2000

Low Potassium Enhances Sodium Uptake in Red-Beet Under Moderate Saline Conditions

G. V. Subbarao

Dynamac Corporation, Kennedy Space Center, FL

R. M. Wheeler

NASA Biomedical Office, Kennedy Space Center, FL

G. W. Stutte

Dynamac Corporation, Kennedy Space Center, FL

L. H. Levine

Dynamac Corporation, Kennedy Space Center, FL

Follow this and additional works at: <http://digitalcommons.unl.edu/nasapub>

Subbarao, G. V.; Wheeler, R. M.; Stutte, G. W.; and Levine, L. H., "Low Potassium Enhances Sodium Uptake in Red-Beet Under Moderate Saline Conditions" (2000). *NASA Publications*. 197.
<http://digitalcommons.unl.edu/nasapub/197>

This Article is brought to you for free and open access by the National Aeronautics and Space Administration at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in NASA Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Low Potassium Enhances Sodium Uptake in Red-Beet Under Moderate Saline Conditions

G. V. Subbarao,^a R. M. Wheeler,^b G. W. Stutte,^a
and L. H. Levine^a

^aDynamac Corporation, Kennedy Space Center, FL 32899

^bR.M. Wheeler, NASA Biomedical Office, Kennedy Space Center, FL 32899

ABSTRACT

Due to the discrepancy in metabolic sodium (Na) requirements between plants and animals, cycling of Na between humans and plants is limited and critical to the proper functioning of bio-regenerative life support systems, being considered for long-term human habitats in space (e.g., Martian bases). This study was conducted to determine the effects of limited potassium (K) on growth, Na uptake, photosynthesis, ionic partitioning, and water relations of red-beet (*Beta vulgaris* L. ssp. *vulgaris*) under moderate Na-saline conditions. Two cultivars, Klein Bol, and Ruby Queen were grown for 42 days in a growth chamber using a re-circulating nutrient film technique where the supplied K levels were 5.0, 1.25, 0.25, and 0.10 mM in a modified half-strength Hoagland solution salinized with 50 mM NaCl. Reducing K levels from 5.0 to 0.10 mM quadrupled the Na uptake, and lamina Na levels reached -20 g kg⁻¹ dwt. Lamina K levels decreased from -60 g kg⁻¹ dwt at 5.0 mM K to -4.0 g kg⁻¹ dwt at 0.10 mM K. Ruby Queen and Klein Bol responded differently to these changes in Na and K status. Klein Bol showed a linear decline in dry matter production with a decrease in available K, whereas for cv. Ruby Queen, growth was stimulated at 1.25 mM K and relatively

insensitive to a further decreases of K down to 0.10 mM. Leaf glycinebetaine levels showed no significant response to the changing K treatments. Leaf relative water content and osmotic potential were significantly higher for both cultivars at low-K treatments. Leaf chlorophyll levels were significantly decreased at low-K treatments, but leaf photosynthetic rates showed no significant difference. No substantial changes were observed in the total cation concentration of plant tissues despite major shifts in the relative Na and K uptake at various K levels. Sodium accounted for 90% of the total cation uptake at the low K levels, and thus Na was likely replacing K in osmotic functions without negatively affecting the plant water status, or growth. Our results also suggest that cv. Ruby Queen can tolerate a much higher Na tissue concentration than cv. Klein Bol before there is any growth reduction.

INTRODUCTION

Potassium is ubiquitous in all higher plants and plays a vital role in a wide range of biochemical and biophysical processes (Flowers and Lauchli, 1983). Potassium concentrations as high as 120 to 140 g kg⁻¹ dwt, can occur in hydroponically grown salad crops such as lettuce (Wheeler et al., 1994). In contrast, Na is commonly found in most plants but is not normally considered essential element for most plants (Marschner, 1986; Subbarao et al., 2001a), but Na is required for a range of functions in humans (Harrison, 1991). Nutrient cycling from humans to plants and vice versa will be important for the maintenance of bio-regenerative life support systems (BLSS) currently being studied for long-term space missions, where plants could be used to recycle waste water while producing food and oxygen (MacElroy and Brendt, 1985). Because very little Na is found in the edible portions of most crop plants, efforts are underway to develop management approaches to increase the Na concentration of the edible tissues of the BLSS plants, which would facilitate recycling of Na between the plants and humans. In these closed life support systems, human wastes (such as urine), which can be high in N and Na, could be integrated into the hydroponic food production systems as a source of nutrients (Subbarao et al., 1999a, 2000). Our earlier studies indicated that red-beet has an enormous potential to take up Na without negatively affecting its metabolism (Subbarao et al., 1999b). One approach to facilitate Na uptake would be to reduce the K levels in nutrient medium (e.g., <5.0 mM) (Marschner et al. 1981b; Subbarao et al., 1999b). Because Na levels would likely vary in a life support food production system linked to waste recycling, the consequences of minimal K levels on growth and Na uptake of red-beet need to be understood (Subbarao et al., 1999a, 2000). The present investigation was undertaken to assess the effects of a range of nutrient solution K levels on Na, and K uptake, water relations, glycinebetaine accumulation, photosynthesis, growth and productivity of two red-beet cultivars under moderate Na-saline conditions.

MATERIALS AND METHODS

Experimental Setup

Plants were grown in white vinyl plastic trays using a re-circulating 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 m² reach-in growth chamber. Trays were connected to four 20-L nutrient solution reservoirs, from which a solution flow of 1 to 2 L min⁻¹ was maintained. Each tray provided a basal rooting area of 0.08 m². Each nutrient reservoir supplied two culture trays. Solution pH was continuously monitored and maintained between 5.0 to 5.8 by automatically adding 0.1 M HNO₃ 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): 2.6 Ca (NO₃)₂, 1.0 MgSO₄, 0.5 NH₄H₂PO₄, 0.06 Fe-EDTA, 0.0071 H₃BO₃, 0.0074 MnCl₂, 0.00096 ZnSO₄, 0.001 CuSO₄, and 0.00001 (NH₄)₆ Mo₇O₂₄, plus the treatment levels of K and Na.

Electrical conductivity (EC) and pH data were logged continuously throughout the experiment by means of a custom data acquisition software program. Water levels were kept constant in the nutrient solution tanks using a simple gravimetric water supply system that added approximately 60 mL of demonize 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 using ICP spectrometry.

Treatments

Two red-beet (*Beta vulgaris*) cultivars, Ruby Queen (RQ) (open pollinated cv.) and Klein Bol (KB) (hybrid cv.) (obtained from Dr. Irvin Goldman, University of Wisconsin) were used in this study. Plants were grown at four K treatments where the initial nutrient solution K concentration was (mM) (K as KCl): 5.0 mM, 1.25mM, 0.25mM, and 0.10mM. The total concentration of monovalent cations (i.e., K+Na) was the same, 55 mM for all K treatments. Thus, the decrease in K levels for various K treatments were compensated by an increase in equivalent amounts of Na. The sodium levels for four K treatments were: 50.00, 53.75, 54.75, and 54.90 mM NaCl, respectively. The osmotic potential of the nutrient solutions was 0.27 MPa for all treatments. The experiment was replicated twice over time. The experiment was laid out as split-plot design where K treatments were main-plots and cultivars were treated as sub-plots.

Cultural Approach

Seeds were soaked for about 18 h in aerated demonize water, which was replaced with fresh demonize 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 demonize 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 the first 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. 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 a 12/12 h photoperiod, 22°C constant air temperature. Relative humidity was 85% for the first 7 days, followed by 65% for the rest of the growing period. Carbon dioxide concentrations were near 1200 $\mu\text{mol mol}^{-1}$ (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, N.J.; or GE Lucalox, General Electric Co., Cleveland, OH) with the photosynthetic photon flux (PPF) averaging about 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the top of the canopy.

Relative Water Content

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

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

Osmotic Potential of the Leaf Sap

Ten leaf discs were obtained from plants in each tray at 41 DAP. Discs were placed 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 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:

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

Chlorophyll Estimation

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

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

Photosynthetic Rate

Single leaf net photosynthetic rates were measured at 31 and 39 DAP using a LICOR-6200 (LICOR, Lincoln, NE). Two plants from each treatment were used for making photosynthetic measurements; three photosynthesis measurements from each plant 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 an area meter (LICOR LI 3100, Lincoln, NE). Plant tissues were separated into lamina, petiole, storage root and fibrous roots, and fresh weights were recorded. One plant per tray was freeze dried and the samples analyzed for glycinebetaine content. The remaining samples were oven dried at 70°C for 48 h 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 (ICP) analysis using a Perkin-Elmer Plasma 40 (Perkin-Elmer, 1981). For nitrate analysis, nearly 0.2 g of finely ground plant sample was extracted with 20 mL of 2N KCl solution, and analyzed using a Technicon auto analyzer (Technicon Industrial Systems, 1987). Chloride in the plant tissue samples were estimated using the methodology of Blaedel and Meloche (1960), where about 0.5 g of the ground plant tissue was extracted with 20 mL of 2% CaNO₃ and chloride estimated using potentiometric titration.

Glycinebetaine Analysis

Glycinebetaine in leaf tissue was quantified by HPLC using a photodiode array detector using a modified procedure of Naidu (1998). Approximately 0.1 g 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 1000 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 1 N NaOH) ion exchange resins (1:1 of amberlite CG-50 and Dowex 1x2-100). Ion-exchange resins were removed by centrifugation

and the supernatant was filtered through a 0.45- μ m membrane filter into an HPLC auto-sampler vial. The extract (injection volume: 20 μ L) was subjected to HPLC analysis. An analytical column of SUPELCOGEL K (SEPELCO 5-9342), 300x7.8 mm preceded by a SUPELGUARD K, 50x7.8 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

Growth and Dry Matter Production

Leaf dry matter in cv. KB decreased linearly ($r=0.86$; $n=4$) with a decrease in K levels of the nutrient solution, with a 50% loss in leaf dry mass between 5.0 and 0.10 mM K. The cv. RQ, however, showed no decrease in leaf growth with the reduced K levels (Table 1). The cultivar treatment interaction was highly significant.

The storage root dry weight in cv KB decreased linearly ($r=0.96^*$; $n=4$) with a reduction in K levels with nearly a 85% decrease in storage root dry weight occurring at 0.1 mM K compared to the K-control (i.e., 5.0 mM) (Table 1). For cv. RQ, there was a significant stimulation of storage root growth with the 1.25 mM K treatment (compared to the K-control), while the storage root dry matter at 0.25 and 0.10 mM K were similar to the K-control. Total dry matter for cv. KB decreased with decreased K in the nutrient solution, with total dry matter at 0.10 mM K only $\approx 60\%$ that of K-control. In contrast, total dry weight of RQ was stimulated (about 50% higher) at 1.25 mM K, and approximately equal at 5.0, 0.25 and 0.10 mM K (Table 1).

Leaf Chlorophyll and Photosynthesis

Total chlorophyll levels in cv. KB declined with a decrease in K of the nutrient medium; with 0.1 mM levels about 40% that of K-control (Table 2). For cv. RQ, however, there was no significant change in chlorophyll levels at 1.25 mM K, but chlorophyll decreases were observed at the 0.25 and 0.10 mM K levels (Table 2). The leaf photosynthetic rates showed no significant difference to K between 31 or 39 DAP for either cultivar (Table 2).

Water Relations

Leaf relative water contents (RWC) were significantly higher for the low-K treatments in both of the cultivars (Table 3). Leaf sap osmotic potentials were also significantly higher (about 40%) at low-K treatments in both cultivars (Table 3).

TABLE 1. Dry matter production of red-beet grown under different levels of K (42 DAP).

Cultivar	K concentration (mM)			
	5.0	1.25	0.25	0.10
<u>Leaf Dry Weight (g/tray)*</u>				
Klein Bol	46.1	32.7	35.4	23.2
Ruby Queen	30.5	37.6	28.2	35.8
SE (treat)	1.84*			
SE (cultivar)	1.3ns			
SE (cultivarXtreat)	2.6**			
<u>Storage Root Dry Weight (g/tray)</u>				
Klein Bol	28.2	16.7	8.5	5.0
Ruby Queen	11.0	25.6	11.4	12.4
SE (treat)	3.36ns			
SE (cultivar)	1.68ns			
SE (cultivarXtreat)	4.11*			
<u>Total Dry Weight (g/tray)</u>				
Klein Bol	77.6	52.3	46.4	29.6
Ruby Queen	44.2	67.3	41.9	50.7
SE (treat)	5.01*			
SE (cultivar)	2.29ns			
SE (cultivarXtreat)	5.97**			
<u>Storage Root Dry Weight/Total Dry Weight Ratio</u>				
Klein Bol	0.36	0.32	0.17	0.16
Ruby Queen	0.24	0.37	0.27	0.24
SE (treat)	0.04ns			
SE (cultivar)	0.02ns			
SE (cultivarXtreat)	0.05ns			

*Four plants per tray.

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

Glycinebetaine Levels

Leaf glycinebetaine levels showed no significant change in response to K treatment in either cultivar (Table 3). However, cv KB had significantly higher leaf glycinebetaine levels than cv. RQ. Glycinebetaine levels in petioles and storage roots also showed no significant change with K treatments (data not presented).

TABLE 2. Leaf chlorophyll levels and photosynthetic rates of red-beet grown under different levels of K.

Cultivar	K concentration (mM)			
	5.0	1.25	0.25	0.10
<hr/>				
	<u>Total Chlorophyll ($\mu\text{g chlorophyll/mg leaf fr. wt.}$)</u>			
Klein Bol	1.43	0.98	0.90	0.83
Ruby Queen	1.43	1.65	1.00	1.05
SE (treat)	0.16*			
	<u>Photosynthetic Rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)</u>			
At 31 DAP				
Klein Bol	17.5	16.4	14.1	18.1
Ruby Queen	16.9	18.0	16.9	15.2
SE (treat)	0.98ns			
At 39 DAP				
Klein Bol	16.1	15.7	14.8	14.1
Ruby Queen	13.8	15.0*	15.1	15.8
SE (treat)	3.52ns			

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

Note: Photosynthetic rate values are mean of 4 plants (2 plants from each replication).

*Based on photosynthetic measurements of two plants only.

The differences in betaine levels between the two cultivars in petiole and storage root were not significant (data not presented). Mean glycinebetaine levels for both cultivars were 57.1, 54.5, and 63.4 $\mu\text{moles g}^{-1}$ dwt for lamina, petiole, and storage root, respectively.

Tissue Sodium Levels

The amount of K available in the nutrient medium had a significant effect on Na uptake and distribution (Table 4). Distribution of Na in the plant tissues was relatively uniform although Na levels were higher in lamina and petiole than storage roots at higher levels of K (5 and 1.25mM) (Table 4). Sodium levels in lamina, petiole, and storage roots increased substantially for both cultivars with a decrease in available K. Sodium levels in plant tissues were nearly four fold higher in the 0.10 mM K treatment compared to the 5.0 mM K-control. When expressed on a tissue water basis, Na levels in laminae increased from 142 mM in 5.0 mM K-

TABLE 3. Leaf relative water content, osmotic potential and glycinebetaine levels of red-beet grown under different levels of K (42 DAP).

Cultivar	K concentration (mM)			
	5.0	1.25	0.25	0.10
<u>Leaf Relative Water Content (%)</u>				
Klein Bol	68.9	80.0	84.2	74.0
Ruby Queen	73.6	86.4	82.9	82.7
SE (treat)	3.5*			
<u>Leaf Osmotic Potential at Full Turgor (MPa)</u>				
Klein Bol	0.63	0.80	0.98	0.78
Ruby Queen	0.68	0.93	0.82	0.99
SE (treat)	0.05**			
<u>Leaf Glycinebetaine ($\mu\text{mol g}^{-1}$ dwt)</u>				
Klein Bol	59.3	62.7	66.9	64.4
Ruby Queen	49.2	56.4	48.1	49.7
SE (treat)	15.8ns			
SE (cultivar)	3.41*			
SE (cultivarXtreat)	16.5ns			

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

control to 436 mM in the 0.10 mM K treatment for cv. KB; for cv. RQ, the Na levels increased from 137 mM to 383 mM in the leaf laminae (Table 5). Similar trends were observed for Na tissue levels in the petiole and storage root when expressed on a tissue water basis (Table 5).

Tissue Potassium Levels

Tissue K decreased as the K in the nutrient medium decreased. The lamina K concentrations decreased from 59.5 g kg⁻¹ dwt in 5.0 mM K-controls to 2.1 g kg⁻¹ dwt at 0.10 mM K for cv. KB; for cv. RQ, the lamina K levels decreased from 64.8 g kg⁻¹ dwt to 4.5 g kg⁻¹ dwt (Table 6). Similar reductions in tissue K were observed for petiole and storage root with decreases K concentrations in the nutrient medium (Table 6). When expressed on tissue water basis, the lamina K decreased from 136 mM in 5.0 mM K-controls to about 4.5 mM at 0.10 mM K for cv. KB; for cv. RQ, the lamina K levels decreased from 153 mM to 9.4 at 0.10 mM K

TABLE 4. Sodium concentrations in red-beet grown under different levels of K (42 DAP).

Cultivar	K concentration (mM)			
	5.0	1.25	0.25	0.10
<u>Sodium Concentration (g kg⁻¹ dwt)</u>				
<u>Lamina</u>				
Klein Bol	36.6	63.1	93.0	118.6
Ruby Queen	34.1	61.6	85.5	106.0
SE (treat)	6.7**			
SE (cultivar)	2.4ns			
SE (treatXcultivar)	7.6ns			
<u>Petiole</u>				
Klein Bol	24.3	68.2	112.1	119.6
Ruby Queen	33.3	72.4	120.5	117.8
SE (treat)	9.8**			
SE (cultivar)	4.4ns			
SE (treatXcultivar)	11.6ns			
<u>Storage Root</u>				
Klein Bol	13.9	31.8	59.3	81.0
Ruby Queen	11.2	18.7	53.5	68.1
SE (treat)	4.8**			
SE (cultivar)	3.4ns			
SE (treatXcultivar)	6.7ns			

*P≤0.05; **P≤0.001; ns=non-significant.

treatment (Table 5). Similar responses were noticed in K levels of petioles and also in storage root of both cultivars when expressed on tissue water basis (Table 5).

Tissue Calcium and Magnesium Levels

Calcium levels in laminae of both cultivars significantly decreased with a decrease in solution K levels (Table 7). However, there was no significant change in calcium levels in petioles with a decreased K in solution. Magnesium (Mg) levels in laminae and petioles were not significantly affected in either cultivar with a decrease in solution K (data not presented).

TABLE 5. Sodium and potassium concentrations in tissue water of red-beet grown under different levels of K (42 DAP).

Cultivar	K concentration (mM)			
	5.0	1.25	0.25	0.10
<u>Sodium Concentration in Tissue Water (mM)</u>				
Lamina				
Klein Bol	142	251	326	436
Ruby Queen	137	234	251	383
Petiole				
Klein Bol	67	191	244	281
Ruby Queen	92	205	231	263
Storage Root				
Klein Bol	54	133	189	347
Ruby Queen	55	90	198	231
<u>Potassium Concentration in Tissue Water (mM)</u>				
Lamina				
Klein Bol	136.0	63.3	9.1	4.5
Ruby Queen	153.1	77.5	12.1	9.4
Petiole				
Klein Bol	189.9	65.0	7.0	3.8
Ruby Queen	163.7	84.9	11.5	6.3
Storage Root				
Klein Bol	138.2	51.4	10.1	4.8
Ruby Queen	134.7	69.6	21.3	7.4

Note: Concentrations in tissue water basis were calculated based on fresh weight, dry weights and inorganic analysis done on the oven dried samples.

Tissue Nitrate and Chloride Levels

Nitrate levels in laminae and storage roots significantly increased with a decrease in K of the nutrient solution. Nitrate levels in low-K treatments (0.25 and 0.10 mM) were nearly double those in K-control in both cultivars. There was no significant change in petiole nitrate level due to K treatments (Table 8). Chloride levels in laminae, petioles, and in storage roots were not affected by K treatments or cultivars (Table 8).

TABLE 6. Potassium concentrations in red-beet grown under different levels of K (42 DAP).

Cultivar	K concentration (mM)			
	5.0	1.25	0.25	0.10
Potassium Concentration (g kg⁻¹ dwt)				
<u>Lamina</u>				
Klein Bol	59.5	27.1	4.4	2.1
Ruby Queen	64.8	34.7	7.0	4.5
SE (treat)	7.9*			
SE (cultivar)	1.28*			
SE (treatXcultivar)	8.13ns			
<u>Petiole</u>				
Klein Bol	117.2	39.5	5.5	2.7
Ruby Queen	101.2	51.1	10.2	4.8
SE (treat)	9.8**			
SE (cultivar)	2.2ns			
SE (treatXcultivar)	10.2*			
<u>Storage Root</u>				
Klein Bol	60.4	20.9	5.4	1.9
Ruby Queen	47.2	24.6	9.8	3.7
SE (treat)	3.5**			
SE (cultivar)	3.1ns			
SE (treatXcultivar)	5.6ns			

*P_≤0.05; **P_≤0.001; ns=non-significant.

Total Cations in Plant Water and Proportion of Sodium to the Total Cations

Total cation concentration (Na, K, Mg, and Ca) in plant tissue was reasonably constant across all K treatments, irrespective of the wide fluctuations in tissue Na and K levels at the various K treatments (Table 9). The proportion of Na to the total cations in leaf laminae increased from about 40% in the 5.0 mM K-control to about 90% in the 0.10 mM K treatment, showing that Na can be the dominant monovalent cation for red-beet when K is limiting. Similar responses were observed in petioles and storage roots (Table 9). The contribution of cations to the osmotic potential in laminae seems to be substantial for red-beet, as they accounted for 80 to 90% of the osmotic potential of the leaf sap (data not presented).

TABLE 7. Calcium concentration in red-beet grown under different levels of K (42 DAP).

Cultivar	K concentration (mM)			
	5.0	1.25	0.25	0.10
<u>Calcium Concentration (g kg⁻¹ dwt)</u>				
Lamina				
Klein Bol	9.8	8.3	6.5	6.1
Ruby Queen	9.8	7.3	7.8	7.6
SE (treat)	0.64*			
Petiole				
Klein Bol	2.8	2.1	2.2	2.0
Ruby Queen	2.4	1.7	1.8	2.5
SE (treat)	0.41ns			

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

DISCUSSION

Decreasing K availability from 5.0 mM to 0.10 mM quadrupled the Na tissue concentrations, suggesting that low K enhanced the Na uptake even though solution Na levels were nearly the same across all K treatments. Although Na uptake trends were similar for both cultivars, the two cultivars responded differently in their growth. For cv. KB, decreased K in the nutrient medium resulted in a linear decline in growth, whereas for cv. RQ, there was a stimulation of growth when K decreased from 5.0 to 1.25 mM, and the growth at 0.25 and 0.10 mM K treatments were similar to the K-control (i.e., 5.0 mM).

The tissue level of Na and K required to express the full growth potential of red-beet may vary for different genotypes. It is interesting to note that for cv. KB, an increase of lamina Na from 37 g kg⁻¹ dwt to 63 g kg⁻¹ dwt accompanied a loss of 40% dry matter production, whereas for the cv. RQ, a similar doubling of internal Na levels accompanied a 50% increase in dry matter production (Figure 1). Why dry matter production increased in cv. RQ with increased Na in this range is unclear, but seems to suggest that an elevated level of tissue Na is required to express the full growth potential of cv. RQ. Interestingly, even 37 g kg⁻¹ dwt of tissue Na (control treatment) was insufficient to support maximum growth. If Na is required for specific metabolic functions (which are still unknown at this stage), the tissue concentrations in the control plants would seem to be more than adequate. Similar results in yield were seen in cv. RQ under low-Na external

TABLE 8. Nitrate and chloride levels in red-beet grown under different levels of K (42 DAP).

Cultivar	K concentration (mM)			
	5.0	1.25	0.25	0.10
Nitrate Concentration (g kg⁻¹ dwt)				
Lamina				
Klein Bol	9.6	7.4	15.4	24.2
Ruby Queen	8.4	7.4	14.0	20.2
SE (treat)	1.99*			
Petiole				
Klein Bol	30.9	19.3	28.4	31.5
Ruby Queen	24.2	24.4	36.5	29.7
SE (treat)	2.5ns			
Storage Root				
Klein Bol	9.2	7.5	13.8	23.5
Ruby Queen	9.9	7.7	18.9	23.0
SE (treat)	3.0*			
Chloride Concentration (g kg⁻¹ dwt)				
Lamina				
Klein Bol	14.4	18.6	20.3	31.1
Ruby Queen	16.3	25.5	20.4	34.4
SE (treat)	4.6ns			
Petiole				
Klein Bol	63.2	60.8	87.3	43.3
Ruby Queen	50.0	60.6	69.5	71.6
SE (treat)	6.7ns			
Storage Root				
Klein Bol	23.7	22.6	26.5	28.5
Ruby Queen	16.1	15.2	17.9	27.1
SE (treat)	3.3ns			

*P<0.05; **P<0.001; ns=non-significant.

conditions when tissue Na levels reached to about 64 g kg⁻¹ dwt (Subbarao et al., 1999b). It is possible that Na could be more effective than K in osmotic adjustment, but higher levels of Na (e.g., 80 g kg⁻¹ dwt) did not stimulate growth (Figure 1). The different results indicate that the responses vary between genotypes; genotypic

TABLE 9. Total cation concentration in red-beet grown at different levels of K (42 DAP).

Cultivar	K concentration (mM)			
	5.0	1.25	0.25	0.10
<u>Total cations (Na, K, Mg, and Ca) in Tissue Water (mM)</u>				
Lamina				
Klein Bol	352	378	379	489
Ruby Queen	360	366	304	443
Petiole				
Klein Bol	270	264	257	292
Ruby Queen	266	297	247	278
Storage Root				
Klein Bol	205	193	211	399
Ruby Queen	205	171	230	252
<u>Proportion of Na to Total Cations in Tissue Water (mM)</u>				
Lamina				
Klein Bol	40.3	66.5	85.9	89.1
Ruby Queen	38.0	63.9	82.6	86.3
Petiole				
Klein Bol	24.8	72.3	94.7	96.1
Ruby Queen	34.4	68.9	93.5	94.9
Storage Root				
Klein Bol	26.5	69.1	89.3	86.9
Ruby Queen	26.6	52.8	86.0	91.4

Note: Tissue water was calculated as the difference between fresh and dry weights; the inorganic chemical analysis was done only on the oven dried ground plant material.

differences for Na substitution for K have also been reported in sugarbeet (Marschner et al., 1981a, 1981b).

If uptake of relatively high levels of Na are required to achieve maximum growth in cv. RQ, then why didn't this occur at 5.0 mM K, as there was substantial Na in the nutrient medium. One possibility could have been competition from K on Na uptake. According to dual mechanism concept, mechanism-1, which operates below 5 mM concentration has more affinity for K in plants (Epstein et al., 1963; Maathuis and Sanders, 1993); mechanism-2, which operates up to 50 mM is less

FIGURE 1. Relationship between lamina sodium levels (g kg⁻¹ dwt) and total dry matter production in two red-beet cultivars.

discriminatory for Na. It is believed that K and Na compete against each other for ion transport channels during uptake (Epstein et al., 1963; Maathuis et al., 1997; Wegner and DeBoer, 1997; Dietrich et al., 1998). In certain varieties of sugar beet, maximum ATPase activity was observed when a certain combination of Na and K was present in the reaction medium and not with Na or K alone (Hanson and Kylin, 1969). Also, this optimum combination of Na and K for the maximum activity was hypothesized to vary for different varieties (Kylin and Hanson, 1971; Hanson, 1975).

At 0.25 and 0.10 mM K treatments, leaf Na levels reached to nearly 300 to 450 mM when expressed on a tissue water basis, accounting for nearly 90% of the total leaf cations and thus contributing a major portion of the leaf osmotic potential. Total leaf cations in red-beet showed no trend as a function of decreasing K in the nutrient medium. This suggests that the total cation levels were relatively stable despite changes in the individual cation (Na and K) levels. This is consistent with the concept that red-beets have a strong tendency to maintain a constant total content of cations in their tissues (Troug et al., 1953). Sodium contribution to the total cations increased from about 30% under the K-control to nearly 90% at 0.10 mM K. This suggests that Na is responsible for a substantial portion of the observed osmotic potential of the leaf sap (almost 70 to 80%) and Na was able to functionally replace most of the osmotic functions of the K in red-beet. Though Na levels in the plant were quadrupled at 0.10 mM K treatment, there was no significant change in leaf chloride levels. This suggests that chloride may not be the major anion

providing charge balance for Na in red-beet, but this may depend on the availability of alternate anions such as nitrate.

In red-beet cv. KB, the effect of low-K on storage root dry weight was substantially higher than on leaf dry weight. At 0.10 mM K treatment, storage root dry weight was reduced to 15% of the K-control, whereas leaf dry weights were reduced only to 50%. For cv. RQ, storage root dry weight increased 230% when K was decreased from 5.0 to 1.25 mM, while storage root dry weights at 0.25 mM and 0.10 mM were similar to 5.0 mM. Leaf dry weights in cv. RQ showed no significant change with the reduction of K from 5.0 to 0.10 mM. In sugarbeet, Na application can increase leaf area at the expense of storage root growth, and high Na levels were hypothesized to interfere with the starch synthesis leading to more assimilates diverted to leaves at the expense of storage roots (Draycott et al., 1970).

Glycinebetaine (GB) levels of both red-beet cultivars did not change significantly with K treatments in this study. In previous studies using non-saline conditions, leaf GB levels significantly increased in cv. RQ with increased tissue Na levels up to 90 g kg⁻¹ dwt; whereas GB levels in cv. KB increased only with tissue leaf Na levels of up to 50 g kg⁻¹ dwt (Subbarao et al., 1999b). In the present study where plants were subjected to moderate saline conditions, leaf GB levels were higher at 5.0 mM K than were reported for non-saline conditions (Subbarao et al., 1999b), and there was no significant change in whole plant GB levels despite many-fold differences in tissue Na. Glycinebetaine is reported to accumulate in plants both under water deficits and salinity stress, where specific ionic stress such as Na toxicity could be a factor (Greenway and Munns, 1980; Wyn Jones and Storey, 1981; Subbarao and Johansen, 1995; Subbarao et al., 2001b). One hypothesis could be that the moderate saline conditions in the present study stimulated GB accumulation, which could be independent from the internal Na levels acting as a trigger. Also, the site of synthesis for GB is reported to be in chloroplasts (Hanson et al., 1985), but the distribution of GB levels were uniform in various plant parts (i.e., lamina, petiole, storage root), suggesting that GB was translocated freely from the site of synthesis to the entire plant.

Leaf relative water content increased significantly at low-K treatments when compared to the controls in both cultivars. This supports previous reports that high Na levels in tissue can increase leaf water content (Subbarao et al., 1999b). In addition, leaf turgid osmotic potential increased at low-K treatments compared to the controls. This could be due to a slight increase in total cation levels in the lamina, particularly at 0.10 mM K treatment where Na levels in leaf water reached to 400 mM. These results support the notion that Na effectively replaced K as an osmoticum in red-beet without affecting water relations.

Total chlorophyll levels were significantly decreased in both the cultivars of red-beet when K levels were decreased from 5.0 to 0.10 mM, which was not the case under non-saline conditions (Subbarao et al., 1999b). This may be due to

substantially higher Na uptake at 0.25 and 0.10 mM K treatments in the present study. However, there was no significant change in the leaf photosynthetic rates for either cultivars across the K treatments indicating that this was not a limiting factor. In sugarbeet, K deficiency ($<10 \text{ g kg}^{-1} \text{ dwt}$) was shown to reduce Hill reaction activity (Spencer and Possingham, 1960) and diminish the rate ATP and NADPH reduction in beet chloroplasts (Tombesi et al., 1969). Also, K-deficiency can cause chlorophyll loss (Rabinowitch, 1945) and Na was not able to replace K functions in chlorophyll synthesis and photosynthesis in sugarbeet (Terry and Ulrich, 1973a, 1973b). But based on these results with red-beets, it appears that photosynthesis is tolerant to low tissue K as there was no significant change in leaf photosynthetic rates across leaf K concentrations ranging from $60 \text{ g kg}^{-1} \text{ dwt}$ to $2 \text{ g kg}^{-1} \text{ dwt}$.

Nitrate levels were nearly doubled in leaf laminae and storage roots at low-K (0.25 and 0.10 mM), suggesting that high internal Na levels may have interfered with the nitrate reduction in leaves. Adequate K is required for the stimulation and functioning of nitrate reductase activity in leaves (Suelter, 1970; Kirkby et al., 1981). It remains to be seen whether the high Na levels in laminae may have interfered with nitrate reductase, resulting in higher nitrate levels in the low-K treatments. Also, K has been reported to act as a counter-ion for the transport of nitrates from root to the shoot (Dijkshoorn et al., 1968; Ben-Zioni et al., 1970). Our results indicate that this may be a function that is non-specific in its ion requirements (Marschner, 1971). Since there was no decrease in leaf nitrate at K, this suggests that Na may have replaced K in this function. This is supported by studies with sugarbeet, where Na acted as a long-distance carrier for translocating NO_3 from root to shoot (Marschner, 1971).

Though lamina Ca levels were significantly decreased with a decreased K, this decrease was not substantial and likely had no effect on the metabolism. The petiole Ca levels showed no significant change due to a decrease in K of the nutrient medium, and this was also true for the Mg levels in the plant, indicating that the uptake of the other major cations were not influenced by this decrease in K of the nutrient medium.

CONCLUSIONS

Reducing K to marginal levels in the nutrient medium had a powerful effect on Na uptake in red-beet under moderate saline conditions. Cultivar differences in growth occurred despite similar tissue levels of Na and K, indicating that cultivars of red-beet may differ in their ability to utilize Na in place of K for many metabolic functions. Despite the observed stimulating effect of increased tissue Na on the growth of cv. RQ, the question of whether Na has a specific function in the metabolism of red-beet is still speculation at this stage. Previous results with red-beet indicated that replacing K in nutrient solution with Na caused an increase in

Na uptake in red-beet under non-saline conditions. The present results indicate that reducing K levels even under moderate saline conditions has a similar effect. These results have important implications for Na management in closed life support systems where Na recycling may be difficult because of the limited amount of tissue Na found in plants grown at normal levels of K found in nutrient solutions. Our results indicate that Na can satisfy a major portion of the non-specific cation requirements in red-beet and contributed up to nearly 90% of the total cations taken up by this crop. It is also interesting to note that despite its ability to replace a major portion of the plants K requirements, Na still does not meet Arnon and Stout's criteria for being an essential element.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical help of Neil Yorio and Lisa Ruffe while conducting this experiment. Also, we would like to thank Ms. Teresa Englert, for assistance in the elemental analysis of the plant tissues. We would like to thank Drs. Wade Berry, University of California, Los Angeles, M.C. Shannon, USDA Salinity Laboratory, Riverside, CA for the critical review of this manuscript. This research was supported by National Research Council and the Life Science Support Contract at Kennedy Space Center (NASA-12180).

REFERENCES

- Ben-Zioni, A., Y. Vaadia, and S.H. Lips. 1970. Correlations between nitrate reduction, protein synthesis and malate accumulation. *Physiol. Plant.* 23:1039-1047.
- Blaedel, W.J. and V.W. Meloche. 1960. *Elementary Quantitative Analysis*. Harper and Row, New York, NY.
- Dietrich, P.I., I. Dreyer, P. Wiesner, and R. Hedrich. 1998. Cation sensitivity and kinetics of guard-cell potassium channels differ among species. *Planta* 205:277-287.
- Dijkshoorn, W., D.D. Lathwell, and C.T. De Wit. 1968. Temporal changes in carboxylate content of ryegrass with stepwise changes in nutrition. *Plant Soil* 29:369-390.
- Draycott, A.P., A.P. Marsh, and P.B.H. Tinker. 1970. Sodium and potassium relationships in sugarbeet. *J. Agric. Sci.* 74:567-573.
- Epstein, E., D.W. Rains, and O.E. Elzam. 1963. Resolution of dual mechanisms of potassium absorption by barley roots. *Proc. Nat. Acad. Sci. USA* 49:684-692.
- Flowers, T.J. and A. Lauchli. 1983. Sodium versus potassium: Substitution and compartmentation. pp. 651-681. In: A. Pirson, and M.H. Zimmermann, (eds.), *Encyclopedia of Plant Physiology*. Vol. 15B, New Series. Springer Verlag, Berlin, Germany.

- Graves, C.J. 1981. The nutrient film technique. Hort. Rev. 5:1-43.
- Greenway, H. and R. Munns. 1980. Mechanisms of salt tolerance in nonhalophytes. Annu. Rev. Plant Physiol. Plant Mol. Biol. 31:149-190.
- Hanson, G. 1975. Patterns of ionic influences on sugar beet ATPases. Ph.D. Thesis University of Stockholm, Stockholm, Sweden.
- Hanson, G. and A. Kylin. 1969. ATP-ase activities in homogenates from sugar-beet roots, relation to Mg^{++} and $(Na^{+} + K^{+})$ stimulation. Z. Pflanzenphysiol. 60:270-275.
- Hanson, A.D., A.M. May, R. Grumet, J. Bode, G.C. Jamieson, and D. Rhodes. 1985. Betaine synthesis in chenopods: Localization in chloroplasts. Proc. Nat. Acad. Sci. USA 82:3678-3682.
- Harrison, T.R. 1991. Harrison's Principles of Internal Medicine. 12th ed. McGraw-Hill, Inc., New York, NY.
- Kirkby, E.A., M.J. Armstrong, and J.E. Leggett. 1981. Potassium recirculation in tomato plants in relation to potassium supply. J. Plant Nutr. 3:955-966.
- Kylin, A. and G. Hansson. 1971. Transport of sodium and potassium, and properties of (sodium/potassium) - activated adenosine triphosphatase: Possible connection with salt tolerance in plants. Proc. 8th Coll. Int. Potash Inst., Berlin, Germany.
- Maathuis, F.J.M. and D. Sanders. 1993. Energization of potassium uptake in *Arabidopsis thaliana*. Planta 191:302-307.
- Maathuis, F.J.M., A.M. Ichida, D. Sanders, and J.I. Schroeder. 1997. Roles of higher plant K channels. Plant Physiol. 114:1141-1149.
- MacElroy, R.W. and J. Brendt. 1985. Controlled ecological life support system. Life support functions in space travel: Current concepts and future directions of CELSS. NASA Conf. Pub. 2378 XXV. COSPAR Meeting, Graz, Austria.
- Marschner, H. 1971. Why can sodium replace potassium in plants? Proc. 8th Colloq. Int. Potash Inst. Berlin, Germany.
- Marschner, H. 1986. Mineral Nutrition of Higher Plants. Academic Press, San Diego, CA.
- Marschner, H., A. Kylin, and P.J.C. Kuiper. 1981a. Differences in salt tolerance of three sugar beet genotypes. Physiol. Plant. 51:234-238.
- Marschner, H., P.J.C. Kuiper, and A. Kylin. 1981b. Genotypic differences in the response of sugar beet plants to replacement of potassium by sodium. Physiol. Plant. 51:239-244.

- Naidu, B.P. 1998. Separation of sugars, polyols, proline analogues, and betaines in stressed plant extracts by high performance liquid chromatography and quantification by ultra violet detection. *Aust. J. Plant Physiol.* 25:793-800.
- Perkin-Elmer. 1981. Analytical methods development for inductively coupled plasma spectrometry. Perkin-Elmer Corporation, Norwalk, CT.
- Prince, R.P. and W.M. Knott. 1989. CELSS Breadboard project at the Kennedy Space Center. pp. 155-163. In: D.W. Ming and D.L. Henninger (eds.), *Lunar base agriculture*. American Society of Agronomy, Madison, WI.
- Rabinowitch, E. 1945. *Photosynthesis and Related Processes*. Vol. I. Chemistry of Photosynthesis, Chemosynthesis, and Related Processes. pp. 336-337. Interscience Publishers, New York, NY.
- Spencer, D. and J.V. Possingham. 1960. The effect of nutrient deficiencies on the Hill reaction of isolated chloroplasts from tomato. *Aust. J. Biol. Sci.* 13:441-455.
- Subbarao, G.V. and C. Johansen. 1995. Physiological mechanisms relevant to genetic improvement of salinity tolerance in crop plants. pp. 807-831. In: M. Pessarakli (ed), *Handbook of Plant and Crop Physiology*. Marcel Dekker, Inc., New York, NY.
- Subbarao, G.V., C. Mackowiak and R.M. Wheeler. 1999a. Recycling of sodium in advanced life support—Strategies based on crop production systems. *Life Support and Biospheric Sci.* 6:153-160.
- Subbarao, G.V., R.M. Wheeler, and G.W. Stutte. 2000. Feasibility of substituting sodium for potassium in crop plants for advanced life support systems. *Life Support and Biospheric Sci.* (in press).
- Subbarao, G.V., R.M. Wheeler, G.W. Stutte, and L.H. Levine. 1999b. How far can sodium substitute for potassium in red beet? *J. Plant Nutr.* 22:1745-1761.
- Subbarao, G.V., R.M. Wheeler, W. Berry, and G.W. Stutte. 2001a. Sodium—A Functional Nutrient. In: M. Pessarakli (ed.), *Handbook of Plant and Crop Physiology*. 2nd ed. Marcel Dekker, Inc., New York, NY.
- Subbarao, G.V., L.H. Levine, R.M. Wheeler, and G.W. Stutte. 2001b. Glycine Betaine Accumulation—Its Role in Stress Resistance. In: M. Pessarakli (ed.), *Handbook of Plant and Crop Physiology*. 2nd ed. Marcel Dekker, Inc., New York, NY.
- Suelter, C.H. 1970. Enzymes activated by monovalent cations. *Science* 168:789-795.
- Technicon Industrial Systems. 1987. Nitrate/nitrite in water and waste water. TrAAcs 800 method, Industrial method no. 824-87T. Bran+Luebbe/Technicon Industrial Systems. Elmsford, New York, NY.

- Terry, N. and A. Ulrich. 1973a. Effects of potassium deficiency on the photosynthesis and respiration of leaves of sugar beet. *Plant Physiol.* 51:783-786.
- Terry, N. and A. Ulrich. 1973b. Effects of potassium deficiency on the photosynthesis and respiration of leaves of sugar beet under conditions of low sodium supply. *Plant Physiol.* 51:1099-1101.
- Tombesi, L., M.T. Cale, and B. Tiborne. 1969. Effects of nitrogen, phosphorus and potassium fertilizers on the assimilation capacity of *Beta vulgaris* chloroplasts. *Plant Soil* 31:65-76.
- Troug, E., K.C. Berger, and O.J. Attoe. 1953. Response of nine economic plants to fertilization with sodium. *Soil Sci. Soc. Am. J.* 76:41-50.
- Wegner, L.H. and A. DeBoer. 1997. Two inward K⁺ channels in the xylem parenchyma cells of barley roots are regulated by G-protein modulators through a membrane-delimited pathway. *Planta* 203:506-516.
- Wheeler, R.M., S.H. Schwartzkopf, T.W. Tibbitts, and R.W. Langhans. 1985. Elimination of toxicity from polyurethane foam plugs used for plant culture. *Hort. Sci.* 20:448-449.
- Wheeler, R.M., C.L. Mackowiak, J.C. Sager, N.C. Yorio, and W.M. Knott. 1994. Growth and gas exchange by lettuce stands in a closed, controlled environment. *Am. Soc. Hort. Sci.* 119:610-615.
- Wilson, J.R., M.J. Fischer, E.D. Schulze, G.R. Dolby, and M.M. Ludlow. 1979. Comparison between pressure-volume and dew point hygrometry techniques for determining the water relations characteristics of grass and legume leaves. *Oecologia* 41:77-88.
- Wintermans, J.F.G.M. and A. De Mots. 1965. Spectrophotometric characteristics of chlorophylls a and b and their pheophytins and ethanol. *Biochem. Biophys. Acta.* 109:448-453.
- Wyn Jones, R.G. and R. Storey. 1981. Betaines. pp. 171-204. In: L.G. Paleg, and D. Aspinall (eds.), *Physiology and Biochemistry of Drought Resistance*. Academic Press, Sydney, Australia.