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Sodium–potassium synergism in *Theobroma cacao*: stimulation of photosynthesis, water-use efficiency and mineral nutrition

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In ecological setting, sodium (Na^+) can be beneficial or toxic, depending on plant species and the Na^+ level in the soil. While its effects are more frequently studied at high saline levels, Na^+ has also been shown to be of potential benefit to some species at lower levels of supply, especially in C4 species. Here, clonal plants of the major tropical C3 crop *Theobroma cacao* (cacao) were grown in soil where potassium (K^+) was partially replaced (at six levels, up to 50% replacement) by Na^+, at two concentrations (2.5 and 4.0 $mmol_c\ dm^{-3}$). At both concentrations, net photosynthesis per unit leaf area (A) increased more than twofold with increasing substitution of K^+ by Na^+. Concomitantly, instantaneous (A/E) and intrinsic (A/g_s) water-use efficiency (WUE) more than doubled. Stomatal conductance (g_s) and transpiration rate (E) exhibited a decline at 2.5 mmol dm^{-3}, but remained unchanged at 4 mmol dm^{-3}. Leaf nitrogen content was not impacted by Na^+ supplementation, whereas sulfur (S), calcium (Ca^{2+}), magnesium (Mg^{2+}) and zinc (Zn^{2+}) contents were maximized at 2.5 mmol dm^{-3} and intermediate (30–40%) replacement levels. Leaf K^+ did not decline significantly. In contrast, leaf Na^+ content increased steadily. The resultant elevated Na^+/K^+ ratios in tissue correlated with increased, not decreased, plant performance. The results show that Na^+ can partially replace K^+ in the nutrition of clonal cacao, with significant beneficial effects on photosynthesis, WUE and mineral nutrition in this major perennial C3 crop.

Introduction

Plants absorb more potassium (K^+) than any other mineral element with the exception of nitrogen (Tisdale and Nelson 1975, Mäser et al. 2002, Britto and Kronzucker 2008, Szczerba et al. 2009). It is the only monovalent cation that is essential for all higher plants, and is involved in three major functions: enzyme activation, charge balance and osmoregulation (Mengel 2007, Szczerba et al. 2009). On the other hand, sodium (Na^+) is a mineral element that may be beneficial (Brownell 1979) or toxic for plant growth (Munns and Tester 2008, Kronzucker and Britto 2011), depending on concentration and species. In some C4 plant species, such as members of the *Panicum*, *Atriplex* and *Kochia* genera, Na^+ has been considered an essential micronutrient by some (Brownell 1965, Brownell and Crossland 1972) and a 'functional nutrient' by others (Subbarao et al. 2003). In these species, it can stimulate photosynthesis and be involved in the Na^+-coupling

Abbreviations – DAT, days after transplanting; WUE, water-use efficiency.

of trans-membrane transport events (Ohta et al. 1988, Matoh and Murata 1990, Ohnishi et al. 1990, Murata and Sekiya 1992), although this does not appear to apply to the major crop species corn, sorghum and sugarcane (Ohnishi et al. 1990, Murata and Sekiya 1992). In a variety of other, non-C4 species, Na^+, albeit not required for growth, can still have beneficial effects, especially so in the Chenopodiaceae (Lehr 1953, El-Sheikh and Ulrich 1967, 1970, Draycott and Durrant 1976, Marschner et al. 1981, Subbarao et al. 2003), but also in other, commercially important, species, such as flax, ryegrass and the cereals such as oat, wheat and barley (Lehr 1953, Montasir et al. 1966, Hylton et al. 1967, Leigh et al. 1986). Variably positive effects have been recorded, typically with Na^+ additions in the low-millimolar range, on vegetative growth, yield, sugar production and the accumulation of some nutrient elements. Growth advantages of adding Na^+ can furthermore be particularly pronounced in halophytes, although this classically occurs at much higher levels of Na^+ supply (Flowers et al. 2010, Kronzucker and Britto 2011, Shabala and Mackay 2011).

Several studies have shown that Na^+ can replace some functions of K^+ in the plant (for summaries, see: Marschner 1995, Subbarao et al. 2003). Indeed, such replacement may explain the reduced manifestation of symptoms of K^+ deficiency in plant cultivations in coastal regions (Laclau 2003). Na^+ can replace K^+ nearly completely in its osmotic function in the vacuole (Shabala and Mackay 2011). Thus, under K^+ deficiency, the addition of Na^+ or its presence in solution may have a positive effect (Ali et al. 2006). Further, under conditions of elevated external Na^+, some species tolerant to salinity are especially prone to replacing K^+ by Na^+ (Kronzucker et al. 2008, Kronzucker and Britto 2011). In assessing the effects of high Na^+ concentration on the growth of *Oryza sativa* at different levels of K^+, Yoshida and Castaneda (1969) observed that application of Na^+ altered the leaf habit, from 'flaccid' to 'erect', in plants deficient in K^+. Such observations support the hypothesis that Na^+ is replacing K^+ in the vacuole, allowing cells to maintain turgor.

For other, non-osmotic, functions of K^+, replacement by Na^+ may not be as straightforward. Protein synthesis (Hall and Flowers 1973, Wyn Jones et al. 1979) and oxidative phosphorylation (Flowers et al. 1974) depend more intimately on K^+ and are both equally inhibited by high Na^+ in vitro, regardless of whether enzymes or organelles are isolated from glycophytes or halophytes (Greenway and Osmond 1972). One enzyme that has received particular attention in this regard is starch synthetase. Starch synthetase, in vitro, has a requirement of about 50 mM K^+ for optimal activity (Nitsos and Evans 1969). Other monovalent cations, such as Rb^+, Cs^+ and NH_4^+, are about 80% as effective as K^+, while Na^+ is only about 20% as effective at maintaining starch synthetase activity (Nitsos and Evans 1969). In *Beta vulgaris*, K^+ deficiency has been shown to cause the accumulation of soluble carbohydrates and reducing sugars due to the inhibition of starch synthesis, and sodium was unable to replace K^+ in this situation (Evans and Soger 1966).

In perennial species, the effects of replacement of K^+ by Na^+ are much less well known. More fundamentally, unlike in C4 plants (Ohnishi et al. 1990, Murata and Sekiya 1992), direct effects of K^+ substitution by Na^+ on photosynthesis have been rarely examined in C3 plants. Furthermore, the effects of Na^+ supplementation on water-use efficiency (WUE) are not well studied in any species, despite the well-recognized importance of K^+ to plant water relations. The model system chosen in this study, to address these issues, is the major C3 crop *Theobroma cacao* (cacao). Cacao, the source of chocolate, is one of the most important tropical crops worldwide (Belsky and Siebert 2003, Micheli et al. 2010), and interest in understanding both its genomic variation and physiological requirements is intense (Isaac et al. 2007, Bae et al. 2009, Micheli et al. 2010, Trognitz et al. 2011). In cacao plantations, sodium is commonly introduced by rainfall (Souza et al. 2006), providing a mixture of sodium and potassium in soil, and rendering the study of the elemental interactions and possible synergisms especially important. Orchard (1978) showed that young cacao plants reacted to high K^+ supply by increasing leaf area without any effect on whole plant biomass, and that there was an inverse relationship between transpiration rate (E) and K^+ supply. In adult cacao, K^+ also promoted tolerance to adverse effects of water stress (Bosshart and Uexkhull 1987). Given the documented effects of Na^+–K^+ co-provision in a substantial number of species, and given the natural occurrence of Na^+ in many cacao plantations, this study was designed to assess photosynthetic performance, WUE and mineral status in clonal cacao plants, submitted to partial replacement of K^+ by Na^+ in soil. We documented the effect of increasing substitutions of K^+ by Na^+ upon net photosynthesis, intrinsic and instantaneous WUE, transpiration, stomatal conductance and leaf mineral status, and examined the role of the tissue Na^+/K^+ ratio established in the plants under the various provision regimes. The aim of the study was to identify potential beneficial effects of K^+–Na^+ co-provision in this major perennial C3 crop, and to provide insight into the physiological target points of sodium–potassium interaction in general.

Materials and methods

Plant material and cultivation conditions

Experiments were conducted in a greenhouse, at the Campus of Universidade Estadual de Santa Cruz (UESC), Ilhéus, BA (14°48′53 ′′S/39°02′01′′W), with clonal plants of *Theobroma cacao,* PH-16 clone, grown in soils supplemented with two K^+ concentrations (2.5 and 4.0 $mmol_c\ dm^{-3}$), and six replacement ratios of K^+ by Na^+ (0, 10, 20, 30, 40, 50% replacement, mol/mol). KCl and NaCl salts were used as the sources of Na^+ and K^+. Pots (10 dm^3) were filled with substrate composed of the B horizon of an Alic Clayey Oxisol (Table 1). Young plants were produced by rooting cuttings taken from plagiotropic branches of donor plants of 5 to 10 years, by the Instituto Biofábrica de Cacau. Young plants, 6 months old, were selected for uniformity judged by height, stem diameter and absence of leaf flushes. The substrate was supplemented with N, P, S, Cu, Mn, Mo, Zn and B, and with a mixture of $CaCO_3$ and $MgCO_3$ needed to achieve a Ca^{2+}:Mg^{2+} ratio of 4:1, and raising the value of base saturation to 80%, that results in pH increasing to the optimum soil pH for this plant species (Table 2). Plants were arranged on aluminum benches 1.2 m in height. Every 12 days, plants were randomly redistributed to minimize the effects of heterogeneity inherent in greenhouse environments. Urea fertilizer (45% N; 25 mg dm^{-3}) was applied as topdressing at 30 and 60 days after transplanting (DAT), every 30 days, to the 60th day. Subsequently, the same dosage was maintained at regular intervals of 15 days until the end of the experiment (180 DAT). During this period, the young plants were watered daily with deionized water.

Table 1. Physical and chemical characteristics of the substrate for growth of *Theobroma cacao*. P, Na, K, Fe, Zn, Mn, Cu (extracted by Mehlich 1), Ca, Mg, Al (extracted by KCl, 1 *M*) H + Al (extracted by Ca-acetate 0.5 *M*, pH 7.0), B (extracted by hot water), S (extracted by monocalcium phosphate in acetic acid). SB, sum of bases; t, effective cation exchange capacity; T, cation exchange capacity (pH 7.0); V, base saturation; m, Al saturation; NaSI Na, saturation index; OM, organic matter = Org C. × 1.724; P-rem, remaining phosphorus;. CS, coarse sand; FS, fine sand.

pH	(H_2O)	4.36
P	(mg dm^{-3})	0.8
K	(mg dm^{-3})	12
Na	(mg dm^{-3})	0.0
Ca^{2+}	($mmol_c\ dm^{-3}$)	3.4
Mg^{2+}	($mmol_c\ dm^{-3}$)	2.2
Al^{3+}	($mmol_c\ dm^{-3}$)	8.6
H + Al	($mmol_c\ dm^{-3}$)	60
N	(dag kg^{-1})	0.04
SB	($mmol_c\ dm^{-3}$)	5.9
(t)	($mmol_c\ dm^{-3}$)	14.5
(T)	($mmol_c\ dm^{-3}$)	65.9
Zn	(mg dm^{-3})	0.32
Fe	(mg dm^{-3})	55.8
Mn	(mg dm^{-3})	8.5
Cu	(mg dm^{-3})	0.64
B	(mg dm^{-3})	0.06
S	(mg dm^{-3})	24
V	(%)	9.0
m	(%)	59.3
NaSI	(%)	0.0
OM	(dag kg^{-1})	2.56
P-rem	(mg L^{-1})	14.5
CS	(dag kg^{-1})	20
FS [j]	(dag kg^{-1})	13
Silt	(dag kg^{-1})	8
Clay	(dag kg^{-1})	59

Table 2. Fertilization of the substrate just before transplanting clonal plants of *Theobroma cacao*. [a] Analytical standard.

Nutrient	Dose (mg dm^{-3})	Fertilizer
P	400	MAP purified
N	169	MAP purified
S	4	Copper sulfate and Zinc sulfate AS[a]
B	0.8	Boric acid AS
Cu	2.0	Copper sulfate AS
Mn	10.0	Manganese cloride AS
Mo	0.3	Ammonium molybdate AS
Zn	6.0	Zinc sulfate AS

Leaf gas exchange

At the end of the growth period (180 DAT), leaf gas exchange was evaluated using a portable photosynthesis meter LICOR; model Li-6400 (Nebraska, USA). The measurements were performed under saturation irradiance (800 µmol photons $m^{-2}\ s^{-1}$), using the second or third fully matured leaf of the most vigorous branch containing only fully matured leaves. The minimum time for acclimation of leaves was 60 s, and the time limit for saving each reading was 120 s. The maximum coefficient of variation allowed for recording of each reading was 0.3%. Mean values from three measurements in each replicate for all treatments were recorded. The rates of net photosynthesis (A) and transpiration (E) per unit leaf area and stomatal conductance to water vapor (g_s) were estimated from the values of the variation of CO_2 and humidity inside the chamber, determined by the infrared gas analyzer. The ratio between internal and atmospheric concentrations of CO_2 (C_i/C_a), the instantaneous WUE, where WUE = A/E and the intrinsic WUE (A/g_s) were also calculated.

Macro and micronutrient contents

All leaves of the plant of each replicate and treatment were harvested, dried in a forced air oven at

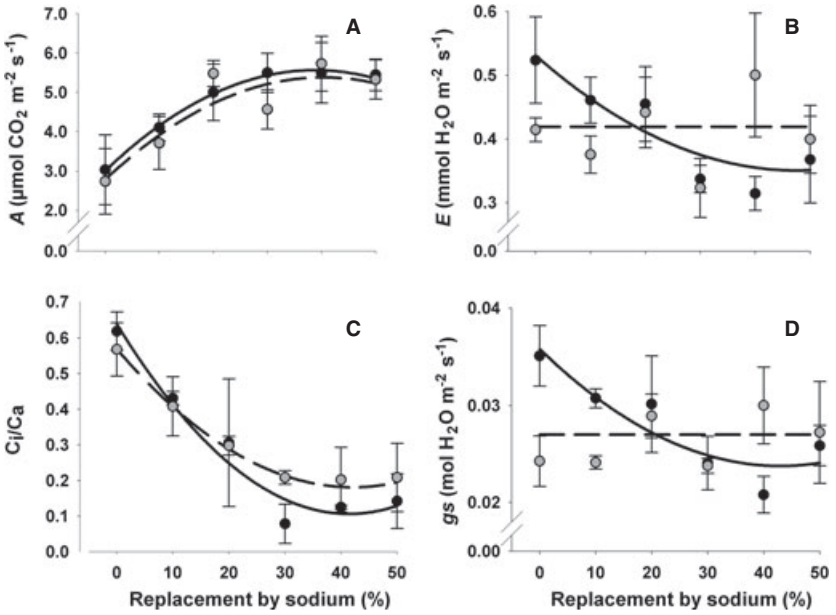

Fig. 1. Net photosynthesis per unit area (A), leaf transpiration rate (B), ratio of internal to atmospheric CO_2 concentration (C), and stomatal conductance to water vapor (D), in leaves of clonal *Theobroma cacao* plants (PH-16 clone), cultivated for 180 days under two soil K^+ treatments (2.5 $mmol_c$ dm^{-3}, continuous line and black circles as means; and 4.0 $mmol_c$ dm^{-3}, dashed line and gray circles as means) replaced gradually by Na^+.

65°C to constant weight. Dried leaves were ground and subjected to nitric-perchloric digestion (3:1). After digestion, Ca, Mg, Fe, Zn, Cu and Mn were determined by atomic absorption spectrophotometry, P by colorimetry, K by flame emission photometry and S by turbidimetry of sulfate (EMBRAPA 1997). Nitrogen was determined by the Kjeldahl method after digestion by sulfosalicylic acid (Jones et al. 1991).

Statistical analysis

The experimental design was completely randomized in a 2 × 6 factorial arrangement, totaling 12 treatments of two soil K^+ levels and six ratios of K^+ and Na^+, with five replicate clonal plants per treatment combination. The results were subjected to factorial analysis of variance (ANOVA). Polynomial regressions were performed, and accepted models with the highest adjusted R^2 and all significant coefficients up to 10% probability by *F* test.

Results

Leaf gas exchange

A highly significant increase in photosynthetic rates (A) was observed at both soil K^+ levels examined (2.5 and 4.0 $mmol_c$ dm^{-3}), and the response followed the shape of an optimum curve, with optimal rates being achieved at 38.8 and 40.3% of replacement of K^+ by Na^+, respectively (Fig. 1). At 40% of replacement of K^+ by Na^+, A was double that recorded in the control treatments (0% replacement). At higher soil K^+, stomatal conductance to water vapor (g_s) did not vary with increasing replacement of K^+ by Na^+ (Fig. 1). However, at the lower soil K^+, g_s in plants without replacement by Na^+ (0%) was 25% higher than that obtained at higher K^+; and then decreased with progressive replacement by Na^+, with a minimum point reached at 46.7% of replacement. The ratio of internal to atmospheric CO_2 concentration (C_i/C_a) showed a decrease with increasing K^+ replacement by Na^+ at both K^+ levels. Minimum values were observed at 41.5 and 42.1% of replacement, at 2.5 and 4.0 $mmol_c$ dm^{-3} external K^+, respectively (Fig. 1). As with g_s, mean transpiration rate (E), at higher K^+ supply, showed no statistically significant difference ($P < 0.05$) at any of the Na^+ replacement treatments (Fig. 1). However, in the lower K^+ treatments, E was 26.5% higher when there was no replacement by Na^+, compared to the average obtained at the high external K^+ level (Fig. 1). In the same treatment, there was a reduction in E of up to 47.5% (minimum point estimated) at high levels of replacement of K^+ by Na^+. The increase of A with increasing K^+ replacement coincided with a decrease in g_s and E, revealing increases in both instantaneous (A/E) and intrinsic (A/g_s) efficiencies of water use (Fig. 2). These variables showed an increase

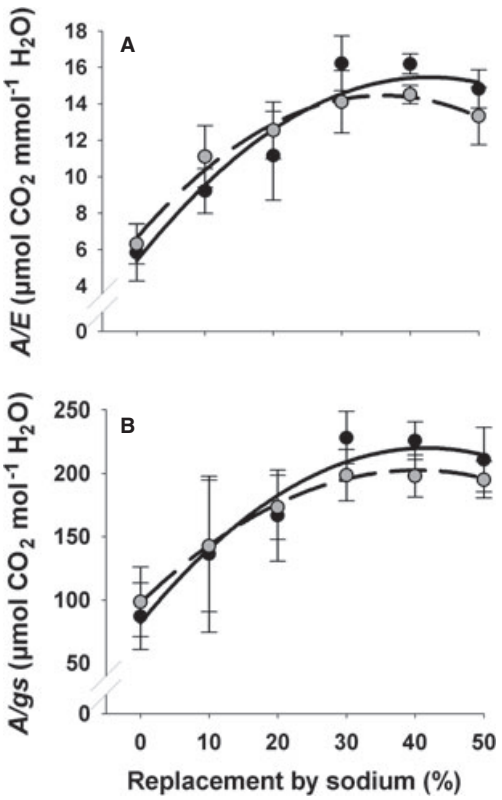

Fig. 2. Instantaneous (A) and intrinsic (B) water-use efficiencies in leaves of clonal *Theobroma cacao* plants (PH-16 clone), cultivated for 180 days under two soil K^+ treatments (2.5 $mmol_c$ dm^{-3}, continuous line and black circles as means; and 4.0 $mmol_c$ dm^{-3}, dashed line and gray circles as means) replaced gradually by Na^+.

up to 42.8 and 43.6% (maximum points estimated) with increasing replacement levels of K^+ by Na^+, respectively, at 2.5 $mmol_c$ dm^{-3} K^+; while at 4.0 $mmol_c$ dm^{-3} K^+, the same variables were increased to 36.0 and 40.2%; at the highest percentages of replacement by Na^+, WUEs were reduced (Fig. 2), revealing a clear optimum pattern. For both measures of WUE (A/E; A/g_s), plants under lower soil K^+ showed slightly lower WUE ($P < 0.01$) at lower levels of replacement by Na^+, compared with those under high soil K^+, whereas, at higher levels of replacement by Na^+, plants under lower soil K^+ slightly exceeded WUE of plants grown under high soil K^+ (Fig. 2).

Leaf mineral composition

Leaf N content was reduced slightly, by less than 10%, in plants under lower soil K^+ as replacement of K^+ by Na^+ exceeded 20% (Fig. 4A). However, at the higher soil K^+, there was no significant difference ($P < 0.10$) in leaf N content. There was a reduction in K^+ content of leaves with increasing replacement of K^+ by Na^+ in the lower soil K^+ treatments. However, there were no significant differences ($P < 0.05$) among leaf K^+ contents in the higher soil K^+ treatments (Fig. 3). Neither of the soil K^+ levels yielded a significant difference ($P < 0.05$) in the P contents in response to replacement of K^+ by Na^+ (Table 3). Differences in the leaf contents of Ca^{2+}, Mg^{2+} and S were not significant ($P < 0.05$) in response to replacement of K^+ by Na^+, in plants grown at higher K^+, but showed significant differences ($P < 0.05$)

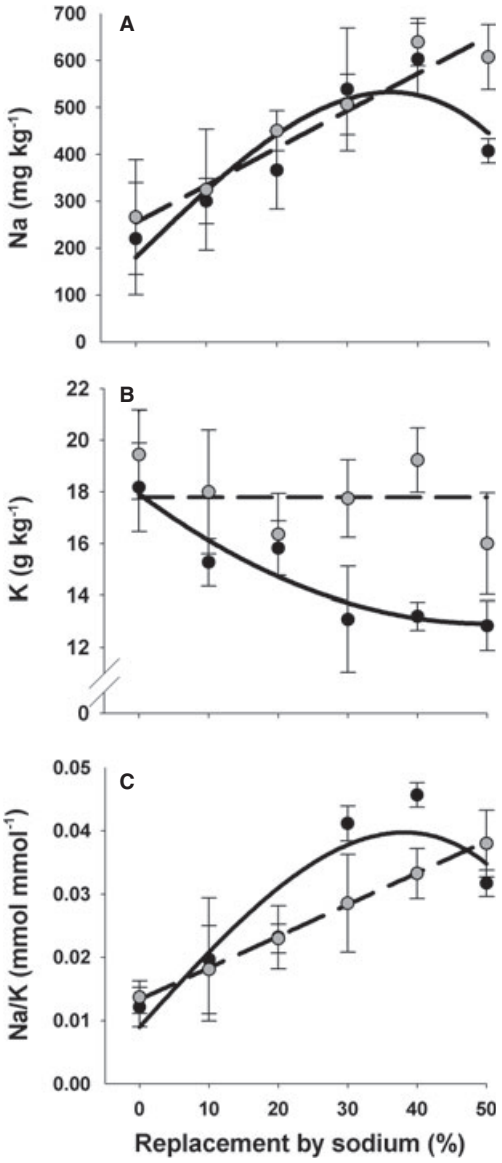

Fig. 3. Leaf N (A), K (B) contents and Na/K (C) ratio in clonal *T. cacao* plants (PH-16 clone), cultivated for 180 days under two soil K^+ treatments (2.5 $mmol_c$ dm^{-3}, continuous line and black circles as means; and 4.0 $mmol_c$ dm^{-3}, dashed line and gray circles as means) replaced gradually by Na^+.

Table 3. Regression models for leaf photosynthetic variables. lw, lower soil K concentration (2.5 $mmol_c\ dm^{-3}$); h, higher soil K concentration (4.0 $mmol_c\ dm^{-3}$); ns, not significant. ** 0.01; * 0.05; '.' 0.1; F test.

A (lw)	$\hat{y} = 3.0 + 0.132^{**}x - 0.0017^{**}x^2$	$R^2 = 0.93$
A (h)	$\hat{y} = 2.78 + 0.129^{**}x - 0.0016^{**}x^2$	$R^2 = 0.80$
E (lw)	$\hat{y} = 0.53 - 0.0076^{**}x + 0.00008^{**}x^2$	$R^2 = 0.81$
E (h)	$\hat{y} = 0.419$	ns
C_i/C_a (lw)	$\hat{y} = 0.64 - 0.0257^{**}x + 0.00031^{**}x^2$	$R^2 = 0.97$
C_i/C_a (h)	$\hat{y} = 0.57 - 0.0185^{**}x + 0.00022^{**}x^2$	$R^2 = 0.94$
g_s (lw)	$\hat{y} = 0.036 - 0.0006^{**}x + 0.00001^{**}x^2$	$R^2 = 0.89$
g_s (h)	$\hat{y} = 0.027$	ns
A/E (lw)	$\hat{y} = 5.37 + 0.471^{**}x - 0.0055^{**}x^2$	$R^2 = 0.89$
A/E (h)	$\hat{y} = 6.62 + 0.435^{**}x - 0.0060^{**}x^2$	$R^2 = 0.96$
A/g_s (lw)	$\hat{y} = 81.74 + 6.64^{**}x - 0.0796^{**}x^2$	$R^2 = 0.90$
A/g_s (h)	$\hat{y} = 97.56 + 5.22^{**}x - 0.065^{**}x^2$	$R^2 = 0.98$

under lower K^+, where increases were observed up to 33.79, 35.5 and 29.15% replacement of K^+ by Na^+ (maximum points estimated using the fitted equations) for Ca^{2+} Mg^{2+}, and S, respectively, after which point there was a reduction (Fig. 4). There were no significant effects ($P < 0.05$) in concentrations of the micronutrients Mn^{2+}, Fe^{2+}, regardless of soil K^+ level and degree of replacement by Na^+, or any interaction between these factors (Table 4). However, although no significant difference ($P < 0.05$) was detected in the contents of Zn^{2+} and Cu^{2+} amongst treatments at the higher external K^+, there was a significant effect ($P < 0.05$) of K^+ replacement on the contents of these micronutrients in plant leaves under the lower K^+ level (Fig. 4). Leaf Cu^{2+} content showed a decrease with replacement of K^+ by Na^+, especially at replacement percentages above 20%, while leaf Zn^{2+} content increased until 32.4% replacement of K^+ by Na^+ was reached, and, above this point, it decreased. Leaf Na^+ content increased linearly at 4.0 $mmol_c\ dm^{-3}$ soil K^+, with increasing replacement of K^+ by Na^+, while at 2.5 $mmol_c\ dm^{-3}$ K^+, leaf Na^+ content increased up to approximately 40% replacement (Fig. 3). There was an increase of 291% in the leaf content of Na^+, with increasing replacement at lower soil K^+, while leaf K^+ content, under the same treatment, showed a reduction of approximately 28%. Thus, in the replacement series at lower K^+, about 0.35 g kg^{-1} (0.015 mol kg^{-1}) Na^+ was added to leaf tissue, while there was a reduction of approximately 5.3 g kg^{-1} (0.136 mol kg^{-1}) K^+. On a molar basis, the replacement of K^+ by Na^+ occurred in the proportion 9:1 (mol mol^{-1}), respectively.

Discussion

The increase of the rate of photosynthesis, A, in response to replacement of potassium with sodium shows that the Na^+ ion can act as a beneficial nutritional element in the major perennial C3 crop *Theobroma cacao*. This is the first time photosynthetic response to Na^+ addition has been measured in a major perennial crop. Previous studies identified the potential for an increase in photosynthetic capacity in C4 plants (Matoh and Murata 1990, Murata and Sekiya 1992), where Na^+ has been considered essential by several workers and where the stimulation has been attributed to enhanced conversion of pyruvate to phosphoenolpyruvate (Johnston et al. 1988), and in part to the facilitation of Na^+/pyruvate cotransport at the chloroplast envelope (Ohnishi et al. 1990). In a previous study on non-perennial C3 species, no stimulatory influence of Na^+ addition on photosynthesis was seen (Subbarao et al. 1999). In our study, however, a progressive increase in A was observed up to the point where the K^+:Na^+ ratio reached 1.5. Indeed, the increase in photosynthetic capacity seen here rivals that observed in C4 plants upon Na^+ addition, and, thus, stimulations in A do not appear to be contingent upon the overcoming of limitations in pyruvate supply, transport and enzymatic conversion, but may well be more general. Coincident with the increase in photosynthetic capacity, we observed optimum-curve responses in sulfur, calcium, magnesium and zinc, albeit only at the lower level of K^+ supply. All four elements are critical to photosynthetic function (Murata 1952, Randall and Bouma 1973, Debus 1992, Wulff-Zottele et al. 2010), but, as changes in their accumulation patterns were only seen at one of the K^+ levels examined whereas changes in A were seen throughout, these offer at best a partial explanation for the photosynthetic enhancements seen. The Na^+ ion itself is typically implicated as a photosynthetic toxicant in C3 species (Munns and Tester 2008, Kronzucker and Britto 2011), and it is understood that genotypes more tolerant of Na^+ possess the capacity to sequester the ion away from the cytosol, in the vacuolar compartment (Blumwald et al. 2000, Wu et al. 2011). This sequestration is accomplished by the activity of Na^+/H^+ antiporters at the tonoplast membrane, preventing toxicity in the cytosol and imparting osmotic capacity on the vacuole, rendering plants more resistant to water stress (Blumwald et al. 2000, Kronzucker and Britto 2011, Wu et al. 2011). It is likely that the increased shoot Na^+ content seen in cacao with increasing replacement of K^+ by Na^+ (Fig. 3) also manifests predominantly as an increased vacuolar pool, including in guard cells (Terry and Ulrich 1973). Indeed, significant increases in cytosolic Na^+ above 20–30 mM are rarely observed, even under toxic, high Na^+, conditions (Munns and Tester 2008). Thus, with Na^+ available for osmotic functions in the vacuole, K^+ ions may be targeted to metabolic pathways,

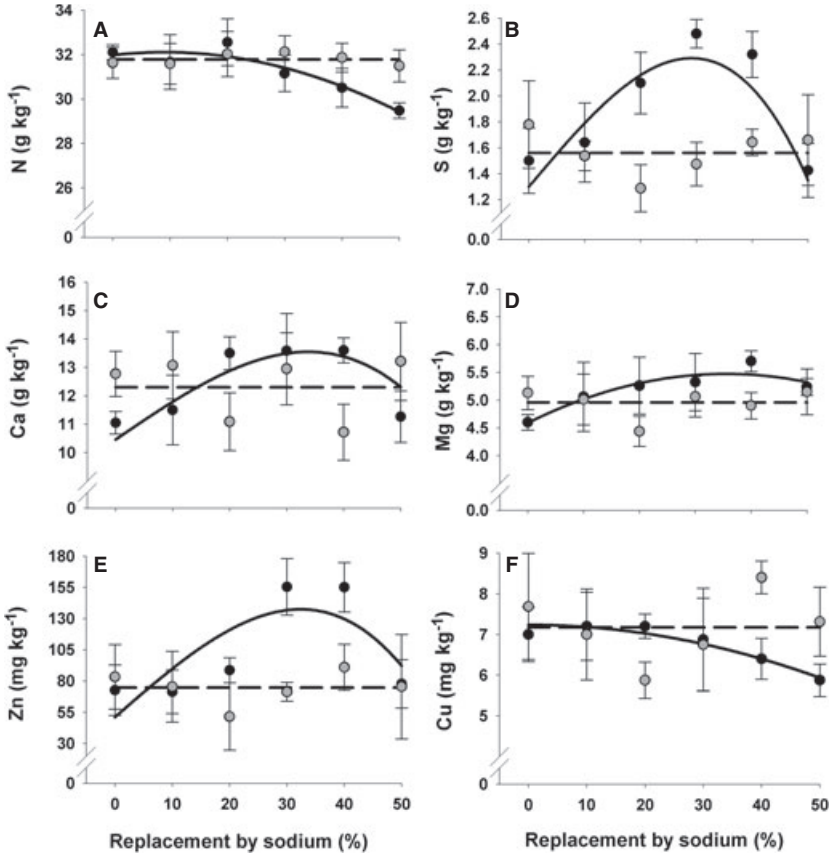

Fig. 4. Leaf N (A), S (B), Ca (C), Mg (D), Zn (E) and Cu (F) contents in clonal *Theobroma cacao* plants (PH-16 clone), cultivated for 180 days under two soil K^+ treatments (2.5 $mmol_c$ dm^{-3}, continuous line and black circles as means; and 4.0 $mmol_c$ dm^{-3}, dashed line and gray circles as means) replaced gradually by Na^+.

promoting an increase in A (see also Speer and Kaiser 1991). In our study, we have also observed a highly significant decrease in g_s, the stomatal conductance to water vapor, as well as leaf transpiration rate (E), in the lower, although not the higher, soil K^+ treatment. Changes in g_s relate to the control of both water loss and CO_2 assimilation (Taiz and Zeiger 2003). The substantial decline in g_s, and E at lower soil K^+ mirrored the increase in photosynthesis. While decreases in g_s may restrict the rate of CO_2 fixation, associated with the reduction of its concentration in the sub-stomatal cavities and intercellular spaces (Daley et al. 1989), more important are likely the effects of efficient stomatal closure, allowing maintenance of cellular water potential and turgor (Henson et al. 1982). Na^+ addition, thus, at least at lower (however ecologically highly relevant) K^+ concentrations in soils (Subbarao et al. 2003), may promote higher resistance to water loss during dry periods, and possibly enhanced tolerance to sudden onset of salinity (Kronzucker and Britto 2011). This is of particular importance to cacao, which is known to be especially intolerant of even brief drought episodes, cutting yields significantly, and strategies to overcome this limitation are being sought actively (Belsky and Siebert 2003, Bae et al. 2009).

Given the lack of direct action of low-level Na^+ on the metabolic processes of C3 plants, it is suggested that the observed increase in the content of Na^+ in leaf tissue is primarily directed toward osmotic functions in the vacuole. In contrast, nutrients acting on the metabolism of CO_2 assimilation, in particular potassium, whose content was not affected much by Na^+ co-presence in *Theobroma*, are expected to remain available in the cytoplasm and, thus, the chloroplast (Speer and Kaiser 1991). Unlike under high Na^+ supply (Speer and Kaiser 1991), there is no evidence in the literature of suppression of key cytosolic components under low Na^+ supply. Our hypothesis is less consistent, but not invalid, for plants grown under lower soil K^+, where there was a reduction of g_s that mirrored the decline in C_i/C_a and E. (Fig. 1). Variations in transpiration rate cause changes in various aspects of physiology, such as leaf temperature

Table 4. Regression models for leaf mineral content. lw, lower soil K concentration (2.5 $mmol_c\ dm^{-3}$); h, higher soil K concentration (4.0 $mmol_c\ dm^{-3}$); ns, not significant. ** 0.01; * 0.05; '.' 0.1; *F* test.

N (lw)	$\hat{y} = 31.97 - 0.029^{**}x - 0.0016^{**}x^2$	$R^2 = 0.83$
N (h)	$\hat{y} = 31.78$	ns
P (lw)	$\hat{y} = 1.46$	ns
P (h)	$\hat{y} = 1.50$	ns
K (lw)	$\hat{y} = 17.92 - 0.20^{**}x + 0.002^{**}x^2$	$R^2 = 0.82$
K (h)	$\hat{y} = 17.79$	ns
Ca (lw)	$\hat{y} = 10.46 + 0.137^{**}x - 0.00004^{**}x^3$	$R^2 = 0.92$
Ca (h)	$\hat{y} = 12.30$	ns
Mg (lw)	$\hat{y} = 4.59 + 0.0497^{**}x - 0.0007^{**}x^2$	$R^2 = 0.60$
Mg (h)	$\hat{y} = 4.96$	ns
S (lw)	$\hat{y} = 1.30 + 0.051^{**}x + 0.00002^{**}x^3$	$R^2 = 0.90$
S (h)	$\hat{y} = 1.56$	ns
Cu (lw)	$\hat{y} = 7.23 - 0.00052^{**}x^2$	$R^2 = 0.97$
Cu (h)	$\hat{y} = 7.17$	ns
Fe (lw)	$\hat{y} = 117.56$	ns
Fe (h)	$\hat{y} = 117.52$	ns
Zn (lw)	$\hat{y} = 51.07 + 4.0^{**}x - 0.00127^{**}x^3$	$R^2 = 0.82$
Zn (h)	$\hat{y} = 74.71$	ns
Mn (lw)	$\hat{y} = 1056$	ns
Mn (h)	$\hat{y} = 1230$	ns
Na (lw)	$\hat{y} = 179.92 + 14.60^{**}x - 0.0037^{**}x^3$	$R^2 = 0.86$
Na (h)	$\hat{y} = 254.4 + 7.94^{**}x^2$	$R^2 = 0.84$
Na/K(lw)	$\hat{y} = 0.0089 + 0.00121^{**}x - 0.00000028^{**}x^3$	$R^2 = 0.85$
Na/K(h)	$\hat{y} = 0.01332 + 0.0005^{**}x^2$	$R^2 = 0.88$

and water potential (Farquhar and Sharkey 1982). The two main resistances to transpiration are the air boundary layer and stomatal opening (Taiz and Zeiger 2003). It is suggested, from our data, that reduced stomatal opening promoted the variations in E. The initial increase and subsequent reduction in instantaneous (A/E) and intrinsic (A/g_s) efficiencies of water use with replacement of K^+ by Na^+ in both soil K^+ treatments suggest that Na^+ can significantly improve the regulation of water use in the leaves of *T. cacao*. It is suggested that there may well be greater efficiency of Na^+, compared to K^+, in the osmotic function of stomatal closure. The amount of Na^+ that replaced K^+ in our study, on a molar basis, was about one ninth of the amount of leaf K^+. It has been previously suggested that the Na^+ ion can replace the K^+ ion in the process of osmoregulation in vacuoles, for the generation of turgor and cell expansion (Jeschke 1977; Nunes et al. 1984). Indeed, Marschner and Possingham (1975) demonstrated Na^+ superiority in this important regard by studying the expansion of leaf segments of *Beta vulgaris* both in vitro and in intact plants; leaf area, leaf thickness and succulence were also shown to be greater at increased replacement levels of K^+ by Na^+ (Milford et al. 1977). Results obtained in these, and our, studies may be due to the difference between the ionic hydrated radii of K^+ and Na^+, and the impact this has on hydrated bulk volumes. While the non-hydrated Na^+ ion is smaller than non-hydrated K^+, the hydrated radius of Na^+ is nearly 1.5 times that of K^+ (Kielland 1937, see also: Horne 1971, Conway 1981, Israelachvili 1992, Jakli 2007), with a consequent volume difference in aqueous media of 4.02 $cm^3\ mol^{-1}$ between the two ions. However, whether this would render equimolar quantities of Na^+ potentially more osmotically effective than K^+ has not been examined at the physico-chemical level hitherto.

Progressive replacement of K^+ by Na^+ affected the mineral status of cacao shoot tissue significantly, and this may in part underpin the improved photosynthetic performance and WUE. Interestingly, nitrogen (N), the principal growth-limiting nutrient (Kronzucker et al. 1997), was affected only slightly by Na^+ introduction in cacao leaves, and only at one of the two soil K^+ levels examined. Even where a slight reduction in leaf N was seen (by less than 10%), it did not fall below 23 g kg^{-1}, content deemed critical for cacao plants under high nutritional supplementation and rapid growth (Machicado and Boyton 1961, Souza Junior and Carmello 2008). Photosynthesis is, thus, not expected to experience nitrogen deficiency when Na^+ replacement occurs. However, Na^+ supplementation also clearly does not enhance nitrogen capture, and the stimulation of photosynthesis does not occur via this route (Haxeltine and Prentice 1996). A further surprise was that leaf K^+ content in plants under higher soil K^+ was maintained more or less constant, even when soil K^+ decreased with progressive replacement by Na^+. This is in agreement with older studies that show that, in the millimolar range of supply of the two ions, root K^+ acquisition is only marginally suppressed by Na^+ (Rains and Epstein 1967), while K^+ transporters can be upregulated successfully in response to falling external K^+ at both molecular and functional levels (for review, see Britto and Kronzucker 2008). At lower soil K^+ in our study, leaf K^+ content did decrease when replacement by sodium was increased; however, with no resultant symptoms of K^+ deficiency. It has been suggested by others that Na^+ has the ability to reduce critical levels of leaf K^+ (Greenwood and Stone 1998), and, at least at high concentrations of Na^+, this suppression has been shown to come about, in part, through enhanced release of K^+ from root tissues (Shabala and Cuin 2008; Britto et al. 2010). In *Beta vulgaris*, the K^+ content of leaf tissue (for 95% of maximum yield) decreased from 100 to 4 g kg^{-1}, when 98% of K^+ was replaced by Na^+ (Subbarao et al. 1999). This decrease in K^+ content occurred without affecting short-term growth, suggesting that 4 g kg^{-1} was still above the critical level of K^+ in that species. This critical level was much higher, at 30 g kg^{-1}, in *Spinacea oleracea*, and was 65 g kg^{-1} in *Lactuca sativa* (Subbarao et al. 2002). It is suggested that, even with

rather significant declines in total-tissue K^+ content, the concentration of K^+ in the cytoplasm is maintained near its steady-state set point of 100 mM, which is required to maintain enzyme activities (Walker et al. 1996, Wyn Jones 1999, Britto and Kronzucker 2008). Thus, any changes in K^+ content are expected to predominantly reflect changes in vacuolar K^+, which, together with other solutes, is accumulated in that compartment to maintain osmotic potential (Wyn Jones 1999). When other cations are abundant in tissue, the critical content of K^+ typically varies between 10 and 20 g kg^{-1}, but when concentrations of other ions are low, the critical K^+ content can increase from 40 to 70 g kg^{-1}, depending on the species (Hylton et al. 1967, Smith et al. 1982). In treatments with higher replacements by Na^+, leaf K^+ contents in plants under lower soil K^+ provision fell slightly below those considered sufficient by Malavolta (2006) and Raij et al. (1997) for adult cacao plants, but this appears to be more than compensated for by the concomitant increase in tissue Na^+. We should point out that, in the Na^+–K^+ replacement series used here (as in other studies), K^+ levels remain high enough to favor low-affinity K^+ uptake (Britto and Kronzucker 2008), and it will be of interest in the future to test whether Na^+ can also exert its beneficial effects when K^+ uptake proceeds predominantly via high-affinity systems.

Typically, following a general pattern of cation competition at the whole-tissue level (van Beusichem et al. 1988, Speer and Kaiser 1991), Na^+ absorption increases and uptake of Ca^{2+} decreases in plant cells and tissues when the availability of external Na^+ is high (Montasir et al. 1966, Rengel 1992, Cramer 1997, Lazof and Bernstein 1999). Moreover, under high external Ca^{2+} availability, absorption and Na^+ content often decrease, and the absorption and Ca^+ content increase. Some studies have shown that Ca^{2+} attenuates the reduction in growth effected by Na^+ when saline levels are reached (Chapman 1968, Lahaye and Epstein 1969, Zekri and Parsons 1990, Bañuls et al. 1991, Bañuls and Primo-Millo 1992). Therefore, given the relationship of Ca^{2+} as a mitigator of injuries caused by salts, and the high correlation between tissue Ca^{2+} and Na^+ especially at higher levels of supply (Rengel 1992), the peak of foliar Ca^{2+} content obtained in our study at intermediate replacement levels of K^+ by Na^+ may, in fact, predispose cacao plants to enhanced tolerance to salinity. In other words, lower levels of Na^+ under such circumstances may be seen as hormetic doses that may enhance Na^+ tolerance at higher doses. In our study, a similar trend as that for Ca^{2+} was observed for Mg^{2+}, whose effects are often similar to those of Ca^{2+} in stabilizing membranes and mitigating effects in the toxic range for Na^+ (Kronzucker and Britto 2011). Our results contrast with those in an early study by Montasir et al. (1966), who reported declines in both Ca^{2+} and Mg^+ accumulation in non-perennial species with increasing Na^+ supplementation. The tissue levels of other cations displayed either no significant suppression by Na^+ supplementation, or these contents were in fact maximized, especially so for S, Mn^{2+} and Zn^{2+}. All these responses can be seen as potentially beneficial to photosynthesis, although they cannot, in and of themselves, explain the optimization of photosynthesis, as the pattern of response was dependent on the total combined level of potassium and sodium in the growth medium.

Of particular interest to us was the examination of the development of the tissue Na^+/K^+ ratio as Na^+ supplementation increased. The Na^+/K^+ ratio has received much recent attention especially in the literature on salt stress responses (Munns and Tester 2008), and is frequently cited as an excellent predictor of plant performance. Frequently, reference is made to this ratio specifically in the cytosol of cells, but this parameter is, in fact, rarely measured, and, rather, total-tissue measurements are drawn upon in most studies for this purpose (Kronzucker and Britto 2011). More specifically, it is commonly held that increases in the tissue Na^+/K^+ ratio correlate with decreased photosynthetic performance and biomass (see Kronzucker et al. 2006, for detailed discussion), although this has also recently been challenged (Kronzucker and Britto 2011). Fig. 3C shows that, in cacao, the tissue Na^+/K^+ ratio increased four- to fivefold in the course of Na^+ supplementation. Rather than incurring negative consequences, the key processes of photosynthesis and WUE both experienced the maximization development discussed above. To illustrate this further, in Figs. S1 and S2 of the supporting information, we plotted the physiological variables evaluated in our study against the tissue Na^+/K^+ ratio. It is clear that relating a rise in the ratio with declining plant performance, as has become common practice, is not as universally tenable, and certainly does not apply to the major crop species under investigation here, under provision levels of Na^+ and K^+ in soil solution that fall into the low-millimolar range (see also Marschner et al. 1981, Subbarao et al. 1999, 2003). It is, indeed, rather possible, as argued here, that enhanced plant performance can be achieved by raising the Na^+/K^+ ratio upward, which is not only relevant to cultivation conditions where natural infiltration of Na^+ may already occur, or may be encouraged by soil amendment (Subbarao et al. 2003), but may furthermore be of benefit in preconditioning plants for the onset of stresses that affect plant water relations such as salinity and drought. This important implication will be a topic for future study.

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References

Ali L, Rahmatullah, Ranjha AM, Aziz T, Maqsood MA, Ashraf M (2006) Differential potassium requirement and its substitution by sodium in cotton genotypes. Pak J Agric Sci 43: 3–4

Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, Melnick RL, Bailey BA (2009) The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. J Exp Bot 60: 3279–3295

Balkos KD, Britto DT, Kronzucker HJ (2010) Optimization of ammonium acquisition and metabolism by potassium in rice (*Oryza sativa* L. cv. IR-72). Plant Cell Environ 33: 23–34

Bañuls J, Legaz F, Primo-Millo E (1991) Salinity–calcium interactions on growth and ionic concentration of *Citrus* plants. Plant Soil 133: 39–46

Bañuls J, Primo-Millo E (1992) Effects of chloride and sodium on gas exchange parameters and water relations of *Citrus* plants. Physiol Plant 86: 115–123

Belsky JM, Siebert SF (2003) Cultivating cacao: implications of sun-grown cacao on local food security and environmental sustainability. Agric Hum Val 20: 277–285

Blumwald E, Aharon GS, Apse MP (2000) Sodium transport in plant cells. Biochim Biophys Acta 1465: 140–151

Bosshart RP, Von Uexkull HR (1987) Some occasionally overlooked criteria for assessing fertilizer requirements of high yielding cocoa. In: Kernel P (ed) Seminar on Palm Kernel Utilization and Recent Advances in Cocoa Cultivation. Sawan, Sabah, Malaysia

Britto DT, Kronzucker HJ (2008) Cellular mechanisms of potassium transport in plants. Physiol Plant 133: 637–650

Britto DT, Ebrahimi-Ardebili S, Hamam AM, Coskun D, Kronzucker HJ (2010) ^{42}K analysis of sodium-induced potassium efflux in barley: mechanism and relevance to salt tolerance. New Phytol 186: 373–384

Brownell PF (1965) Sodium as an essential micronutrient element for a higher plant (*Atriplex vesicaria*). Plant Physiol 40: 460–468

Brownell PF (1979) Sodium as an essential micronutrient element for plants and its possible role in metabolism. Adv Bot Res 7: 117–224

Brownell PF, Crossland CJ (1972) The requirement for sodium as a micronutrient by species having the C4 dicarboxylic photosynthetic pathway. Plant Physiol 49: 794–797

Chapman HD (1968) The mineral nutrition of citrus. In: Reuther W, Batchelor LD, Webber HJ (eds) The Citrus Industry II. University of California Press, Berkeley and Los Angeles, CA, pp 127–289

Conway BE (1981) Ionic Hydration in Chemistry and Biophysics. Elsevier, New York, NY.

Cramer GR (1997) Uptake and role of ions in salt tolerance, In: Jaiwal PK, Singh RP, Gulati A (eds) Strategies for Improving Salt Tolerance in Higher Plants. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, pp 55–86

Daley PF, Raschke K, Ball JY, Berry JA (1989) Topography of photosynthetic activity of leaves obtained from video images of chlorophyll fluorescence. Plant Physiol 90: 1233–1238

Debus RJ (1992) The manganese and calcium ions of photosynthetic oxygen evolution. Biochim Biophys Acta 1002: 269–352

Draycott AP, Durrant MJ (1976) Response by sugar beet to potassium and sodium fertilizers, particularly in relation to soils containing little exchangeable potassium. J Agric Sci 87: 105–112

El-Sheikh AM, Ulrich A (1970) Interactions of rubidium, sodium, and potassium on the nutrition of sugar beet plants. Plant Physiol 46: 645–649

El-Sheikh AM, Ulrich A, Broyer TC (1967) Sodium and rubidium as possible nutrients for sugar beet plants. Plant Physiol 42: 1202–1208

Embrapa (1997) Manual de métodos de análise de solo. Embrapa – Centro nacional de pesquisa de solos, Rio de Janeiro

Evans HJ, Sorger GJ (1966) Role of mineral elements with emphasis on the univalent cations. Annu Rev Plant Physiol 17: 47–76

Farquhar GD, Sharkey TD (1982) Stomatal conductance and photosynthesis. Annu Rev Plant Physiol 33: 317–345

Greenway H, Osmond CB (1972) Salt responses of enzymes from species differing in salt tolerance. Plant Physiol 49: 256–259

Greenwood DJ, Stone DA (1998) Prediction and measurement of the decline in the critical-K, the maximum-K and total cation plant concentrations during the growth of field vegetable crops. Ann Bot 82: 871–881

Hall JL, Flowers TJ (1973) The effect of salt on protein synthesis in the halophyte *Suaeda maritima* Planta 110: 361–368

Haxeltine A, Prentice IC (1996) A general model for the light-use efficiency of primary production. Func Ecol 10: 551–561

Henson IE, Alagarswamy G, Bidinger FR, Mahalakshmi V (1982) Stomatal responses of pearl millet (*Pennisetum americanum* [L.] Leeke) to leaf water status and

environmental factors in the field. Plant Cell Environ 5: 65–74
Horne RA (1971) Water and Aqueous Solutions. Wiley-Inter Science, New York, NY
Hylton LO, Ulrich A, Cornelius DR (1967) Potassium and sodium interrelations in growth and mineral content of Italian ryegrass. Agron J 59: 311–314
Isaac ME, Ulzen-Appiah F, Timmer VR, Quashie-Sam SJ (2007) Early growth and nutritional response to resource competition in cocoa-shaded intercropped systems. Plant Soil 298: 243–254
Israelachvili JN (1992) Intermolecular and Surface Forces. Academic Press, London, UK
Jakli G (2007) The H_2O-D_2O solvent isotope effects on the molar volumes of alkali-chloride solutions at T = (288.15, 298.15, and 308.15) K. J Chem Therm 12: 1589–1600
Jeschke WD (1977) K and Na exchange and selectivity in barley root cells: effect of Na^+ on the Na^+ fluxes. J Exp Bot 28: 1289–1305
Johnston M, Grof CPL, Brownell PF (1988) The effect of sodium nutrition on the pool size of intermediates of the C_4 photosynthetic pathway. Austr J Plant Physiol 15: 749–760
Jones JB Jr, Wolf B, Mills HA (1991) Plant Analysis Handbook. Micro-Macro publishing, Inc., Athens, GA
Kielland J (1937) Individual activity coefficients of ions in aqueous solutions. J Am Chem Soc 59: 1675–1678
Kronzucker HJ, Britto DT (2011) Sodium transport in plants: a critical review. New Phytol 189: 54–81
Kronzucker HJ, Siddiq MY, Glass ADM (1997) Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature 385: 59–61
Kronzucker HJ, Szczerba MW, Moazami-Goudarzi M, Britto DT (2006) The cytosolic Na^+:K^+ ratio does not explain salinity-induced growth impairment in barley: a dual-tracer study using $^{42}K^+$ and $^{24}Na^+$. Plant Cell Environ 29: 2228–2237
Kronzucker HJ, Szczerba MW, Schulze LM, Britto DT (2008) Non-reciprocal interactions between K^+ and Na^+ ions in barley (*Hordeum vulgare* L.). J Exp Bot 59: 2973–2981
Laclau JP, Ranger J, Bouillet JP, Nizla JD, Deleporte P (2003) Nutrient cycling in a clonal stand of Eucalyptus and an adjacent savanna ecosystem in Congo. 1. Chemical composition of rainfall. Throughfall and stemflow solutions. For Ecol Manage 176: 105–119
Lahaye PA, Epstein E (1969) Salt toleration by plants: enhancement with calcium. Science 166: 395–396
Lazof DB, Bernstein N (1999) The NaCl induced inhibition of shoot growth: the case for disturbed nutrition with special consideration of calcium. Adv Bot Res 29: 113–189
Lehr JJ (1953) Sodium as a plant nutrient. J Sci Food Agric 4: 460–471
Leigh RA, Chater M, Storey R, Johston AE (1986) Accumulation and subcellular distribution of cations in relation to the growth of potassium-deficiency barley. Plant Cell Environ 9: 595–604
Machicado M, Boynton D (1961) Effect of three nitrogen sources and two light intensities on the nitrogen constituents of cocoa seedling leaves of three different ages. Proceedings of Inter-American Cacao conference, Trinidad e Tabago, pp 345–354
Malavolta E (2006) Manual de nutrição mineral de plantas. Ceres Press, São Paulo, SP
Marschner H (1995) Mineral Nutrition of Higher Plants, 2nd Edn. Academic Press, London
Marschner H, Possingham JV (1975) Effect of K^+ and Na^+ on growth of leaf discs of sugar beet and spinach. Z Pflanzenphysiol 75: 6–16
Marschner H, Kuiper PJC, Kylin A (1981) Genotypic differences in the response of sugar beet plants to replacement of potassium by sodium. Physiol Plant 51: 239–244
Mäser P, Gierth M, Schroeder JI (2002) Molecular mechanisms of potassium and sodium uptake in plants. Plant Soil 247: 43–54
Matoh T, Murata S (1990) Sodium stimulates growth of *Panicum coloratum* through enhanced photosynthesis. Plant Physiol 92: 1169–1173
Mengel K (2007) Potassium. In: Barker AV, Pilbeam DJ (eds) Handbook of Plant Nutrition, 1st Edn. Taylor & Francis, London, UK, pp 91–120
Micheli F, Guiltinan M, Gramacho KP, Wilkinson MJ, Figueira AVD, Cascardo JCD, Maximova S, Lanaud C (2010) Functional genomics of cacao. Adv Bot Res 55: 119–177
Milford GFJ, Cormack WF, Durrant MJ (1977) Effects of sodium chloride on water status and growth of sugar beet. Exp Bot 28: 1380–1388
Montasir AH, Sharoubeem HH, Sidrak GH (1966) Partial substitution of sodium for potassium in water cultures. Plant Soil 25: 181–194
Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59: 651–681
Murata N (1952) Control of excitation transfer in photosynthesis. 2. Magnesium ion-dependent distribution of excitation energy between 2 pigment systems in spinach chloroplasts. Biochim Biophys Acta 189: 171
Murata S, Sekiya J (1992) Effects of sodium on photosynthesis in *Panicum coloratum*. Plant Cell Physiol 33: 1239–1242
Nitsos VN, Evans HJ (1969) Effect of univalent cations on activity of particulate starch synthetase. Plant Physiol 44: 1260–1266
Nunes MA, Dias MA, Correia M, Oliveira MM (1984) Further studies on growth and osmoregulation of

sugarbeet leaves under low salinity conditions. J Exp Bot 35: 322–331
Ohnishi J, Flügge UI, Heldt HW, Kanai R (1990) Involvement of Na^+ in active uptake of pyruvate in mesophyll chloroplasts of some C_4 species. Plant Physiol 94: 950–959
Ohta D, Matoh T, Takahashi E (1988) Sodium-stimulated NO_3 uptake uptake in *Amaranthus tricolor* L. Plant Physiol 87: 223–225
Orchard JE (1978) Efeito do K na transpiração, na resistência difusiva de folha e crescimento em plântulas de *Theobroma cacao* L. In: Informe técnico Cepec/Ceplac. Ilhéus-Brasil, pp 61–64
Raij B van Cantarella H, Quaggio JA (1997) Estimulantes. In: van Raij B, Cantarella H, Quaggio JA, Furlani AMC (eds) Recomendação de adubação e calagem para o Estado de São Paulo, 2nd Edn.
Rains DW, Epstein E (1967) Sodium absorption by barley roots: Its mediation by mechanism 2 of alkali cation transport. Plant Physiol 42: 319–323
Randall PJ, Bouma D (1973) Zinc deficiency, carbonic anhydrase, and photosynthesis in leaves of spinach. Plant Physiol 52: 229–232
Rengel Z (1992) The role of calcium in salt toxicity. Plant Cell Environ 15: 625–632
Shabala S, Cuin T (2008) Potassium transport and plant salt tolerance. Physiol Plant 133: 651–669
Shabala SN, Mackay AS (2011) Ion transport in halophytes. In: Kader J, Delseny M (eds) Advances in Botanical Research. Academic Press, Elsevier Ltd, Burlington, MA, pp 151–199
Smith GS, Lauren DR, Cornforth IS, Agnew MP (1982) Evaluation of putrescine as a biochemical indicator of potassium requirements of lucerne. New Phytol 91: 419–428
Souza PA, Mello WZ, Maldonado J, Evangelista H (2006) Composição química da chuva e aporte atmosférico na Ilha Grande, RJ Quím Nova 29: 471–476
Souza JO Jr, Carmello QAC (2008) Forms and doses of urea to fertilize clonal cocoa tree cuttings cultivated in substrate. Rev Bras Ciên Solo 32: 2367–2374
Speer M, Kaiser WM (1991) Ion relations of symplastic and apoplastic space in leaves of *Spinacea oleracea* L. and *Pisum sativum* L. under salinity. Plant Physiol 97: 990–997
Subbarao GV, Wheeler RM, Stutte GW, Levine LH (1999) How far can sodium substitute for potassium in redbeet? J Plant Nutr 22: 1745–1761
Subbarao GV, Stutte GW, Wheeler RM, Berry WL (2002) Sodium: a functional nutrient in plantsIn: Pessarakli M (ed) Handbook of Plant and Crop Physiology, 2nd Edn. Marcel Dekker, New York, NY, pp 583–613
Subbarao GV, Ito O, Berry WL, Wheeler RM (2003) Sodium – a functional plant nutrient. Crit Rev Plant Sci 22: 391–416
Szczerba MW, Britto DT, Kronzucker HJ (2008) K^+ transport in plants: physiology and molecular biology. J Plant Phyiol 166: 447–466
Taiz L, Zeiger E (2003) Fisiologia Vegetal, 3rd Edn. Artmed, São Paulo, SP
Ten Hoopen F, Cuin TA, Pedas P, Hegelund JN, Shabala S, Schjoerring JK, Jahn TP (2010) Competition between uptake of ammonium and potassium in barley and *Arabidopsis* roots: molecular mechanisms and physiological consequences. J Exp Bot 61: 2303–2315
Terry N, Ulrich A (1973) Effects of potassium deficiency on the photosynthesis and respiration of leaves of sugar beet. Plant Physiol 51: 1099–1101
Tisdale SL, Nelson WL (1975) Soil Fertility and Fertilizers, 3rd Edn. Macmillan, New York, NY
Trognitz B, Scheldeman X, Hansel-Hohl K, Kuant A., Grebe H, Hermann M (2011) Genetic population structure of cacao plantatings within a young production area in Nicaragua. PLOS One 6: e16056
Van Beusichem ML, Kirkby EA, Baas R (1988) Influence of nitrate and ammonium nutrition on the uptake, assimilation, and distribution of nutrients in *Ricinus communis*. Plant Physiol 86: 914–921
Walker DJ, Leigh RA, Miller AJ (1996) Potassium homeostasis in vacuolate plant cells. Proc Natl Acad Sci USA 93: 10510–10514
Wu GQ, Xi JJ, Wang Q, Bao AK, Ma Q, Zhang JL, Wang SM (2011) The ZxNHX gene encoding tonoplast Na^+/H^+ antiporter from the xerophyte *Zygophyllum xanthoxylum* plays important roles in response to salt and drought. J Plant Physiol 168: 758–767
Wulff-Zottele C, Gatzke N, Kopka J, Orellana A, Hoefgen R., Fisahn J, Hesse H (2010) Photosynthesis and metabolism interact during acclimation of *Arabidopsis thaliana* to high irradiance and sulphur depletion. Plant Cell Physiol 33: 1974–1988
Wyn Jones RG (1999)Cytoplasmic potassium homeostasis: review of the evidence and its implications. In: Oosterhuis D, Berkowitz G (eds) Frontiers in Potassium Nutrition: New Perspectives on the Effects of Potassium on Physiology of Plants. Potash and Phosphate Institute of Canada, Saskatoon, Canada, pp 13–22
Wyn Jones RG, Brady CJ, Speirs J (1979) Ionic and osmotic relations in plant cells. In: Laidman DC, Wyn Jones RG (eds) Recent advances in the Biochemistry of Cereals. Academic Press, New York, NY, pp 63–103
Yoshida S, Castaneda L (1969) Partial replacement of potassium by sodium in the rice plant under weakly saline conditions. Soil Sci Plant Nutr 15: 183–186
Zekri M, Parsons LR (1990) Calcium influences growth and leaf mineral concentration of citrus under saline conditions. HortScience 25: 784–786

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Net photosynthesis per unit area (A and B), stomatal conductance to water vapor (C and D), and ratio of internal to atmospheric CO_2 concentration (E and F) in relation with Na/K ratio (mmol/g:mmol/g) in leaves of clonal *Theobroma cacao* plants.

Fig. S2. Leaf transpiration rate (A and B) and efficiencies instantaneous (C and D) and intrinsic (E and F) of water use in relation with Na/K ratio (mmol/g:mmol/g) in leaves of clonal *Theobroma cacao* plants.

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