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The Requirement for Sodium as a Micronutrient by Species Having the C_4 Dicarboxylic Photosynthetic Pathway

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ABSTRACT

Six species having characteristics of plants with the C_4 dicarboxylic photosynthetic pathway, *Echinochloa utilis* L. Ohwi et Yabuno (Japanese millet), *Cynodon dactylon* L. (Bermuda grass), *Kyllinga brevifolia* Rottb., *Amaranthus tricolor* L. cv. Early splendour, *Kochia childsii* Hort., and *Portulaca grandiflora* Hook (rose moss), responded decisively to 0.1 milliequivalent per liter NaCl supplied to their culture solutions initially containing less than 0.08 microequivalent per liter Na. Chlorosis and necrosis occurred in leaves of plants not receiving sodium. *Portulaca* failed to set flower in the sodium-deficient cultures. Under similar conditions *Poa pratensis* L. (Kentucky blue grass) having characteristics of the C_3 photosynthetic pathway made normal growth and did not respond to the addition of sodium. It is concluded from these results and previously reported work that sodium is generally essential for species having the C_4 pathway but not for species with the C_3 pathway.

Sodium was first shown to be essential for the blue-green alga *Anabaena cylindrica* Lemm. in 1954 (1), when it was demonstrated that small amounts of the element are specifically required for growth. Subsequently, sodium was shown to be essential for the angiosperm, *Atriplex vesicaria* Heward ex Benth (Bladder saltbush) (6), and more recently other species have been shown to respond to sodium (3, 16). Significant responses to sodium have been reported in barley (3) and tomatoes (17), but the responses were small and no deficiency symptoms were observed.

Only certain species of *Atriplex* responded, decisively, to small quantities of sodium whereas other species of the same genus showed no response (3). Subsequently, it was observed that the species of *Atriplex* which required sodium had characteristics of plants with the C_4 photosynthetic pathway whereas those not requiring sodium had features of the C_3 photosynthetic pathway (9-11).

This communication gives results of experiments in which six species from five families (*Echinochloa utilis, Cynodon dactylon, Kyllinga brevifolia, Amaranthus tricolor, Kochia childsii*, and *Portulaca grandiflora*) with features of C_4 plants (7, 9) were shown to have a sodium requirement whereas *Poa pratensis*, a species with C_3 characteristics, showed no response to sodium. These results in conjunction with previously published data are discussed to demonstrate a correlation between the essentiality of sodium and the possession of the C_4 dicarboxylic photosynthetic pathway.

MATERIALS AND METHODS

Seeds of *Echinochloa utilis* L. Ohwi et Yabuno (Japanese millet), *Cynodon dactylon* L. (Bermuda grass), *Poa pratensis* L. (Kentucky blue grass), *Amaranthus tricolor* L. cv. Early splendour, *Kochia childsii* Hort., and *Portulaca grandiflora* Hook (rose moss) were obtained from commercial seed suppliers. Seeds of *Kyllinga brevifolia* Rottb. were collected locally. The procedures for germination and growth of plants under conditions of low sodium have been described previously (2). The basal culture solution had the following composition expressed in μmoles/liter: KNO_3, 5,000; $Ca(NO_3)_2$, 4,000; KH_2PO_4, 1,000; $MgSO_4$, 1,000; $(NH_4)_2HPO_4$, 1,000; H_3BO_3, 46; $MnSO_4 \cdot 7H_2O$, 9.1; $CuSO_4 \cdot 5H_2O$, 0.31; $ZnSO_4 \cdot 7H_2O$, 0.76; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.1; NH_4Cl, 350. Iron was supplied as ferric ammonium ethylenediaminetetraacetate at 90 μmoles/liter in the basal culture solution. Less than 0.07 μeq/liter sodium was derived from the purified salts of the culture solution as an impurity, and silica-distilled water contained less than 0.0087 μeq/liter, giving a total sodium concentration of approximately 0.08 μeq/liter. Culture vessels of 2-liter capacity were of sodium-free plastic material. Plants were grown in a naturally illuminated cabinet, slightly pressurized by a continual supply of filtered air. This prevented the entry of dust particles and other atmospheric contaminants which could be

Table I. *Responses of Various Plants to Sodium*

Species[1]	Age at Harvest	Lesions in Plants Not Receiving Sodium	Yield		Significance of Difference
			No addition	0.1 meq liter NaCl	
	days		*g dry wt/plant*		%
Gramineae					
Poa pratensis L. (Kentucky blue grass)	52	None	0.0236	0.0206	NS[2]
Echinochloa utilis L. Ohwi et Yabuno (Japanese millet)	22	Chlorosis and necrosis	0.404	0.713	0.1
Cynodon dactylon L. (Bermuda grass)	47	Chlorosis	0.178	0.337	1
Cyperaceae					
Kyllinga brevifolia Rottb.	57	Chlorosis	0.628	1.245	1
Amaranthaceae					
Amaranthus tricolor L. cv. Early Splendour	40	Chlorosis	0.884	2.099	0.1
Chenopodiaceae					
Kochia childsii Hort.	21	Chlorosis	0.125	0.442	1
Portulacaceae					
Portulaca grandiflora Hook (rose moss)	29	Chlorosis, necrosis, and failure to set flower	0.242	0.789	1

[1] Names given in this table are those under which the seeds were received.
[2] Not significant.

Table II. *Sodium Requirement in Relation to C_4 Pathway Characteristics*

Species	Lesions in Plants Not Receiving Sodium	Yield		Significance of Difference	Reference	C_4 Pathway Characteristics[1]	Reference	Probable Pathway
		No addition	0.1 meq/liter NaCl or Na_2SO_4					
		g dry wt/plant		%				
Gramineae								
Hordeum vulgare L. cv. Pallidium (barley)	None	0.77 3.82	0.99 4.01	5	(3)	N, H	(8)	C_3
Poa pratensis L. (Kentucky blue grass)	None	0.024	0.021	NS		N		C_3
Echinochloa utilis L. Ohwi et Yabuno (Japanese millet)	Chlorosis and necrosis	0.404	0.713	0.1		K		C_4
Cynodon dactylon L. (Bermuda grass)	Chlorosis	0.178	0.337	1		K, $H^{14}C_4$	(7)	C_4
Cyperaceae								
Kyllinga brevifolia	Chlorosis	0.628	1.245	1		K		C_4
Amaranthaceae								
Amaranthus tricolor L. cv. Early Splendour	Chlorosis and necrosis	0.884	2.099	0.1		K		C_4
Chenopodiaceae								
Chenopodium capitatum L. Aschers	None	12.25	14.37	NS	(3)	N	(15)	C_3
Beta vulgaris L. (sugar beet)	None	3.86	5.07	NS	(3)	N, H, $L^{14}C_4$	(8)	C_3
Atriplex nummularia Lindl. (oldman, giant saltbush)	Chlorosis	0.166	0.830	0.1	(3)	K, L, $H^{14}C_4$, $L^{13}C$	(11, 13, 14)	C_4
Atriplex paludosa R.Br. (marsh saltbush)	Chlorosis and necrosis	0.215	2.789	5	(3)	K		C_4
Atriplex quinii Fv.M.	Chlorosis and necrosis	0.116	0.815	5	(3)	K		C_4
Atriplex semibaccata R.Br. (berry saltbush)	Chlorosis and necrosis	0.104	0.653	1	(3)	K, $L^{13}C$	(13, 15)	C_4
Atriplex inflata Fv.M.	Chlorosis and necrosis	0.202	9.745	0.1	(3)	K, $H^{14}C_4$	(11, 14)	C_4
Atriplex leptocarpa Fv.M.	Chlorosis and necrosis	0.050	1.329	1	(3)	K		C_4
Atriplex spongiosa Fv.M. (pop saltbush)	Chlorosis and necrosis	0.570	12.472	0.1	(3)	K, L, P.carb	(10, 14)	C_4
Atriplex semilunalaris Aellen	Chlorosis and necrosis	0.098	0.526	0.1	(3)	Unknown		...
Atriplex lindleyi Moq.	Chlorosis and necrosis	0.093	0.560	5	(3)	Unknown		...
Atriplex vesicaria Heward ex Benth (bladder saltbush)	Chlorosis and necrosis	0.013	0.129	0.1	(2)	K, $H^{14}C_4$, P. carb, $L^{13}C$	(11, 13)	C_4
Atriplex hortensis L. var. Atrosanguineae (garden orache)	None	2.849	3.677	5	(3)	N, H, $L^{14}C_4$, $H^{13}C$	(14, 15)	C_3
Atriplex angustifolia Sm.	None	0.531	0.377	NS	(3)	Unknown		...
Atriplex glabriuscula Edmonton	None	24.804	24.100	NS	(3)	N, H, $H^{13}C$	(14)	C_3
Atriplex albicans Ait.	None	8.818 9.237	11.688 9.401	NS	(3)	Unknown		...
Kochia pyramidata Benth.	None	35.390	33.990	NS	(3)	N, $L^{14}C_4$	(11)	C_3
Kochia childsii Hort.	Chlorosis and necrosis	0.125	0.442	1		K, L, $L^{13}C$	(13, 14)	C_4
Exomis axyrioides Fenzl ex Moq.	None	0.864	0.925	NS	(3)	Unknown		...
Halogeton glomeratus (Bieb) Meyer	Smaller curved leaves, tendency to wilting	0.285	0.800	1	(16)	K	(15)	C_4
Cruciferae								
Brassica oleracea L. cv. "Savoy" (cabbage)	None	14.244	17.766	NS	(3)	N		C_3
Leguminosae								
Trifolium repens L. cv. "Palestine" (white clover)	None	2.299	2.897	NS	(3)	N		C_3
Solanaceae								
Lycopersicum esculentum Mill.								
cv. "Grosse Lisse"	None	4.84 6.93	4.76 7.83	NS	(3)	N		C_3
cv. "Marglobe"	None	13.76	15.40[2]	1	(17)	N		C_3

Table II—*Continued*

Species	Lesions in Plants Not Receiving Sodium	Yield		Significance of Difference	Reference	C_4 Pathway Characteristics[1]	Reference	Probable Pathway
		No addition	0.1 meq/liter NaCl or Na_2SO_4					
		g dry wt/plant		%				
Compositae								
Lactuca sativa L. cv. "Great Lakes" (lettuce)	None	3.871	6.113	NS	(3)	N		C_3
		0.822	1.228					
		2.230	2.444					
Aster tripolium L.	None	0.282	0.458	NS	(3)	Unknown		...
Portulacaceae								
Portulaca grandiflora Hook (rose moss)	Chlorosis, no flowers	0.242	0.789	1		K		C_4

[1] Leaf anatomy: N, normal (bifacial); K, Kranz. CO_2 compensation: L, low; H, high. ^{14}C in C_4 compounds (malate, aspartate): $L^{14}C_4$, low; $H^{14}C_4$, high. Phosphoenol pyruvate carboxylase activity: P.carb, high. ^{13}C discrimination: $L^{13}C$, low; $H^{13}C$, high.

[2] Cultures contained 1 meq/liter NaCl.

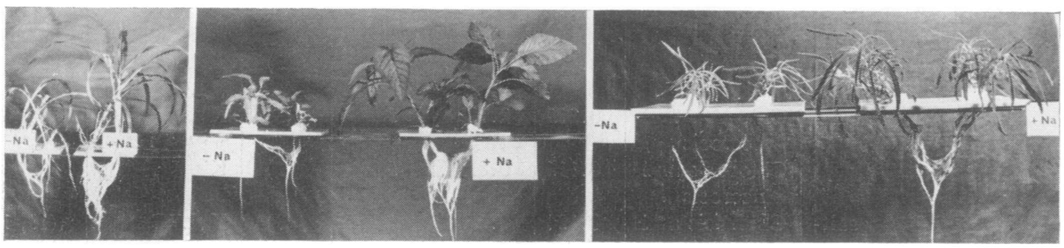

FIG. 1. Comparisons between plants of *Echinochloa utilis* (left), *Amaranthus tricolor* (center), and *Kochia childsii* (right) which received no addition (−Na) and 0.10 meq/liter sodium chloride (+Na).

a source of sodium to the plants. Seeds were thoroughly washed many times with distilled water before placing them in germination cultures. The seedlings were transferred to their cultures when sufficiently developed, and the sodium chloride treatment was supplied to the appropriate cultures.

RESULTS

Echinochloa utilis, Cynodon dactylon, Kyllinga brevifolia, Amaranthus tricolor, Kochia childsii, and *Portulaca grandiflora* showed significant dry weight responses when 0.1 meq/liter NaCl was supplied to their culture solutions (Table I). Plants of each species growing in cultures not receiving sodium made little growth compared to those supplied with sodium and had the characteristic leaf lesions described previously in sodium-deficient *Atriplex* species (Fig. 1) (2, 3, 6). Only the *Portulaca grandiflora* plants which received the sodium treatment set flower. Plants of *Poa pratensis* grew normally in "sodium-free" cultures and did not respond to the sodium treatment.

DISCUSSION

In Table II, the responses of 32 species to 0.10 meq/liter of sodium chloride or sodium sulfate are summarized. Only certain species of *Echinochloa, Cynodon* (Gramineae), *Kyllinga* (Cyperaceae), *Amaranthus* (Amaranthaceae), *Atriplex, Kochia, Halogeton* (Chenopodiaceae), and *Portulaca* (Portulacaceae) have been shown to respond to sodium by marked increases in dry weight. Plants grown in sodium-free cultures exhibited the leaf lesions previously described (2, 3, 6). These species have characteristics of plants with the C_4 dicarboxylic pathway which include the "Kranz type" specialized leaf anatomy (9), a low CO_2 compensation value, and reduced ^{13}C discrimination (13). The known C_4 pathway characteristics for each species are shown in Table II. No data relating to the C_4 pathway was available for certain species, including *Atriplex semilunalaris, Atriplex lindleyi, Atriplex angustifolia, Atriplex albicans, Exomis axyrioides,* and *Aster tripolium,* since their requirement for sodium was determined before the discovery of the C_4 dicarboxylic pathway and material is currently unavailable.

Three other species, barley (3), *Atriplex hortensis* (3), and tomato (17), with characteristics of C_3 pathway plants responded to sodium, but only marginally. The plants of these species in "sodium-free" cultures did not exhibit sodium deficiency lesions.

Within *Atriplex* and *Kochia,* which include both C_3 and C_4 species, only the C_4 species have been shown to respond to sodium; no decisive response was obtained in their C_3 species.

It appears from these data that species having characteristics of the C_4 photosynthetic pathway generally have a requirement for sodium.

Little is known of the role of sodium in plant metabolism. The respiration of leaves of sodium-deficient *Atriplex nummularia* is markedly depressed and restored within a few hours of adding sodium to the deficient culture solution, whereas the leaf symptoms are not alleviated until the 5th day. The output of CO_2 in the dark under anaerobic conditions was restored when sodium was fed to the deficient plants (4). In *Anabaena cylindrica,* nitrate reductase activity was greatly increased in cells grown without sodium. Addition of sodium controlled the activity of this enzyme. Nitrogen fixation was reduced in sodium-deficient cells (5). Sodium chloride compared to potassium chloride at 1 mM concentration has been shown to stimulate markedly the uptake of phosphate in the unicellular green alga, *Ankistrodesmus braunii* (12).

The correlation between the possession of the C_4 pathway and the essentiality of sodium could contribute to the understanding of the role of sodium in the metabolism of plants for which it is essential. It would be expected to function in a metabolic system unique to species with the C_4 dicarboxylic photosynthetic pathway.

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