_3^- uptake and NR induction in other C_4 plants or not, the reason why C_4 plants require sodium for growth may be explained at least in part by the study on the Na^+-stimulated ion transport.

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