

Short Communication

Growth Responses to Sodium by *Bryophyllum tubiflorum* under Conditions Inducing Crassulacean Acid Metabolism

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ABSTRACT

The dry weight yield of plants of *Bryophyllum tubiflorum* Harvey, a species with Crassulacean acid metabolism characteristics, increased significantly ($P < 1\%$) in response to added sodium (0.1 milliequivalents per liter NaCl was supplied to the culture solution initially containing less than 0.08 microequivalents per liter of Na) when grown under short day (8 hours) conditions but not when grown under long day conditions (16 hours).

From results of other work with Crassulacean acid metabolism species, it appears likely that under long day conditions, the plants assimilate CO_2 by the C_3 pathway but under short day conditions by the Crassulacean acid metabolism pathway in which metabolic processes common to those operating in the C_4 dicarboxylic pathway are active. It is suggested that sodium is involved in plants assimilating CO_2 with the C_4 and Crassulacean acid metabolism pathways.

Recent evidence suggests that species having the C_4 dicarboxylic photosynthetic pathway require small quantities of Na in their nutrition (3). Plants in cultures not supplied with Na made little growth compared with those receiving 0.1 meq/l NaCl and acquired leaf lesions of chlorosis and necrotic leaf tips, whereas species having C_3 (Calvin cycle) fixation grew normally in culture solutions to which no Na was supplied. The mechanisms for CO_2 fixation by species undergoing Crassulacean acid metabolism resemble those of species having the C_4 dicarboxylic photosynthetic pathway in certain respects (1, 4-6). These similarities suggested that CAM¹ species might also require small amounts of Na.

In preliminary experiments conducted in a greenhouse under long day conditions, *Bryophyllum tubiflorum* did not show any growth responses to small amounts of Na added to the cultures from which Na had been carefully eliminated. This lack of response to added Na was attributed to the possibility that the plants already contained sufficient Na for normal growth. However, under these conditions it is likely that the plants would have fixed CO_2 by the C_3 system and that the CAM system would not have been involved. Ting (7), reviewing previous literature, concluded that CAM activity is most pro-

nounced when night temperatures are low and day temperatures are high and that short day conditions were also conducive to the CAM type of metabolism. Recent evidence (4) suggests that the activities of the enzymes specifically involved with CAM metabolism in *Kalanchoe blossfeldiana*, a short day plant, are phytochrome-controlled. In short days there was a progressively rapid increase in the activity of all the enzymes of the CAM pathway whereas in long days or in short days with nights interrupted by red light, the pathway was not operative, presumably due to low activity of PEP carboxylase.

In several experiments we found that growth was greatest under conditions of long days and small diurnal temperature variation. Under these conditions there was no growth response to Na. When plants were grown under conditions of short day length and large diurnal temperature variation, significant responses to Na were obtained although the overall growth was still less than that of the former plants.

Plantlets collected locally from plants of *Bryophyllum tubiflorum* Harvey were grown in low Na culture solution under conditions of small diurnal variation in temperature and with long days (approximately 14 hr/day). The plantlets from these plants were thoroughly washed in several changes of silica-distilled water and transferred to cultures (eight plants per culture) for subsequent experiments. The procedures for growth of plants under conditions of low Na have been previously described (2). The basal culture solution had the following composition expressed in $\mu\text{moles/l}$: KNO_3 , 5,000; $\text{Ca}(\text{NO}_3)_2$, 4,000; KH_2PO_4 , 1,000; MgSO_4 , 1,000; $(\text{NH}_4)_2\text{HPO}_4$, 1,000; H_3BO_3 , 46; $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, 9.1; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.31; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.76; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{21} \cdot 4\text{H}_2\text{O}$, 0.1; NH_4Cl , 350. Iron was supplied as ferric ammonium ethylenediaminetetraacetate at 90 $\mu\text{moles/l}$ in the basal culture solution.

Less than 0.07 $\mu\text{eq/l}$ Na was derived from the purified salts of the culture solution as an impurity, and silica-distilled water contained less than 0.0087 $\mu\text{eq/l}$, giving a total Na concentration of approximately 0.08 $\mu\text{eq/l}$. Culture vessels of 2-l capacity were of Na-free plastic material. Plants under long day conditions 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 a source of Na to the plant. Short day conditions were obtained in an artificially illuminated growth cabinet situated in an air-conditioned building with filtered air entering it continuously. The air for culture aeration was passed through an 8 μm air-filter. NaCl was supplied to the appropriate cultures to give a final concentration of 0.1 meq/l.

The results of these experiments (Table I and Fig. 1) suggest that Na is not required by plants grown under conditions

¹ Abbreviation: CAM: Crassulacean acid metabolism.

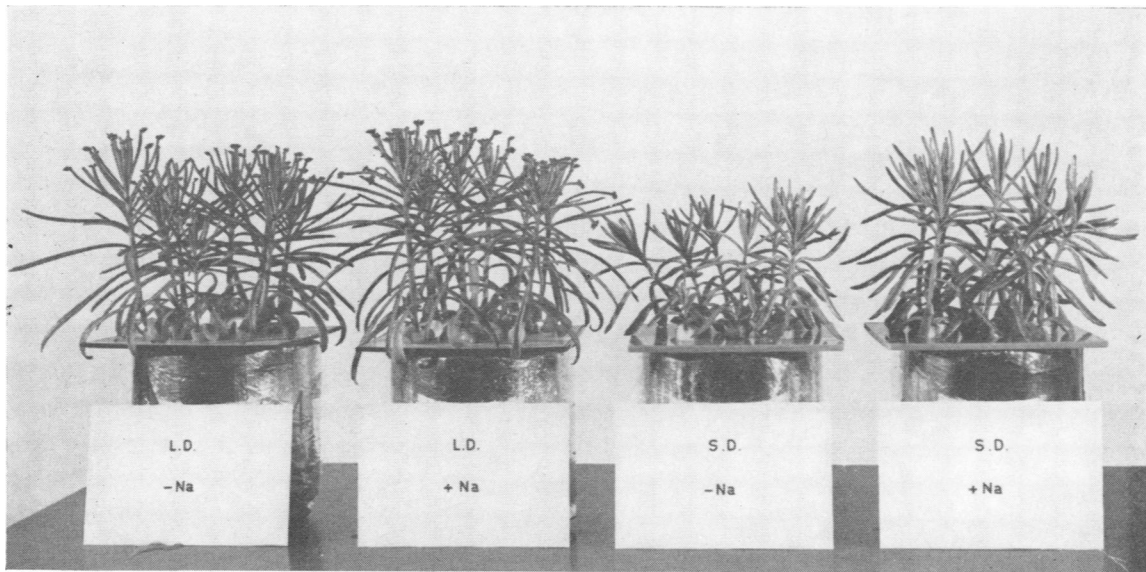


FIG. 1. Comparisons between plants of *Bryophyllum tubiflorum* receiving the following treatments. Left to right: no addition, 0.1 meq/l NaCl (16-hr light period; over-all temperature range, 15 to 38 C); no addition, 0.1 meq/l NaCl (8-hr light period; light temperature 33 C, dark temperature 13 C.

Table I. Responses by *Bryophyllum tubiflorum* to NaCl Grown under Different Conditions

Plants were transferred on day 24 from seedling cultures to culture vessels and received differential NaCl additions under the following conditions. Short days: in artificially illuminated growth cabinet with an 8 hr light period at approximately 2,800 ft-c and 16 hr dark period. Temperature during the light period was 33C and in the dark period 13 C. Long days-short days: in a naturally illuminated cabinet with the normal day length of 11 hr extended to 16 hr per day by a 100 w incandescent lamp giving an intensity of approximately 100 ft-c. The over-all temperature range was 15 to 38 C. On day 65, cultures were transferred to short day conditions in the artificially illuminated growth cabinet. Long days: in the naturally illuminated cabinet with the normal day length of 11 hr extended to 16 hr per day. Plants were harvested on day 100.

Conditions of Growth	Mean Dry Wt per Plant		Significance of Difference
	No addition	0.1 meq/l NaCl	
	<i>g</i>		<i>%</i>
Short days	0.123	0.176	<1
Long days-short days	0.175	0.218	<1
Long days	0.548	0.521	NS

conductive to the C₃ mode of photosynthesis, *i.e.* under long day periods. Plants grew more actively under these conditions

but showed no growth response to Na. Plants taken from the same population, however, when grown under conditions conducive to CAM photosynthesis, *i.e.* under conditions of short days and large diurnal temperature variation, responded significantly to small amounts of Na. Under these conditions of growth, metabolic processes common to those operating in C₄ dicarboxylic photosynthesis are active and this observation would suggest that Na is involved in this area of metabolism both in species having the C₄ photosynthetic system and in members of CAM carbon fixation. This supports the hypothesis that Na may be required for the primary dicarboxylic CO₂-fixation system characteristic of C₄ and CAM plants.

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