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ABSTRACT

Sodium (Na) movement between plants and humans is one of the more critical aspects of bioregenerative systems of life support, which NASA is studying for the establishment of long-term bases on the Lunar or Martian surface. This study was conducted to determine the extent to which Na can replace potassium (K) in red beet (*Beta vulgaris L.* ssp vulgaris) without adversely affecting metabolic functions such as water relations, photosynthetic rates, and thus growth. Two cultivars, Ruby Queen and Klein Bol, were grown for 42 days at 1200 μmol mol^{-1} CO_2 in a growth chamber using a re-circulating nutrient film technique with 0%, 75%, 95%, and 98% Na substitution for K in a modified half-strength Hoagland solution. Total biomass of Ruby Queen was greatest at 95% Na substitution and equal at 0% and 98% Na substitution. For Klein Bol, there was a 75% reduction in total biomass at 98% Na substitution. Nearly 95% of the total plant K was replaced with Na at 98% Na substitution in both cultivars. Potassium concentrations in leaves decreased from 120 g kg^{-1} dwt in 0% Na substitution to 3.5 g kg^{-1} dwt at 98% Na substitution. Leaf chlorophyll concentration, photosynthetic rate, and osmotic potential were not affected in either cultivar by Na substitution for K. Leaf glycinebetaine levels were

doubled at 75% Na substitution in Klein Bol, but decreased at higher levels of Na substitution. For Ruby Queen, glycinebetaine levels in leaf increased with the first increase of Na levels and were maintained at the higher Na levels. These results indicate that in some cultivars of red beet, 95% of the normal tissue K can be replaced by Na without a reduction in growth.

INTRODUCTION

Nutrient recycling is an essential component of bioregenerative systems used for advanced life support, which are being developed by NASA for long-duration space missions, such as lunar or Martian bases (MacElroy and Bredt, 1985). Sodium was essential for human metabolism at relatively high levels, but has not been shown to be essential for all plants. This imbalance in the required levels of Na between humans and plants could result in a Na buildup during hydroponic production of crops, if human wastes were recycled as a source of nutrients for crop production (Subbarao et al., 1999a,1999b). One approach to recycling Na in such closed life support systems might be to use plants that have the ability to utilize Na in place of K for many of their metabolic functions, thereby providing a continual removal of Na from the growing medium. Potassium is taken up in large quantities by plants (up to 130 g kg^{-1} dwt in hydroponically grown plants) (Hoagland and Martin, 1933) and plays an important role as a co-factor for a number of enzymes (Marschner, 1986; Glass, 1989). Though K is essential for a number of metabolic functions in the cytoplasm (e.g., enzyme activation, sustaining protein synthesis, and transporting of organic acids through phloem) (Glass, 1989), only a small fraction of the total plant tissue K is required for these functions. As the cytoplasm typically occupies <10% of the cell volume (Pitman, 1963), a major portion of the plant's K is localized in the vacuole where it apparently functions as an osmoticum to maintain cell turgor (Marschner, 1971). Since this is a non-specific function, Na should be able to substitute for K, provided that the plants have the ability for uptake, translocation and compartmentalize Na in the vacuole (Marschner, 1971; Lindhauer et al., 1990; Subbarao et al., 1999a,1999b). However, high levels of Na in the cytoplasm (>20mM) have been shown to interfere with essential K functions and thus result in Na toxicity (Wyn Jones et al., 1979).

Sodium has been reported to have a stimulatory effect on growth of some *Chenopodiaceae* members (El Sheikh et al., 1967; Draycott et al., 1970; Brownell and Crossland, 1972), but is not generally considered a macronutrient and has not been shown to be essential for all higher plants (Flowers et al., 1977). The extent to which K can be replaced by Na in metabolic processes varies with plant families and species. Within the family *Chenopodiaceae*, this replaceability is generally high (Lehr, 1953; El-Sheikh et al., 1967), although it has not been clear to what extent Na can substitute for K even in salt tolerant *Chenopods*, such as sugarbeet (*Beta vulgaris* L.) (Flowers and Lauchli, 1983). The present investigation was aimed at determining the extent that Na can replace K in red beet without adversely

affecting metabolic functions such as water relations, photosynthetic rates, and thus growth. Beet was selected because of its high tolerance to sodium and its role as a candidate crop for life support systems by NASA.

MATERIALS AND METHODS

Experimental Setup

Plants were grown in white vinyl plastic trays using a recirculating nutrient film technique (NFT) (Graves, 1981). A total of eight trays (0.75 m length, 0.10 m width, and 0.06 m deep) were spaced evenly in a 0.90 m^2 reach-in growth chamber (Environmental Growth Chambers, Chagrin Falls, IN). The trays were connected to four 20-L nutrient reservoirs, from which a nutrient flow of 1 to 2 L min^{-1} was maintained. Each tray provided a basal rooting area of 0.0045 m^2. Each nutrient reservoir supplied to two culture trays. Solution pH was continuously monitored and maintained between 5.0 and 5.8 by automatically adding 0.1 *M* HNO_3 using a peristaltic pump connected to a controller (Model No. 05997-20; Cole-Palmer, Chicago, IL) or manually adding 0.2 *M* of $Ca(OH)_2$ once daily. The total volume of acid and base addition for each treatment was recorded daily. A modified half-strength Hoagland nutrient solution was used with the nutrient composition of (mM): 2.5 $Ca(NO_3)_2$, 1.0 $MgSO_4$, 0.5 $NH_4H_2PO_4$, 0.06 Fe-EDTA, 0.0071 H_3BO_3, 0.0074 $MnCl_2$, 0.00096 $ZnSO_4$, 0.001 $CuSO_4$, and 0.00001 $(NH_4)_6Mo_7O_{24}$.

Electrical conductivity (EC) and pH data were logged continuously throughout the experiments by means of a custom data acquisition software program. Water levels were kept constant in the nutrient tanks using a simple gravimetric water supply system that added approximately 60 mL of water from 4 L jugs (under negative pressure) whenever the system volume decreased through evapotranspiration. Total water used was recorded daily. Solution samples were analyzed for Na and K each week.

Treatments

Two red beet cultivars, Ruby Queen (open pollinated cv.) and Klein Bol (hybrid cv.) (obtained from Dr. Irvin Goldman, Univ. Wisconsin) were used in this study. Plants were grown under four treatments: 5.0 mM KCl + 0 mM NaCl; 1.25 mM KCl + 3.75 mM NaCl; 0.25 mM KCl + 4.75 mM NaCl; and 0.10 mM KCl + 4.90 mM NaCl. These treatments are equivalent to 0%, 75%, 95%, and 98% of the standard concentration of K being replaced by Na. The experiment was replicated twice over time. The experiment was laid out as split-plot design where Na-treatments were main-plots and cultivars were treated as sub-plots.

Cultural Approach

Seeds were soaked for about 18 h in aerated deionized water; water was replaced with fresh deionized water every 6 h, to remove seed coat inhibitors. The sprouted

seeds were planted between two nylon (Nitex) fabric wicks supported 4 cm above the bottom of culture trays as described by Prince and Knott (1989). All new wicks were pre-rinsed with ethanol and deionized water to remove potential phytotoxins (Wheeler et al., 1985). Three seeds were planted at each of the four locations in the culture trays, and trays were covered with white, translucent acrylic covers for 3 days to maintain a high humidity during seedling establishment. Seedlings were thinned to one per position (i.e., four per tray) 7 days after planting (DAP). At 3 DAP, nutrient solutions were introduced into the reservoirs with the treatment concentrations of K and Na. Entire nutrient solution volumes were replaced at 21 DAP and 35 DAP. Plants were harvested at 42 DAP. The growing conditions in the controlled environment chamber were 12/12 h, 22/22°C light/dark, air temperature regime respectively, 85% RH for the first 7 days, followed by 65% for the rest of the growing period. Minimal carbon dioxide concentrations were maintained at 1,200 $\mu mol\ mol^{-1}$ (0.12 kPa) by controlled additions of pure CO_2. Lighting to the chamber was provided with high pressure sodium (HPS) lamps (400-W Philips Ceramalux, Philips Lighting Corp., Bloomfield, NJ or GE Lucalox, General Electric Co., Cleveland, OH) with photosynthetic photon flux (PPF) averaging about 400 $\mu mol\ m^{-2}\ s^{-1}$ at the top of the canopy.

Relative Water Content

Ten leaf disks were obtained from plants in each tray at 41 DAP, and the fresh weights were determined. Discs were then floated on deionized water for 5 h under low irradiance. The turgid tissue was then quickly blotted dried with tissue paper prior to determining turgid weight. Dry weight was then determined after oven-drying at 70°C for 48 h. The relative water content (RWC) was calculated using the following formula:

$$RWC\ (\%)=100x[(\text{fresh weight-dry weight})/(\text{turgid weight-dry weight})] \quad [1]$$

Osmotic Potential of the Leaf Sap

Ten leaf discs were obtained from plants in each tray at 41 DAP and stored in 1 mL centrifuge vials and immediately frozen at -5 C; these samples were thawed and centrifuged for 5 min at 18,000 g, and the osmotic potential (OP) of the expressed sap was measured with a calibrated (with a range of KCl solutions) vapor pressure osmometer (Wescor 5500, Wescor Inc., Logan, UT). Osmotic potential at full turgor (OP_{100}) (turgid OP) was calculated using the formula of Wilson et al. (1979) assuming negligible apoplastic water:

$$OP_{100}=(OP \times RWC)/100 \quad [2]$$

Chlorophyll Estimation

At harvest (i.e., 42 DAP), ten leaf disks were taken randomly from the four plants of each tray and fresh weight was determined. Chlorophyll was extracted with 10

mL of 95% ethanol and determined using a spectrophotometer. Absorbencies were measured at 649 and 665 nm and chlorophyll a, b, total chlorophyll were calculated according to Wintermans and De Mots (1965).

Photosynthetic Rates

Single leaf photosynthetic rates were measured at 32 and 38 DAP using a LICOR-6200 (LICOR, Lincoln, NE). Two plants from each treatment were used for making photosynthesis measurements, with a total of six measurements for each treatment. These six measurements per treatment were averaged and used as one replication for statistical analysis.

Harvest Data

Plants were harvested at 42 DAP and leaf area was measured using a area meter (LiCOR LI 3100, Lincoln, NE). Plant tissues were separated into lamina, petiole, storage root and fibrous root, and fresh weights were recorded. One plant per tray was freeze-dried and the samples were analyzed for glycinebetaine content. The remaining samples were oven dried at 70°C for 48h for dry weight measurements. About 0.5 g of finely ground tissue was digested with 10 mL of concentrated nitric acid in a microwave digestion system. The macro and micro nutrients were analyzed by inductively-coupled plasma analysis using a Perkin-Elmer Plasma 40 (Perkin-Elmer, 1981).

Glycinebetaine Analysis

Glycinebetaine in leaf tissue was quantified by HPLC using a photodiode array detector using a modified procedure of Naidu (1998). Approximately 100 mg of finely ground, freeze dried leaf tissue was extracted with 3.5 mL of ice-cold extraction mixture of methanol, chloroform, and water (MCW 12:5:3) in a vortex mixer. An additional 3.5 mL of deionized water was added to this mixture to break the emulsion formed with MCW. The extraction mixture was refrigerated overnight after thoroughly mixing and then centrifuged at 1,000 g for 10 min at room temperature. One mL of the upper methanol-water phase was transferred to a 1.5 mL microfuge tube and then treated with a predetermined (about 0.5 g each) amount of pre-washed (with 1N NaOH) ion-exchange resins (1:1 of amberlite CG-50 and Dowex 1 x 2-100). Ion-exchange resins were removed by centrifugation and the supernatant was filtered through a 0.45 μm membrane filter into an HPLC auto-sampler vial. The extract (injection volume: 20 μl) was subjected to HPLC analysis. An analytical column of SUPELCOGEL K (SEPELCO 5-9342), 300 x 7.8 mm proceeded by a SUPELGUARD K, 50 x 7.8 mm was used. Glycinebetaine was separated and eluted using 15 mM KH_2PO_4 at 0.8 mL min^{-1} and 80°C and quantified by measuring absorbance at 195 nm.

Data Analysis

All data were analyzed by ANOVA using the GENSTAT statistical package.

RESULTS

Leaf Area

Leaf area was greatest at 75% Na substitution in Klein Bol, and for Ruby Queen, it was at 95%. The cultivar by treatment interaction was significant ($P \leq 0.05$), where Klein Bol suffered nearly a 75% reduction in leaf area at 98% Na substitution, whereas for Ruby Queen, leaf area increased by about 40% over 0% Na control (Table 1).

Fresh and Dry Weight Yields

Total plant dry matter yields were greatest at 75% Na substitution for Klein Bol, and at 95% for Ruby Queen (Table 1). Dry matter yields were significantly ($P \leq 0.05$) reduced at 95% and 98% Na substitution for Klein Bol (Table 1). Storage root dry matter was significantly reduced at 95% and 98% Na substitution for Klein Bol. Harvest index (storage root fresh weight/total fresh weight) was significantly different for the cultivars, and the treatment x cultivar interaction was significant (data not presented).

Storage root fresh weight was significantly affected by Na substitution in both the cultivars, though most growth was observed at 75% Na substitution (data not presented). As with leaf area, a significant cultivar x treatment interaction occurred, where storage root fresh weight was about 68% of the 0% Na control at 98% Na substitution for Ruby Queen, whereas it was only 5% of the 0% Na control for Klein Bol.

Chlorophyll Concentration and Photosynthetic Rate

Chlorophyll concentration was not affected by Na substitution for K (Table 2). Similar responses were found for chlorophyll-a and chlorophyll-b (data not presented). Photosynthetic rates were not significantly affected in either cultivar by the Na substitution treatments. However, cultivar differences were significant ($P \leq 0.001$); Ruby Queen showed higher photosynthetic rates than Klein Bol at 32 and 38 DAP (Table 2).

Water Relations

The substitution of Na for K resulted in significantly ($P \leq 0.05$) higher leaf relative water content in both red beet cultivars (Table 3). The osmotic potential of the leaf at full turgor (OP_{100}), however, was not significantly affected by the replacement of K with Na. There were no significant differences in osmotic potentials between the two cultivars (Table 3).

TABLE 1. Effect of Na substitution for K in nutrient solution on leaf area, and dry matter production of two beet cultivars (42DAP).

Parameter	Cultivar	% Na substituted for K (from a total K+Na of 5 mM)			
		0	75	95	98
			$cm^2\ tray^{-1}$		
Leaf Area	Klein Bol	4497	5599	2478	1105
	Ruby Queen	3585	5076	6483	5058
SE (treat)	739ns				
SE (cultivar)	513*				
SE (treatXcultivar)	1036*				
Total dry weight			$g\ tray^{-1}$		
	Klein Bol	42.4	68.3	20.1	11.6
	Ruby Queen	46.6	55.4	75.8	46.1
SE (treat)	4.2*				
SE (cultivar)	6.1*				
SE (treatXcultivar)	9.6*				
Storage root dry weight			$g\ tray^{-1}$		
Klein Bol		14.2	26.6	3.4	0.8
Ruby Queen		19.0	23.7	19.0	9.0
SE (treat)	3.93*				
SE (cultivar)	1.72*				
SE (treatXcultivar)	4.62ns				

*$P \leq 0.05$; **$P \leq 0.001$; ns=non-significant.

Sodium Uptake

Sodium levels in plant tissue were substantially increased ($P \leq 0.001$) in both red beet cultivars as the K in the nutrient solution was replaced by Na (Table 4). In leaf lamina, Na levels increased from 0.2 g kg^{-1} dry weight (dwt) in 0% Na control to nearly 100 g kg^{-1} dwt at 98% Na substitution (Table 4). Similar response in petiole Na levels were found for both cultivars of red beet to Na substitution for K, with Na levels reached nearly 120 g kg^{-1} and 130 g kg^{-1} dwt for Klein Bol and Ruby Queen, respectively (Table 4). Storage root Na levels showed similar response to Na substitution, but were substantially lower than the leaf tissue Na levels (nearly 50% of leaf Na) (Table 4). The Na levels in fibrous root were the lowest among the various plant parts.

TABLE 2. Effect of Na substitution for K in nutrient solution on chlorophyll levels and photosynthetic rate in two red beet cultivars.

Parameter	Cultivar	% Na substituted for K (from a total K+Na of 5 mM)			
		0	75	95	98
Total chlorophyll (42 DAP)		**µg chlorophyll/mg leaf fresh wt.**			
	Klein Bol	1.75	1.40	1.20	1.10
	Ruby Queen	1.55	2.10	1.50	1.50
SE (treat)	0.26ns				
SE (cultivar)	0.23ns				
SE (treatXcultivar)	0.42 ns				
Photosynthetic Rate (32 DAP)		**µmol m^{-2} s^{-1}**			
	Klein Bol	13.6	16.6	11.3	9.2
	Ruby Queen	17.3	15.9	14.5	14.8
SE (treat)	1.11ns				
SE (cultivar)	0.58**				
SE (treatXcultivar)	1.38ns				
Photosynthetic Rate (38 DAP)		**µmol m^{-2} s^{-1}**			
	Klein Bol	14.7	14.7	7.2	9.8
	Ruby Queen	16.6	16.5	15.0	16.6
SE (treat)	1.30ns				
SE (cultivar)	0.72**				
SE (treatXcultivar)	1.65ns				

*$P \leq 0.05$; **$P \leq 0.001$; ns=non-significant.

Potassium Uptake

Replacement of K with Na in nutrient solution substantially decreased tissue K levels in leaf lamina, petiole, root and tuber (Table 5). In the leaf laminae, K levels decreased from 121 g kg^{-1} dwt in the 0% Na control to 3.5 g kg^{-1} dwt at 98% Na substitution. There were no significant differences between Ruby Queen and Klein Bol in their tissue K levels in any of the K/Na treatments.

Glycinebetaine Accumulation

Substitution of Na for K in nutrient medium had a significant ($P \leq 0.001$) effect on glycinebetaine levels in leaves of red beet (Table 3). The cultivar x treatment

TABLE 3. Effect of Na substitution for K in nutrient solution on leaf relative water content, osmotic potential, and glycinebetaine of two red beet cultivars (42 DAP).

Parameter	Cultivar	% Na substituted for K (from a total K+Na of 5 mM)			
		0	75	95	98
Leaf Relative water content			(%)		
	Klein Bol	78.9	73.8	79.5	84.2
	Ruby Queen	77.2	78.6	82.5	84.1
SE (treat)	1.19*				
SE (cultivar)	0.18**				
SE (treatXcultivar)	1.21**				
Osmotic potential (at full turgor)			MPa_{100}		
	Klein Bol	1.33	1.25	1.33	1.18
	Ruby Queen	1.42	1.16	1.14	1.50
SE (treat)	0.18ns				
SE (cultivar)	0.08ns				
SE (treatXcultivar)	0.21ns				
Glycinebetaine in leaf			μmmole g^{-1} dwt		
	Klein Bol	45.2	76.5	30.1	32.3
	Ruby Queen	31.1	56.4	52.4	50.3
SE (treat)	2.86**				
SE (cultivar)	4.08ns				
SE (treatXcultivar)	6.43*				

*$P \leq 0.05$; **$P \leq 0.001$; ns=non-significant

interaction was significant ($P \leq 0.05$). Glycinebetaine levels were nearly doubled in Klein Bol and Ruby Queen at 75% Na substitution for K in the nutrient medium (Table 3). However, only in Ruby Queen, were the higher glycinebetaine levels maintained at 95 and 98% Na substitution; for Klein Bol, glycinebetaine levels declined and reached levels equivalent to its concentrations in the 0% Na control treatment (Table 3).

DISCUSSION

It is commonly accepted that K acts as a macronutrient in plants. Though, Na has often been shown to have some stimulatory affect on the growth of some *Chenopodiaceae* species, it is not generally thought of as an essential nutrient for

TABLE 4. Effect of Na substitution for K in nutrient solution on sodium levels (g kg^{-1} dwt) in leaf lamina, petiole, tuber and root of red beet cultivars (42 DAP).

Parameter	Cultivar	% Na substituted for K (from a total K+Na of 5 mM)			
		0	75	95	98
		g kg^{-1} dwt			
Leaf lamina	Klein Bol	0.2	51.2	81.1	100.0
	Ruby Queen	0.2	52.9	68.0	89.7
SE (treat)	4.2**				
SE (cultivar)	5.2ns				
SE (treat X cultivar)	8.4ns				
Leaf petiole	Klein Bol	0.7	52.6	91.2	129.5
	Ruby Queen	0.2	44.0	91.4	118.2
SE (treat)	8.452**				
SE (cultivar)	1.73*				
SE (treat X cultivar)	8.79ns				
Fibrous root	Klein Bol	0.3	4.3	8.6	10.9
	Ruby Queen	0.3	4.9	7.1	13.2
SE (treat)	2.28*				
SE (cultivar)	1.48ns				
SE (treatXcultivar)	3.10ns				
Storage root	Klein Bol	0.2	14.5	33.4	54.2
	Ruby Queen	0.2	13.5	38.0	54.7
SE (treat)	1.40**				
SE (cultivar)	2.37ns				
SE (treatXcultivar)	3.63ns				

*$P \leq 0.05$; **$P \leq 0.001$; ns=non-significant.

all plants. Our studies indicate that at least in certain cultivars of red beet, Na can replace a major portion of the tissue K. The positive growth response of red beet to Na in our study is consistent with other reports on *Chenopodiaceae* to Na (El-Sheikh et al. 1967). Ruby Queen can tolerate a much higher Na environment than Klein Bol and still maintain its growth potential. High levels of Na have also been reported to suppress storage root growth, while stimulating shoot growth in sugarbeet (El-Sheikh et al., 1967; Marschner et al., 1981, Hampe and Marschner, 1982). Our results with red beet are in agreement with this general Na effect on storage root growth resulting in an altered storage root/shoot ratio.

TABLE 5. Effect of Na substitution for K in nutrient solution on potassium levels (g kg^{-1} dwt) in leaf lamina, petiole, tuber, and root of red beet cultivars (42 DAP).

Parameter	Cultivar	% Na substituted for K (from a total K+Na of 5 mM)			
		0	75	95	98
				g kg^{-1} dwt	
Leaf lamina	Klein Bol	120.5	16.50	3.5	3.5
	Ruby Queen	125.5	20.50	5.0	3.5
SE (treat)	1.48**				
SE (cultivar)	2.07ns				
SE (treatXcultivar)	3.28ns				
Leaf petiole	Klein Bol	146.5	26.5	3.5	5.0
	Ruby Queen	150.5	18.5	10.0	3.5
SE (treat)	5.36**				
SE (cultivar)	5.54ns				
SE (treatXcultivar)	9.49ns				
Fibrous root	Klein Bol	37.0	7.0	5.5	6.0
	Ruby Queen	42.5	8.0	5.5	4.5
SE (treat)	8.88ns				
SE (cultivar)	1.55ns				
SE (treatXcultivar)	9.11ns				
Storage root	Klein Bol	62.5	19.5	4.0	1.5
	Ruby Queen	54.5	21.0	9.5	6.0
SE (treat)	7.87*				
SE (cultivar)	1.87ns				
SE (treatXcultivar)	8.30ns				

*$P \leq 0.05$; **$P \leq 0.001$; ns=non-significant.

The maximum growth of red beet in these studies was found at leaf K levels of about 5.0 g kg^{-1} dwt in Ruby Queen, and at 17 g kg^{-1} dwt for Klein Bol. For many plants, the critical leaf concentrations for K deficiency is reported to be in the range of 5 to 20 g kg^{-1} dwt (Leigh and Wyn Jones, 1984 and references therein), while for sugarbeet it was 8.0 g kg^{-1} dwt (El-Sheikh et al., 1967). However, these are average levels and do not take into consideration the influence of different concentrations of Na in the tissues.

Through its contribution as an osmoticum, K is known to play a major role in the maintenance of cell turgor, water uptake, and thus growth of the plants (Cram, 1976; Wyn Jones et al., 1979). Our results with red beet indicate that even when 95% of the plant's leaf tissue K was replaced with Na, the turgid osmotic potential (OP_{100}) of the leaf sap was still similar to the 0% Na control plants (Table 3), indicating that Na can effectively replace K for this function. Moreover, this degree of replacement resulted in no significant reduction in the relative water content of the leaves of either of the two cultivars.

Glycinebetaine concentration in leaves were high even at 0% Na control level, which agrees with its reported constitutive presence in *Chenopodiaceae* members (Wyn Jones and Storey, 1981; Hanson and Wyse, 1982). Glycinebetaine is reported to protect various enzymes, and chloroplasts from Na damage in the cytoplasm (Murata et al., 1992; Rajasekaran et al., 1997; Nomura, et al., 1998). In red beet, glycinebetaine levels were nearly doubled at 75% Na substitution for K, even though the osmotic potential of the nutrient medium was essentially the same for all K/Na replacement treatments. The higher levels of glycinebetaine in Ruby Queen suggest that some protective role from Na damage in the cytoplasm (Hanson and Wyse, 1982; Robinson and Jones, 1986). Higher glycinebetaine levels were maintained only in Ruby Queen at the higher Na substitution treatments 95% and 98%; in contrast, the glycinebetaine levels at the higher Na levels in Klein Bol, where the yield was reduced, returned to the lower levels similar to those of the 0% Na control. Glycinebetaine accumulation, whether constitutive or salt induced, may be a specific adaptation for salt tolerance in wild and cultivated members of the *Chenopodiaceae,* including *Beta* spp (Hanson and Wyse, 1982). It remains to be seen whether the ability to maintain higher glycinebetaine levels at high tissue Na levels of 100 g kg^{-1} dwt has any adaptive significance in red beet and if it is related to the greater growth rates observed in Ruby Queen at 95 and 98% Na substitution in these studies.

Tissue Na levels in shoot and other parts of the plant increased dramatically at 75, 95 and 98% Na substitution in both cultivars. As the nutrient solution K was replaced with Na, the tissue concentration of these two ions followed a similar pattern (Figure 1). At 98% Na substitution, nearly 95% of the plant's tissue K was replaced by Na. Leaf Na increased from 0.2 g kg^{-1} dwt in 0% Na control to 100 g kg^{-1} dwt at 98% Na substitution. At the same time, leaf tissue K decreased from 120 g kg^{-1} dwt in 0% Na control to about 3.5 g kg^{-1} dwt at 98% Na substitution. These results with red beet suggest that there is limited, if any, discrimination against Na under these conditions, as it appears that Na was taken up freely and translocated to the shoot. This can be demonstrated more clearly by plotting tissue Na content with nutrient solution Na content, which shows a slope close to unity (b=0.96) (Figure 1). Sodium concentrations of 100 g kg^{-1} dwt are equivalent to about 500 mM when expressed on leaf tissue water and these levels were similar to those of some halophytes (Berry, 1970; Flowers and Lauchli, 1983). It is interesting to note that even at the extremely high concentrations of Na in leaves, the chlorophyll content

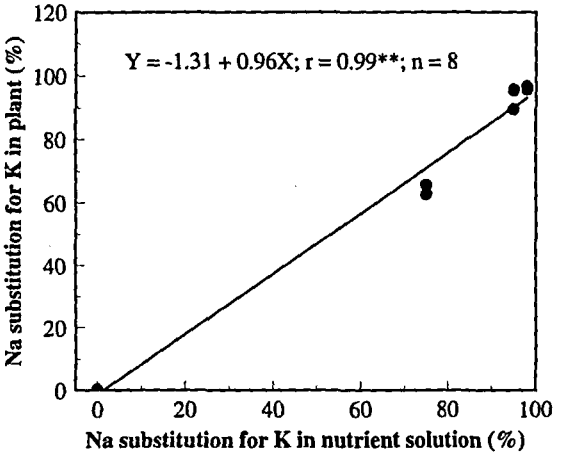

FIGURE 1. Relationship between Na substitution for K in the nutrient solution to Na substitution for K in the plant of red beet.

was not affected, suggesting that Na may be able to substitute for K in more than just osmotic functions in beet. However, Na was unable to replace K for chlorophyll synthesis, despite its stimulating effect in spinach and sugarbeet (Marschner and Possingham, 1975). In our study with red beet, photosynthetic rates were not affected in Ruby Queen even when leaf K levels dropped to 3.5 g kg^{-1} dwt. In contrast, photosynthetic rates were severely reduced in maize leaves below K levels of 15 g kg^{-1} dwt (Peaslee and Moss, 1966; Smid and Peaslee, 1976). In sugarbeet, most of the leaf Na was reported to have concentrated in the chloroplast (Stocking and Ongun, 1962) and has been hypothesized to have been involved in photosynthesis. It is suggested that Na could replace K in guard cells in some plants (Terry and Ulrich, 1973), but at K concentrations below 10 g kg^{-1} dwt, the stomatal conductance and photosynthetic rates were substantially reduced (Humble and Hsiao, 1969).

Nearly 60 or so enzymes require K as a co-factor in the cytoplasm (Marschner, 1986; Glass, 1989). In red beet, K concentration in the leaf decreased to 3.5 g kg^{-1} dwt at 98% Na substitution (i.e., 16 mM on a tissue water basis) for K in the nutrient solutions. For many glycophytes, the optimal cytoplasmic K levels required for normal metabolism lie between 100 and 200 mM, while Na concentrations should be less than 20 mM (Wyn Jones et al., 1979). If K is uniformly distributed inside the cell (i.e., between vacuole and cytoplasm), then it could be inadequate to meet its metabolic requirements. Thus, it is possible that K is compartmentalized in the

cytoplasm, which occupies between 5 and 10% of the cell volume (Pitman, 1963). If so, K concentrations in the cytoplasm are estimated at 160 to 300 mM, sufficient to meet most of the metabolic requirements of the red beet., e.g., Ruby Queen, when 95% of its K was replaced by Na.

CONCLUSIONS

In red beet nearly 95% of the tissue K could be replaced with Na without reducing growth below that of the 0% Na treatment, thus the major use of K during plant growth may be as an osmoticum where it can be replaced by Na. Genetic differences in this capacity to utilize Na indicates the possibility for further genetic improvement by combining this capability with high productivity. This capability to utilize Na for K may very well be linked to their ability to maintain higher levels of glycinebetaine in leaves.

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