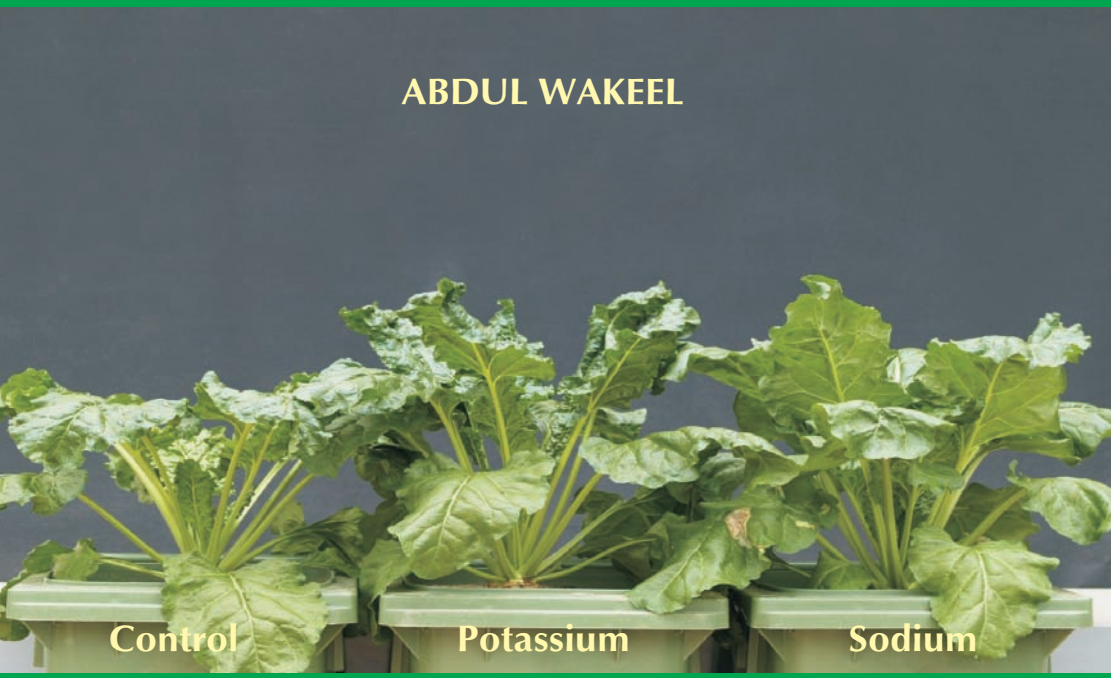
Institute of Plant Nutrition
Justus Liebig University Giessen, Germany
Prof. Dr. Sven Schubert

Substitution of Potassium by Sodium in Sugar Beet (*Beta vulgaris* L.) Nutrition with Special Reference to K-Fixing Soils

ABDUL WAKEEL



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To
My Beloved Parents
May Allah bless them with peace

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1 INTRODUCTION

1.1. Potassium dynamics in soil

Potassium (K) is the most abundant major plant nutrient in most soils. Its concentration in the earth's crust is 2.3%, but the greatest part of this K is bound to primary and secondary clay minerals, thus not readily available for plants. Its availability to plants depends upon the K concentration in the soil solution and transfer of K from exchangeable and fixed form to soil solution. Intensity is the concentration of K in soil solution, whereas capacity is the total amount of K in the soil which can be taken up by plants. The transfer rate from capacity to intensity shows the kinetic factor of renewal of potassium from capacity to intensity (Barber, 1984).

The major natural source of soil potassium is the weathering of K-containing minerals such as micas and alkali feldspars, which contain 6 - 9 and 3.5 - 12% K, respectively. During K uptake plants reduce its concentration in the immediate vicinity of roots which releases K-ions from the minerals (Kuchenbuch and Jungk, 1984). The release of K converts micas to secondary 2:1 clay minerals, illite and then vermiculite Fig. 1.1 (Farmer and Wilson, 1970; Havlin *et al.*, 1999). Application of K fertilizer to soils containing illite and vermiculite clay minerals often leads to fixation of some of its fraction in soil particles. This fraction then becomes unavailable or slowly available to the plants (Scott and Smith, 1987). This fixed K can be made available to plants by its release from soil particles into soil solution when the concentration of K is lowered in soil solution (Cox *et al*., 1999), but in many cases this release is too slow to meet the plant's requirement.

Potassium sorption on exchange sites and its fixation depend on the physicochemical properties of the soil, as well as type and content of clay minerals (von Braunschweig, 1980). The soils containing vermiculite clay minerals are able to fix huge amounts of K. Their cation exchange capacity (CEC) is 1.2 – 1.5 mol (+) kg^{-1} soil. The layer basel distance for these soils is 1.4 – 1.5 nm with a layer charge of 0.6 – 0.9 per half unit-

cell. Other K-fixing clay minerals are smectites with CEC 0.8 – 1.2 mol (+) kg^{-1} soil, layer thickness 1.0 – 2.0 nm and layer charge 0.2 – 0.6 per half unit-cell (Bohn *et al.*, 2001).

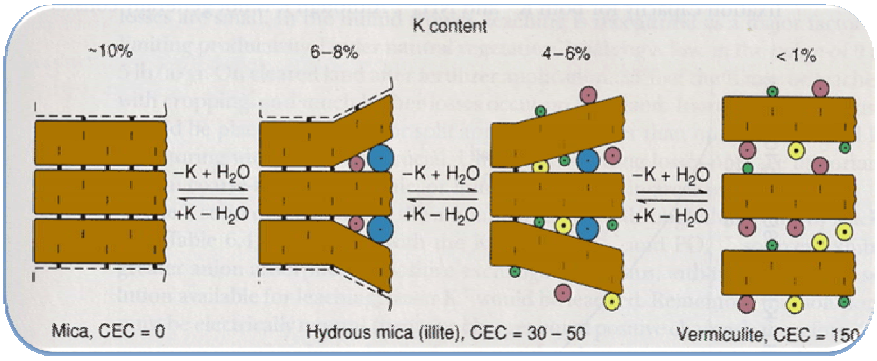


Figure 1.1. K release during mineral weathering and its fixation by clay minerals. CEC = cation exchange capacity. (Havlin *et al.* 1999)

Sometimes plants do not respond to the application of generally recommended levels of K fertilizer in the soils with expandable clay minerals (Mengel and Kirkby, 2001). Doll and Lucas (1973) reported from Michigan that, in a sandy clay loam soil, about 92% of the applied K fertilizers were fixed and 1600 kg K ha^{-1} were applied to make it responsive in tomato production. This soil was rich in illite and vermiculite clay minerals with high CEC and a major part of applied K was fixed and became unavailable to plants immediately. Similar results have been obtained in other parts of the USA (Mengel and Kirkby, 2001).

1.2. Potassium in Plants

Potassium is a major plant nutrient, taken up in a large amount by higher plants. It is almost exclusively present in the ionic form in the plant tissue. It is highly mobile in the plant. Young roots and fleshy fruits are rich in K. In plant cells, the highest concentrations are in the cytosol and are in the range of 130 – 150 mM (Leigh *et al.*, 1999). Vacuolar K concentration ranges from 20 – 100 mM and reflects the K supply (Fernando *et al.*, 1992).

According to Schubert (2006) potassium is involved in:

- enzyme activation
- charge balance
- osmoregulation

Potassium ions activate various enzymes which are involved in many key functions of the cell such as polypeptide synthesis at ribosomes (Jones and Pollard, 1983). In general, it is assumed that K-ions bind to the enzyme surfaces, changing the enzymatic conformation and thus leading to enzyme activation. Specifically, it has been shown that in the enzyme dialkyl-glycine decarboxylase, K is centered in an octahedron with O atoms at the six corners (Fig. 1.2). These O atoms are provided by three amino acyls, one water molecule and O of hydroxyl groups of each of serine and aspartate (Miller, 1993). Up to now, however, it is not clear which particular enzyme of translation or ribosomal site is activated by K-ions (Mengel, 2007). As compared with Na, the K-binding is very selective because the dehydration energy required for K is much lower than for Na. If the latter binds to the enzyme, the natural confirmation of the enzyme is distorted and the access of substrate to the binding site is blocked.

The electrochemical difference between the cytosol and the outer medium is of decisive importance for ion transport. Due to presence of various selective K channels, plant membranes are relatively permeable to K and K is involved in charge balancing in the various processes of the cell. The K-ion is imported into the cell as long as the electrochemical potential in the cytosol is lower than in the outer medium. With the import of K-ions, the electrochemical potential increases and finally attains the equilibrium (Mengel and Kirkby, 2001). The negative charge of cytosol is maintained by the activity of plasmalemma H^+ pump permanently releasing H^+ ions from the cytosol into the apoplast and thus maintaining the negative charge of cytosol in the range of 120 – 200 mV.

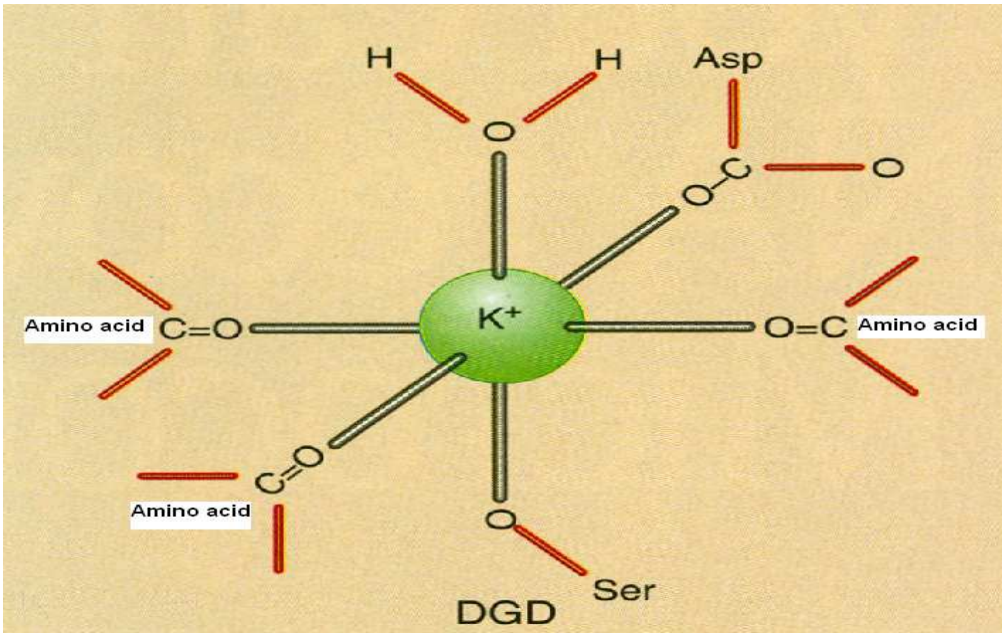


Figure 1.2. Potassium complexed by organic molecules of which the oxygen atoms are oriented to positive charge of K^+ (Adapted from Miller, 1993).

The K ion improves H^+ pumping by the photosynthetic electron transport chain located in the inner membrane of chloroplasts. The H^+ are pumped out of the stroma and thus induce a K influx into the stroma via selective channels for charge balancing (Berkowitz and Peters, 1993).

Potassium may accumulate in vacuoles at high concentration (Hsiao and Läuchli, 1986), where it not only represents K storage but also functions as an osmoticum. As an osmoticum, it plays a decisive role in uptake of water from the soil and maintenance of turgor in the guard and motor cells (Moran *et al*., 1988), and phloem (Mengel and Haeder, 1977; Smith and Milburn, 1980).

1.3. Sodium versus potassium

The roles of K and Na in plant nutrition have sparked numerous investigations, which ultimately led to the conclusion that K is the only monovalent cation that is essential for all higher plants (Flowers *et al.*, 1977). It is possible that some elements such as Na and Si may promote maximal biomass production without meeting the requirements

for essentiality. In addition, not all metabolic functions require a unique nutrient to function.

Many essential metabolic processes can function equally well with a number of chemically and physically similar elements. It appears that it is possible for similar elements such as Na and K to replace each other fully in certain nonspecific metabolic functions. Thus, even though an element may be involved in a vital life activity (and may be even more effectual than any other essential element), it would not be considered an essential nutrient unless it has a unique function that it alone can meet. From agronomic considerations it could be argued that additional levels of essentiality should be differentiated to denote elements that may be required for maximal yield. Moreover, these should be able to replace other nutrients in certain essential metabolic functions, reducing the critical level of an essential element (Subbarao *et al.,* 2003). Despite the fact that Na is not essential for many species, application of Na to the growth medium has been shown to stimulate the growth of asparagus, barley, broccoli, caraway, carrot, cotton, millet, oat, sugar beet, red beet, and turnip (Harmer and Benne, 1945: Larson and Pierre, 1953; Lehr, 1953; Montasir *et al.,* 1966).

On the other hand, Na is toxic for most plants. There is considerable evidence that Na exclusion is the mechanism for survival of important crops to combat salt stress. This exclusion mechanism is contrary to the response of halophytes, which tend to accumulate a lot of salts as a mechanism for osmotic adjustment and nutritional supplementation when exposed to even moderate salinity (Brownell, 1979; Jefferies, 1981). There is substantial evidence that plants of moderate to high salt resistance may accumulate a large amount of Na in cell vacuoles under saline conditions. Sodium can make a significant contribution to both the osmotic relations (El-shourbagy and Ahmed, 1975; Jennings, 1976; Storey and Jones, 1979) and the mineral nutrition of those plants, especially when K is present at sub-optimal concentrations (Besford, 1978; Marschner, 1971). Moreover, Na can have beneficial effects on plant growth and it has been shown to be a functional nutrient for some species. Improvement in growth and productivity in several crops, particularly those in the family

Chenopodeaceae has been noticed (Hylton *et al.*, 1967, Marschner, 1971, Truog *et al.*, 1953). Cytosolic enzymes in halophytic plants are also not adapted to higher concentration of Na (Flowers *et al.*, 1977). Greenway and Osmond (1972) also investigated that Na cannot be accumulated in cytoplasm as it interferes with the metabolic functions of the cell. Thus, halophytic plants respond to elevated Na concentrations by maintaining low cytosolic Na concentration and high K/Na ratio. These plants compartmentalize Na into the vacuoles and use it as an osmoticum. Conversely, Na-sensitive plants are neither able to exclude Na at root surface nor to compartmentalize it in the cell vacuole. So, absorbed Na is translocated to shoot resulting in specific ion toxicity and finally in the death of plants (Greenway and Munns, 1980; Cheeseman, 1988). Recently, it has been discovered that plants need a lower amount of K for specific cytoplasmic functions. A major portion (90%) of K is localized in vacuoles where it functions as an osmoticum (Subbarao *et al.*, 2000). Maintenance of an osmotic equilibrium in vacuole and cytoplasm is a non-specific function of K and can be replaced by some other cations such as Na (Leigh and Jones, 1986; MacRobbi, 1977). Functions of Na and K are closely related and the beneficial effects of Na on plant growth were peculiarly noticed, when potassium supply was limited (Hylton *et al*. 1967, Amin and Joham, 1968). In halophytes, Na cannot only replace K completely for osmotic functions but also stimulates plant growth, which is mainly caused by an Na effect on cell expansion (Nunes *et al.*, 1983). Water balance of plants and growth responses of halophytes to Na are due to the high salt requirements for osmotic adjustment (Flower and Läuchli, 1983). Undoubtedly, Na can do better osmotic adjustments than K (Eshel, 1985). Sodium contributes not only to solute potential, turgor pressure and cell expansion, but it can also suppress K since it accumulates preferentially in vacuoles (Jeschke, 1977). Several members of Chenopodiaceae such as sugar beet, spinach, red beet *etc.* are capable of using Na as an osmoticum (Flowers, 1975; Flowers *et al*., 1977). Even in some crops Na is able to prevent or reduce considerably the occurrence of K deficiency. Thus, the question of substitution of K by Na in physiological processes of plants is not only of academic interest but is also of practical importance in relation to fertilizer application (Mengel

and Kirkby, 2001). A lot of work on this issue has already been done. Nevertheless, the practical application of these findings must be explored.

1.4. Sugar beet - a source of sucrose

Sucrose is a very important carbohydrate. It is a source of energy and sweetness. Mostly, sugar is extracted either from sugarcane (*Saccharum officinarum* L.) or sugar beet (*Beta vulgaris* L.). Sugarcane contains 12 – 20% sucrose of plant dry matter, where as sugar beet contains up to 75% of beet dry weight (Mahn and Hoffmann, 2001). Sucrose is known as table sugar. It is a disaccharide (glucose + fructose). Some other minor commercial sugar crops include date palm (*Phoenix dactylifera*), sorghum (*Sorghum vulgare*) and sugar maple (*Acer saccharum*). Countries with warm climate such as Australia, Brazil, Thailand, India, Pakistan etc. grow sugarcane as a source of sugar, while sugar beet is cultivated in regions of cool climate such as Europe, some part of USA, northern Japan etc. Globally, a total of 140 million tons sugar is produced yearly and about 25% of it is from sugar beet, while 75% is mostly produced from sugar cane (Märländer *et al.*, 2003).

Sugar beet is a biennial plant, which belongs to the family Chenopodeaceae. It is a plant with halophytic behavior and yields 8.6 t ha^{-1} white sugar from 50 – 60 t ha^{-1} of beets. Nonetheless, it has a potential to yield 15 t ha^{-1} white sugar (Märländer *et al.*, 2003). Sugar beet is salt-resistant and can grow well under moderate Na salinity (Zein *et al.*, 2002). It is not only a Na-resistant plant, but its growth is also stimulated by application of Na fertilizer. Positive effects on growth of sugar beet were observed when 95% of K was substituted by Na (Marschner *et al.*, 1981).

1.5. Ion uptake and homeostasis

Nutrients and water can move through root cells to the stele by two ways: through symplast and via apoplast (Fig. 1.3). Due to the presence of the Casparian strip, ions must enter the symplast before being uploaded into the stele. While ions enter in the symplast of cortex or epidermal cells, they are loaded into stele for translocation towards the shoot. The stele is made up of dead tracheary elements and living

parenchyma cells. Because there is no continuity of cytoplasm in dead elements, ions must exit the symplast by crossing the plasma membrane (Taiz and Zeiger, 2002).

Balanced ion concentration in the plant cell is of great importance for ideal plant growth. Homeostasis of ions is a very important feature of natrophilic plants such as sugar beet. Ionic concentration in the plant cell depends upon many factors. Calcium, K and Na compete with each other for uptake. Large reduction in Na/ K + Ca ratios in salt-stressed roots of seedlings grown with supplemental Ca may have significant effects on metabolic plant functions (Kent and Laüchli, 1985).

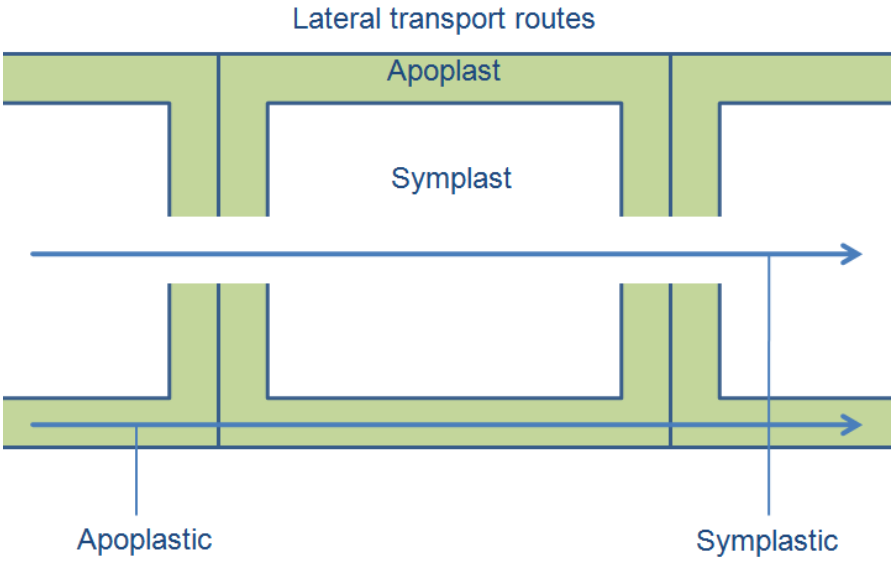


Figure 1.3. Routes of nutrient transport from nutrient medium to xylem vessel.

The ionic ratio in plants is distorted owing to Na influx through K pathways. The similarity of the monovalent Na and K ions makes the discrimination during transport difficult. Sodium enters the plant cell either through high-affinity potassium transporters HKT1 (Rus *et al.*, 2001) or non-selective cation channels NSCC (Amtmann and Sander, 1999). In some of the plants, the third route for Na intake is the transpiration stream via apoplast (Yeo *et al.*, 1987). Calcium is also taken up by the root cells through NSCC. Roberts and Tester (1997a) discovered outward-rectifying cation channels (ORCC) in maize root stelar cells. These channels may control the

transport of cations to xylem. These are highly selective for K, but Na can also move through them to lesser extent. Moreover, these channels are also permeable to Ca. These ion interactions with ORCC are involved in their transport from the root to the shoot in salt-stressed maize (Cramer *et al.*, 1994). Elzam (1971) reported that in barley Na interferes with Ca uptake. While studying the substitution of K by Na in sugar beet nutrition, calcium deficiency symptoms were observed when K was substituted by Na. In that study, Ca concentration in the beets was unaffected but in leaves Ca was significantly decreased (Abd-El-Motagally, 2004).

1.6. Objectives of the study

The intention behind this study was to evaluate the strategies to utilize the beneficial effects of Na nutrition on sugar beet and to investigate and understand the limiting factors for K substitution by Na. Highly K-fixing soils could be more suitable for this substitution because of less response of K-fertilization to plant growth. In such soils an enormous amount of K fertilizer is needed to stimulate the plant growth. Application of a huge amount is very expensive and hardly achievable.

It was hypothesized that Na is able to substitute K to a large extent in sugar beet nutrition without affecting the plant growth and white sugar yield. An attempt was made to identify the limiting processes when K is substituted by Na. Moreover, it was also theorized that in K-fixing soils it would be possible to replace a huge amount of K fertilizer with adequate amount of Na fertilizer, which may lead to the development of an interesting fertilizer strategy for sugar beet in K-fixing soils.

2 MATERIALS AND METHODS

2.1. SOIL EXPERIMENTS

2.1.1. Ahr pot experiment

A soil experiment was carried out in Ahr pots (Fig. 2.1) with 14 kg soil in each pot. Three soils i.e. Kleinlinden, Giessen and Trebur (Tab. 2.1) with different K-fixing capacities were used in the experiment and K-fixing capacities of these soils were 488, 526 and 618 mg K kg^{-1} of soil, respectively. To get soils of low exchangeable K concentration, these soils were diluted with sand at a ratio of 1:1, 1:1 and 1:10, respectively.

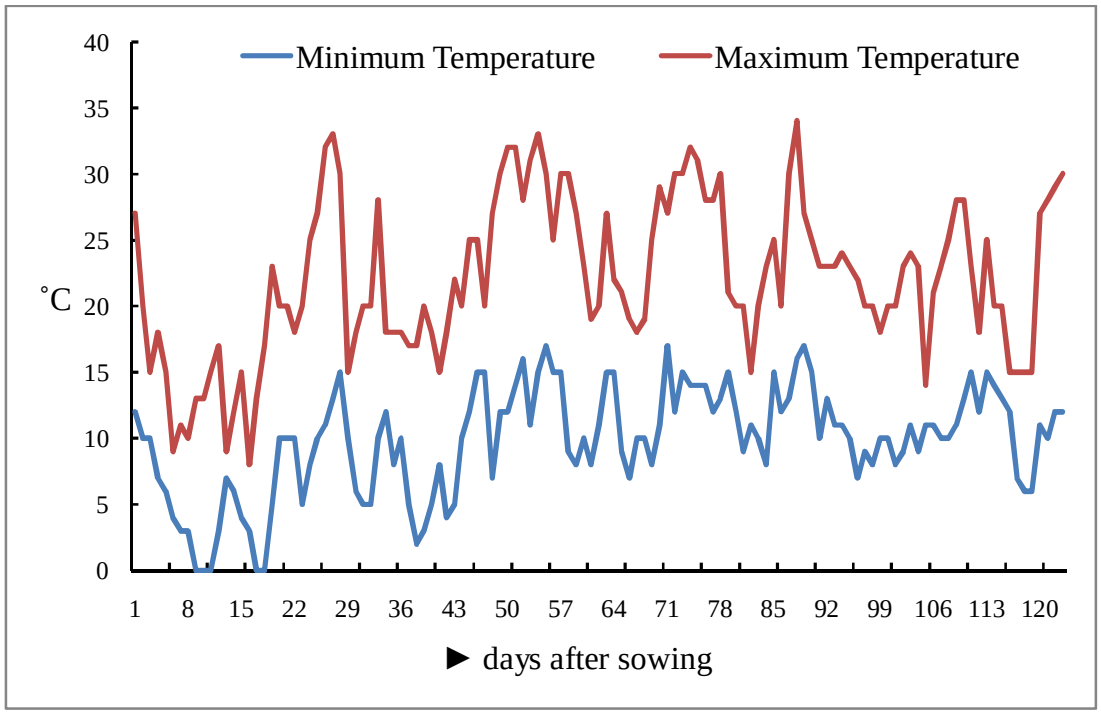
The fertilizers mixed in the soils were:

$MgCO_3$	(0.133 g kg^{-1} soil)
Superphosphate	(1.910 g kg^{-1} soil)
NH_4NO_3	(0.380 g kg^{-1} soil)
H_3BO_3	(0.003 g kg^{-1} soil)

Figure 2.1. Pot used in ahr pot experiment.

Table: 2.1: Physicochemical characteristics of the experimental soils

Soil	pH (0,01M $CaCl_2$)	Clay (g kg^{-1})	Silt (g kg^{-1})	Sand (g kg^{-1})	Exch. Mg (mg kg^{-1})	CAL-P (mg kg^{-1})	CAL-K (mg kg^{-1})	N_t (%)
Kleinlinden	5.8	207	338	455	245	9.0	49.1	0.042
Giessen	5.2	303	631	47	296	18.7	26.9	0.241
Trebur	7.4	446	436	112	229	208.9	254.0	0.432



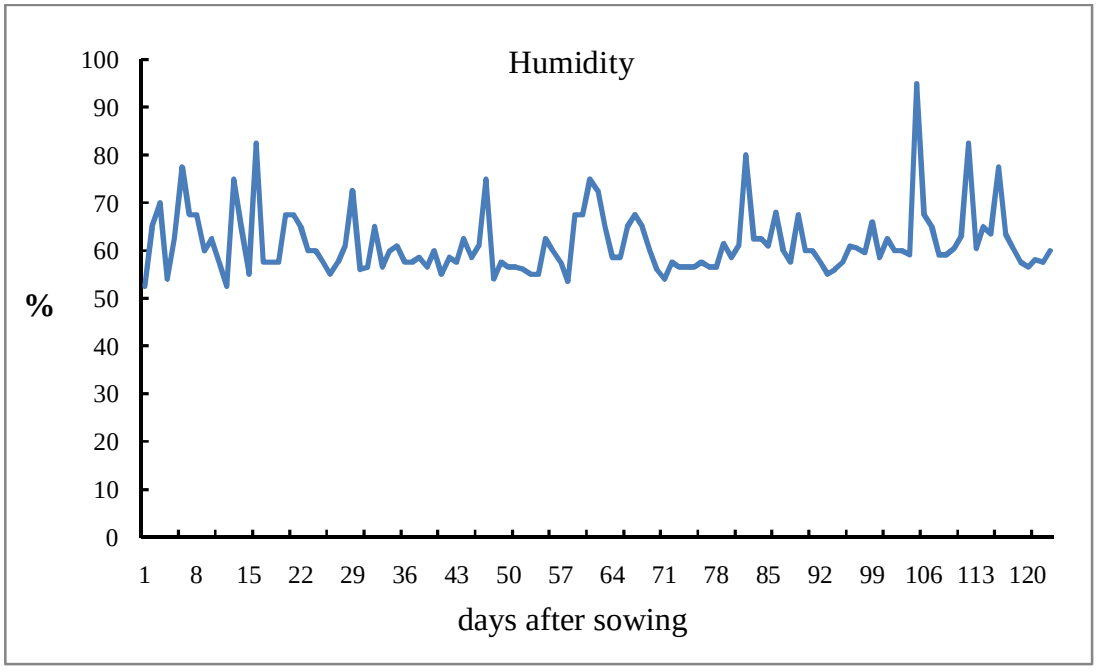


Figure 2.2. Weather data during the Ahr pot experiment conducted at experimental station, Institute of Plant Nutrition, Giessen. Experiment duration: 02-05-05 to 01-09-05

The experiment comprised of following three treatments i.e.

1. No K and Na application (control)
2. K-application equal to K-fixing capacity of soil (as KCl)
3. Na application equivalent to a regular K fertilization (350 kg K ha^{-1} as NaCl).

Sugar beet (*Beta vulgaris* L.; cv. Evita) seeds (five seeds per pot) were sown and the pots were irrigated with distilled water to 60-70% of water-holding capacity (WHC) just after sowing. One month after emergence, the seedlings were thinned to one plant per pot. The experiment was laid out in completely randomized design with four replications.

Plants were harvested at maturity four months after sowing. The plants were washed with distilled water and were separated into young leaves, old leaves and beet for fresh weight measurements. The beets were cut with a knife in 1 cm small pieces to have large surface area for drying. To record dry weight, leaves and beets were oven-dried at 80°C. The oven-dried plant material was ground to pass a 1 mm sieve for further analyses.

2.1.2. Container experiment

Based on the results of the Ahr pot experiment, Kleinlinden soil was selected to carry out a container (Fig. 2.3) experiment. Potassium-fixing capacity of the soil was 431 mg K kg^{-1} soil. The soil was mixed with quartz sand at ratio 1:2 and was filled into the containers each with a capacity of 169 kg of soil and 0.16 m^2 surface area.

Figure 2.3. Container used in container experiment.

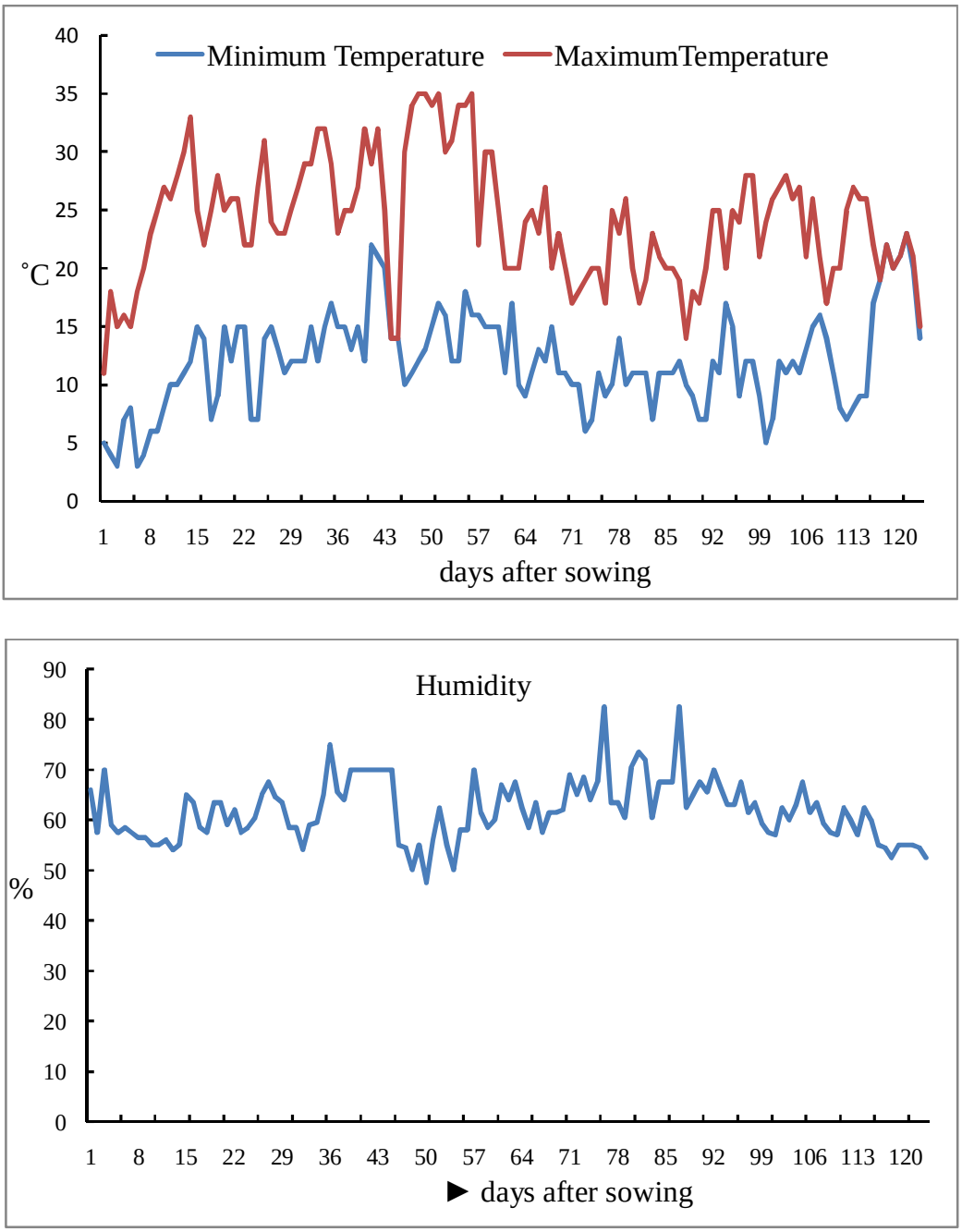


Figure 2.4. Weather data during the Container experiment conducted at experimental station, institute of plant nutrition, Giessen. Experiment duration: 02-06-06 to 02-10-06.

Fertilizers including $MgCO_3$, superphosphate, NH_4NO_3 and H_3BO_3 were mixed with the soil of upper 30 cm layer: 30 kg Mg ha^{-1}, 88 kg P ha^{-1}, 300 kg N ha^{-1} and 2 kg B ha^{-1}, respectively. Whilst, micronutrients Fe (500 g ha^{-1}), Mn (500 g ha^{-1}), Zn (200 g ha^{-1}) and Cu (100 g ha^{-1}) were applied in the form of Fe-EDTA, $MnSO_4$, $ZnSO_4$ and $CuSO_4$, respectively, with irrigation. All the fertilizers applied were calculated on a surface area basis.

Treatments for the container experiment were:

1. No application of Na and K (Control)
2. Potassium according to the regular K fertilization (415 kg K ha^{-1} as KCL).
3. Sodium equivalent to the regular K fertilization (350 kg Na ha^{-1} as NaCl).

One container was taken as experimental unit. The experiment was laid out in a completely randomized design with four replications. Sugar beet (cv. Evita) seeds (five seeds per container) were sown and one month after sowing, the seedlings were thinned to one plant per container. Moisture contents in each container were maintained at 60% of maximum WHC by applying water when required. At maturity, plants were harvested, beet was separated from shoot and leaves were divided into young and old leaves. Shoot and beet fresh mass were recorded and then dry weight was taken after drying at 80°C in a forced-air oven. Samples were ground to pass 1 mm sieve for further analyses.

2.2.3. Field experiment

A field experiment was conducted to affirm the results of pot and container experiments. A field relatively deficient in plant-available potassium with high K-fixing capacity was selected at Trebur, Germany. It was Trebur soil with physico-chemical properties as given in Table 2.2.

Table: 2.2: Physicochemical characteristics of the experimental soil

Soil	pH (0.01M $CaCl_2$)	Clay (g kg^{-1})	Silt (g kg^{-1})	Sand (g kg^{-1})	CAL-K (mg kg^{-1})	CAL-P (mg kg^{-1})	NH_4-acetate K (mg kg^{-1})	Nt (mg kg^{-1})
Trebur	7.4	446	436	112	65.9	58.3	163.0	23.95

Potassium-fixing capacity of the soil was 779 mg K kg^{-1} soil. In this experiment, there were four treatments i.e.

1. No application of Na and K (Control)
2. Potassium application according to the regular K fertilization (400 kg K ha^{-1} as KCl).
3. Sodium equivalent to the regular K fertilization (236 kg Na ha^{-1} as NaCl).
4. Potassium according to half K-fixing capacity of soil (1575 kg K ha^{-1}).

Sugar beet (cv. Felicita) was sown in the field with a plot size of 5 m x 2 m. Seeds were sown with 7-8 kg ha^{-1} and plants were thinned one month after sowing to maintain 50 plants $plot^{-1}$. Row to row distance was 50 cm and average plant to plant distance was 25 cm. Fertilizer treatments were applied in solution form, about one month after sowing. Required amount of fertilizer for each plot was dissolved in 25 L of distilled water and was applied to the soil on the both sides of plant rows. In control treatment, distilled water without any fertilizer was applied in the same way. The experiment was arranged in randomized complete block design with four replications. Plants were harvested at maturity. The leaf and beet fresh weights for each plot were recorded. To estimate the white sugar yield, three medium-size beets from each plot were selected. Sampled beets were oven-dried at 80°C. The fine-ground beet material was analyzed separately for sucrose, cations and α-amino N.

Beet dry weight for each plot was calculated by the formula given below:

Formula:

$$\mathbf{BDW_t = BDW_b / BFW_b \times BFW_t}$$

Where

BDW_t = total beet dry weight per plot

BDW_b = beet dry weight per beet

BFW_b = beet fresh weight per beet

BFW_t = total beet fresh weight per plot

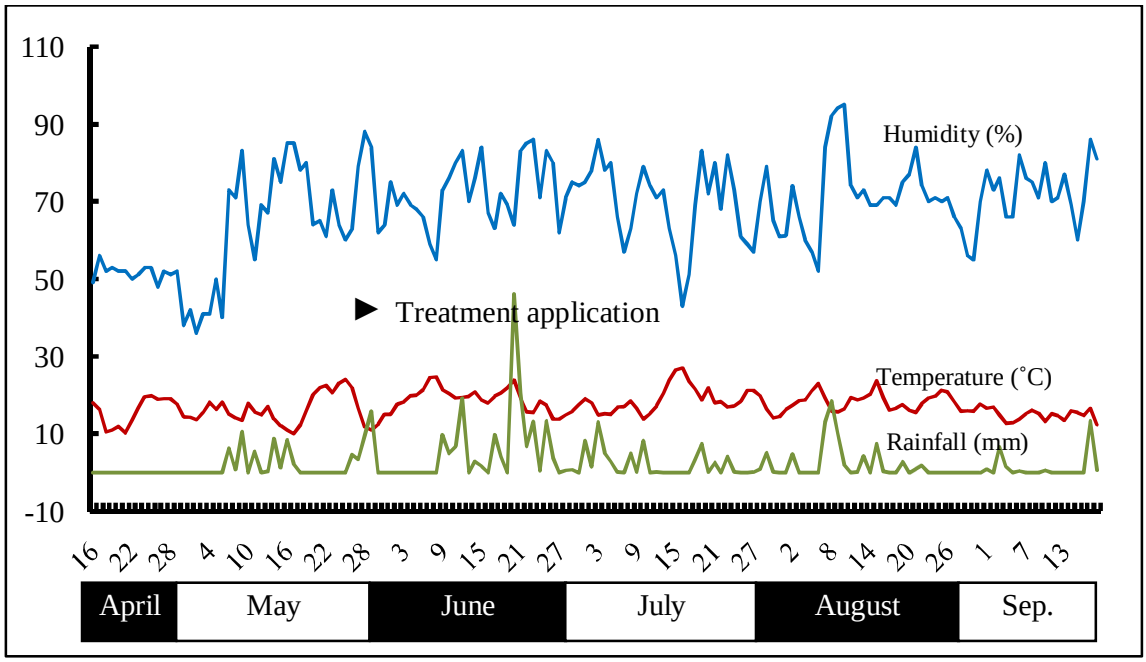


Figure 2.5. Weather report of field experiment conducted at Trebur, Germany. Experimental time period: 16-04-07 to 18-09-07

2.2. NUTRIENT SOLUTION EXPERIMENTS

2.2.1. Plant cultivation

Sugar beet seeds (cv. Evita) were sown in sand at room temperature. The seedlings were irrigated with 10 mM $CaSO_4$ and were allowed to grow for 1 w (up to 3 cm shoot length) in a growth chamber. These seedlings were transferred to $1/4^{th}$ strength nutrient solution in a 50 L plastic container and after 3 d, the concentration was increased to 1/2 strength. After another 3 d, the plants were transferred to the pots having full strength nutrient solution. The full concentration of the nutrients in the solution was:

N (5.3 mM)	as	NH_4NO_3
K (4.0 mM)	as	KCl
P (0.3 mM)	as	$NH_4H_2PO_4$
Mg (0.5 mM)	as	$MgSO_4$
Ca (2.0 mM)	as	Ca $(NO_3)_2$
Mn (0.5 µM)	as	$MnSO_4$
Zn (0.1 µM)	as	$ZnSO_4$
Cu (0.2 µM)	as	$CuSO_4$
B (10.0 µM)	as	H_3BO_3
Mo (0.01 µM)	as	$(NH_4)_6Mo_7O_{24}$ and
Fe (10.0 µM)	as	Fe-EDTA

The experiments were conducted in a growth chamber {photoperiod of 16/8 h light/ dark with photon flux density (PFD) of 40 W m^{-2}; temperature 22/ 20°C light/ dark, 70% relative humidity and 1300 µE m^{-2} s^{-1} photosynthetic active radiation}. The experiment was laid out in completely randomized design with five replications. Nutrient solution was changed after every 3^{rd} day. Once during the growth period, before the change of nutrient solution, the sugar beet roots were dipped into 5 L of 0.3% (w/ v) Benomyl solution for 5 min to avoid infection of fusarium wilt.

2.2.2. Experimental procedures

Two experiments were conducted and plants were grown in plastic pots (4.5 L nutrient solution in each) in both experiments. After 3 w of growth in full-strength nutrient solution, two treatments were established. The first treatment was control with 4 mM of K. In the second treatment, K was substituted with 4 mM of Na. Plants were harvested 3 and 7 d after treatment application in first and second experiment, respectively. Roots were cut from harvested plants and the young leaves were separated from the old ones. Young leaves were defined as 2 to 3 leaves of the plant at the time of harvesting to have approximately the same fresh matter {i.e. 6.0 - 8.0 g (4 plants)$^{-1}$} for each replication. Harvested plant material was weighed and oven-dried at 80°C.

Xylem sap collection

At harvest, xylem sap was collected by de-capping the plants from the hypocotyls with a sharp knife. After 5 min, the xylem sap from the top surface was cleaned with tissue paper and then the xylem sap was collected from the top surface for specific time periods in both experiments with the help of micro-pipette. The collected sap was analyzed immediately or was stored at 4°C. The volume of the collected sap was measured and sap samples were centrifuged to prepare for further analysis. The sap was diluted 20 times with double de-ionized water and the ionic concentrations (K, Na, Ca and Mg) were measured by means of atomic absorption spectrophotometry (SpectrAA 220FS, Varian). Boron (B) was analyzed with a miniaturized curcumin method (Wimmer and Goldbach, 1999).

2.3. CHEMICAL ANALYSES

2.3.1. Inorganic cation concentrations in plants

Ground plant material was used for analyses of K, Na, Ca and Mg ion concentrations. The plant material was ashed at 550°C over night in a forced-air oven. The ash was dissolved in 5 M HNO_3 and was heated prior to boiling. After filtering in 50 mL volumetric flasks, the volume was made up to mark with double de-ionized water. The

ionic concentrations (K, Na, Ca and Mg) were measured by using Atomic Absorption Spectrophotometer (SpectrAA 220FS, Varian).

2.3.2. Boron

2.3.2.1. Analyses of plant samples

Preparation of reagents

a. Buffer solution

Buffer solution was prepared by dissolving 250 g ammonium acetate and 15 g Na-EDTA in 400 mL de-ionized water and then 125 mL glacial acetic acid were added slowly. The solution was filtered and pH was adjusted to 5.1 with H_2SO_4.

b. Color reagent

Color reagent was prepared by dissolving 0.45 g azomethine-H in 100 mL 1% ascorbic acid. The reagent was filtered.

c. Standards

Stock standard solution was prepared by dissolving 0.114 g boric acid in 1 L de-ionized water. Its concentration was 20 mg L^{-1} and the required standards ranging from 0.5 to 3.0 mg L^{-1} were prepared by dilution using the equation:

$$\mathbf{C_1V_1 = C_2V_2}$$

Where,

C_1 = concentration of stock solution

V_1 = volume to be taken from stock solution

C_2 = required concentration and

V_2 = volume to be made

Finely ground oven-dried 225 mg plant material was given into a porcelain crucible and ashed at 550°C overnight. The cooled ash was carefully moistured with 2 mL of double-deionized water and 2.5 mL 5 M HNO_3 were added to the crucible. The

samples were heated prior to boiling followed by cooling and filtrating thorough white band 589 filter paper to 25 mL volumetric flasks and volume was made up to mark.

One mL sample aliquot was given into a 10 mL tube (polypropylene tube), 2 mL buffer solution and 2 mL color reagent were added to the sample in tube and mixed well. Absorption was measured after 30 min using a spectrophotometer (Spektralphototmeter PM7) at 420 nm wavelength. A standard curve was prepared by measuring the absorption for each standard (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg L^{-1}) proceeding in the same way as for samples. A blank was also measured by adding 1 mL de-ionized water (Ryan *et al.* 2001).

2.3.2.2. Analysis of xylem sap

Measurements were carried out with a Lambda 20 UV/ VIS spectrophotometer Perkin Elmer). A quartz microcuvette with a filling volume of 120 µL (Hellma) was used throughout. In order to avoid contamination from glass, plastic laboratory ware was used where possible. Extraction as well as the color-developing reaction were performed in PE Eppendorf microtubes (1.5 mL).

a. Reagents

Unless otherwise stated all reagents were of p.a. grade (Fluka). Demineralized water purified with the ultra-pure water system Milli-Q plus 185 (Millipore GmbH, Eschborn) was treated with boron-specific exchange resin Amberlite IRA 743 (Sigma). For all dilutions, polymethylpentene (Nalgene) measuring flasks were used. Boron stock solution: 0.5716 g H_3BO_3 was dissolved in 1 L of IRA 743 treated millipore water. Standards were prepared by serial dilution of this 100 mg B L^{-1} stock solution and used for no longer than two months after preparation.

Acid: 0.1 N HCl (dilution from Titrisol 1 N, stored in a plastic bottle)
Acid mixture: sulphuric acid (conc.) and acetic acid (conc.) 1:1 (v/v)
Extraction solution: 2-ethyl-1,3-hexanediol 10% (v/v) in chloroform

Curcumin solution: 2.0 g curcumin were dissolved in 1 L of methylisobutylketone (MIBK). The solution was filtered through Blue Ribbon Filter Paper (Schleicher & Schuell 589^3) and used for up to 1 w after preparation.

b. Procedure

A sample volume of 50 µL was acidified with 100 µL of 0.1N HCl to make sure that all boron was present in the non-charged H_3BO_3 form. The sample was then extracted for 1min with 50 µL of the extraction solution. After complete separation of the two phases, 20 µL were pipetted from the lower, apolar phase, transferred to a second micro tube and acidified with 200 µL of acid mixture. The color-forming reaction was started by the addition of 250 µL of curcumin solution. After 1 h, the reaction was stopped with 500 µL of water in order to deprotonize the curcumin surplus. 120 µL of the upper, organic phase were used for the photometric determination after complete separation of the two phases. All steps of the procedure were carried out at room temperature. Extinction of the boron-rosocyanine complex was measured at a wavelength of λ = 550 nm.

Samples of low B content were concentrated using an initial sample volume of 100 mL (two fold concentration) or 150 mL (three fold concentration) and 50 mL of extraction solution. All other additions remained the same as in the normal procedure.

2.3.3. α-amino N concentration

Analyses of α-amino-N concentration were carried out after extracting 200 mg of ground dry material of beet with 20 mL phosphate buffer in 100 mL poly flask with an end-over-end shaker for 1 h and were filtered from Faltenfilter 595$^{1/2}$ (Schleicher and Schüll Co., Dassel, Germany). After filtration, 0.4 mL of extract was mixed with 4 mL of citrate buffer and 4 mL ninhydrin solution and was boiled in a flask for 15 min in a water bath at 100°C. After cooling the flask, the absorption was measured at 570 nm with a spectrophotometer. Glutamine standard was prepared in the same way α-amino-N concentration was then calculated from the standard curve. Samples were analyzed in duplicates for each replicate to get maximum accuracy.

2.3.4. Sugar concentration

300 mg ground plant materials were weighed into a 50 mL volumetric flask and 30 mL of double-deionized water were added. The material was then extracted by incubation in a shaking water bath at 60°C for 30 min. The flask was quickly cooled on ice, and filled up to the mark with double-deionized water followed by filtration with (blue-band) filter paper (Faltenfilter $595^{1/2}$, Scheicher and Schüll Co., Dassel, Germany). Sugars (sucrose, glucose and fructose) were determined using enzymatic tests.

Principle of the determination of sucrose, D-glucose and D-fructose using Enzymatic BioAnalysis

The D-glucose concentration was determined before and after the enzymatic hydrolysis of sucrose; D-fructose was determined subsequently to the determination of D-glucose:

a. Determination of D-glucose before inversion

At pH 7.6, the enzyme hexokinase (HK) catalyzes the phosphorylation of D-glucose by adenosine-5´-triphosphate (ATP) with the simultaneous formation of adenosine-5´-diphosphate (ADP).

- D-Glucose + ATP $\xrightarrow{HK}$ G-6-P + ADP

In the presence of glucose-6-phosphate dehydrogenase (G6P-DH), the D-glucose-6-phosphate (G-6-P) formed is specifically oxidized by nicotinamide-adenine dinucleotide phosphate (NADP) to D-gluconate-6-phosphate with the formation of reduced nicotinamide-adenine dinucleotide phosphate (NADPH).

- G-6-P + $NADP^+$ $\xrightarrow{G6P\text{-}DH}$ D-gluconate-6-phosphate + NADPH + H^+

The NADPH formed in this reaction is equivalent to the amount of D-glucose and is measured by means of its light absorbance at 365 nm.

b. Determination of D-fructose

Hexokinase also catalyzes the phosphorylation of D-fructose to D-fructose-6-phosphate (F-6-P) with the aid of ATP.

- D-Fructose + ATP $\xrightarrow{HK}$ F-6-P + ADP

On the completion of the above reaction F-6-P is converted by phosphoglucose isomerase (PGI) to G-6-P.

- F-6-P $\xrightarrow{PGI}$ G-6-P

G-6-P reacts again with $NADP^+$ with the formation of D-gluconate-6-phosphate and NADPH. The amount of NADPH formed now is stoichiometric to the amount of D-fructose.

- G-6-P + $NADP^+$ $\xrightarrow{G6P\text{-}DH}$ D-gluconate-6-phosphate + NADPH + H^+

c. Enzymatic inversion

At pH 4.6, sucrose is hydrolyzed by the enzyme β-fructosidase (invertase) to D-glucose and D-fructose.

- Sucrose + H_2O $\xrightarrow{\beta\text{-fructosidase}}$ D-glucose + D-fructose

The determination of D-glucose after inversion (total D-glucose) was carried out according to the principle outlined above. The sucrose concentration was calculated from the difference of the D-glucose concentration before and after enzymatic inversion.

The white sugar concentration of the beet was calculated with the New Brunswick Formula (Buchholz *et. al.*, 1995) from the sucrose concentration in the beet.

New Brunswick Formula

WSY= RY x WSC/ 100

Where

WSY = White sugar yield (g)

RY = Beet yield (g)

WSC = White sugar concentration (%)

WSC = Sc. – SFL – SML

Where

Sc. = Sucrose concentration (%)

SFL = Standard factory loss (0.6 %)

SML = Standard molasses loss (%)

$SML = 0.12\ w`_{(K + Na)} + 0.24\ w`_{(\alpha - N)} + 0.48$

Where

W` = concentration in mmol / 100 g beet fresh weight

2.3.5. Soil Analyses

2.3.5.1. Analysis for inorganic cation (K, Na, Ca and Mg).

Ten g finely ground soil with 50 mL 1 M NH_4-acetate were shaken for 1 h on a mechanical shaker. After filtration from white-band 589 filter paper (Schleicher and Schuell Co., Dassel, Germany), cation concentration was determined by means of Atomic Absorption Spectrophotometry (SpectrAA 220FS, Varian).

2.3.5.2. Determination of K-fixing capacity of soil

Ten g fine ground soil were shaken for 1 h on a mechanical shaker with 50 mL 0.005 M KCl in Erlenmyer flask. The sample was oven-dried at 100°C and 50 mL 1 M NH_4-acetate solution were added followed by 1 h shaking on a mechanical shaker. After filtration through white-band 589 filter paper (Schleicher and Schuell Co., Dassel, Germany) the samples were analyzed for K concentration using Atomic Absorption Spectrophotometery (SpectrAA 220FS, Varian).

K fixing capacity was calculated by using following formula:

$K_{fix} = (9800 + K_a - K_r)/10 = \mu g/g = mg\ kg^{-1}$

where

9800 = µg of K in 50 mL of 0.005 M KCl solution

K_a = Exchangeable K

K_r = K concentration in soil filtrate after fixation on soil particles

2.3.5.3. Total cation concentration in the soil

a. Reagents

1. Hydrofluoric acid (HF) 48%

2. Perchloric acid ($HClO_4$), 70 to 72%
3. Hydrochloric acid, 6 N
4. Nitric acid, 70%

Table 2.3. Analysis of the soils used for clay concentration, total cation concentration and K-fixing capacity.

Soils	Clay (g kg^{-1} soil)	Ca_t (g kg^{-1} soil)	Mg_t (g kg^{-1} soil)	Na_t (g kg^{-1} soil)	K_t (g kg^{-1} soil)	K-fixing capacity (mg K kg^{-1} soil)
Kleinlinden	207	3.0	4.2	4.5	14.5	488
Giessen	303	3.4	4.0	4.1	14.9	526
Trebur	446	18.1	11.7	3.9	16.5	618

Samples of finely ground soil (100 mg) were placed in a 30 mL platinum crucible. After addition of few drops of water 5 mL of HF and 0.5 mL of $HClO_4$ were added. Soil acid mixture was heated on a hot plate until fumes of $HClO_4$ appeared. Crucibles were cooled, and then 5 mL of HF was added. The crucibles were placed in a sand bath covered about 9/10 of the crucible top with a Pt lid. Crucibles were heated to 200 to 225 °C and the contents were evaporated to dryness. After cooling the crucibles, 2 mL of water were added followed by few drops of $HCLO_4$. Crucibles were placed again in the sand bath and the contents were evaporated to dryness. The crucibles were removed and 5 mL 6 N HCl and 5 mL of double deionized water were added after allowing it to cool. The crucibles were then heated over a burner until the solution started boiling gently. When the residues were completely dissolved in HCl, the material was filtered into a 100 mL volumetric flask and cation concentration was measured in the filtrate by Atomic Absorption Spectrophotometery (SpectrAA 220FS, Varian) (Jackson, 1958).

2.4. DETERMINATION OF INTERLAYER POTASSIUM

Interlayer potassium was determined by means of electro-ultra-filtration (EUF) technique from the soils used for the various experiments. The principle of EUF technique is shown in Fig. 2.6 (Schubert *et al.,* 1989). The soil suspension is exposed to an electric field and K ions migrate to the cathode, where they are collected. Soil samples were extracted for 60 min at 20˚C and 200 V followed by extraction for 60 min at 80˚C and 400 V. Extraction was carried out at 10 min intervals so that 12 subsamples were obtained. Collected extracts were filled up to mark in 200 mL volumetric flasks and were analyzed for K-ions by Atomic Absorption Spectrophotometry (SpectrAA 220FS, Varian). The dynamics of available K was considered for the six samples obtained in the first 60 min at 200 V and the dynamics of non-exchangeable K release was obtained by considering the data obtained in following 60 min at 400 V. Extracted K was plotted against time to characterize the desorption dynamics.

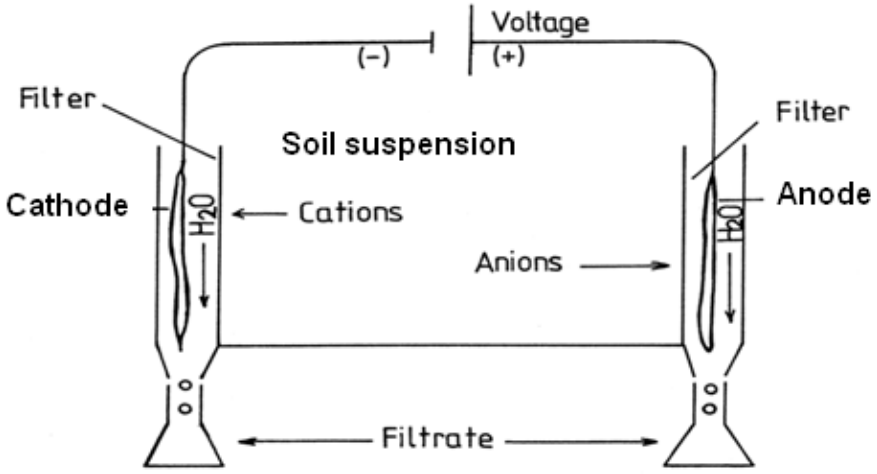


Figure 2.6. Scheme of the electrofiltration (EUF) apparatus. Migration of ions to electrodes results in dilution of ions in the solution and thus promotes the desorption of adsorbed ions (Mengel and Uhlenbecker, 1993).

2.4.1. Comparison of K release for soils used for soil culture experiments

Three different types of soils were used for various soil culture experiments. The soils different in physico-chemical properties.

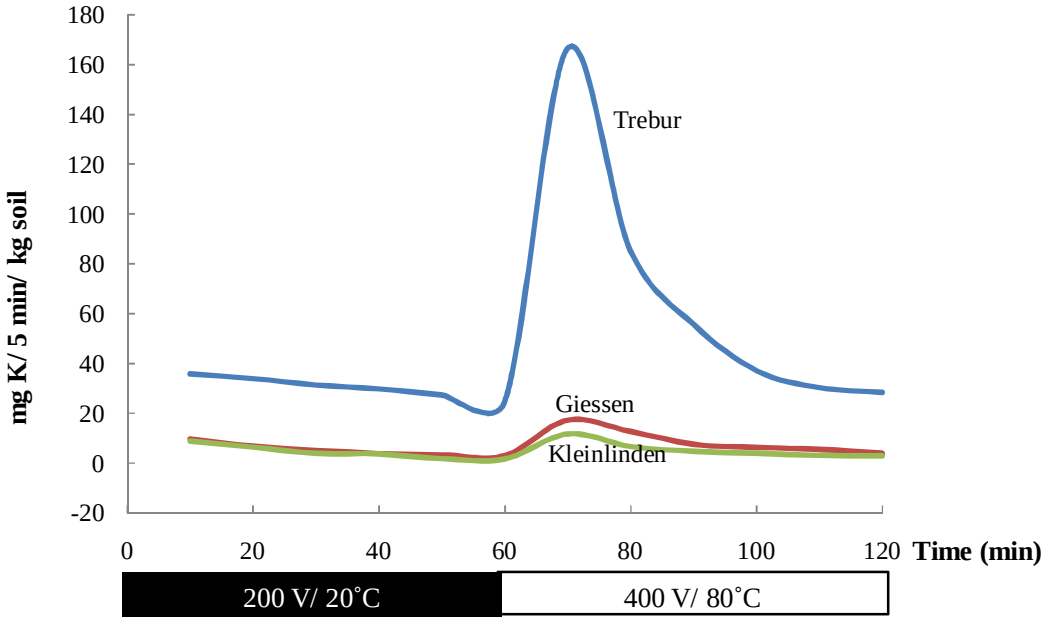


Figure 2.7. Potassium release from the soils (Kleinlinden, Giessen and Trebur) used for soil culture experiments. Potassium was extracted from the soils by electro-ultra-filtration (EUF) technique and K concentration was measured with an atomic absorption spectrophotometer.

Thus it was important to charecterise the K release from the clay minerals of the soils. Figure 2.7 shows that K release was almost similar in soils Kleinlinden and Giessen. However, potassium dynamics were much different in the soil Trebur. Details of the soil characteristics are given in Table 2.4.

In the first 60 min K was extracted from the soil at 200 V and this amount is known to be easily available for plants. If we compare this value obtained in soil Trebur with the other two soils, the easily available K in soil Trebur is >30 mg K kg^{-1} soil which is quite higher than in soils Giessen and Kleinlinden. Similarly, when the soils were exposed to 400 V from 60 - 120 min and K release was measured from the filtrate. This was again almost the same for soil Giessen and Kleinlinden, but the difference with soil Trebur was much higher (about 135 mg K kg^{-1} soil in the start and then about 25 mg K kg^{-1} soil) and afterwards this release was almost constant at the concentration of 40 mg K kg^{-1} soil). Potassium extracted at 400 V is considered as slowly available K.

Table 2.4. Physicochemical properties of soils used for determination of K-dynamics.

Soils	pH (0.01M $CaCl_2$)	Clay (g kg^{-1})	Silt (g kg^{-1})	Sand (g kg^{-1})	Soil textural class (USDA)	Dominant clay minerals
Kleinlinden	5.8	207	338	455	loam	Illite
Giessen	5.2	303	631	47	silty clay loam	Vermiculite & smectite
Trebur	7.4	446	436	112	silty clay	Smectite

2.5. STATISTICAL ANALYSIS

The minimum numbers of replications for each treatment in every experiment were four. The data obtained were analyzed by *t*-test with statistical software SPSS at $p \leq 5\%$ (LSD 5%). Standard errors shown in graphic bars were calculated using Microsoft Excel.

3 RESULTS

3.1. Soil culture experiments

3.1.1. Ahr pot experiment

In this preliminary experiment, substitution of K by Na in sugar beet nutrition was tested in three soils (i.e. Kleinlinden, Giessen and Trebur) differing in their K-fixing capacities. Plants were harvested at maturity and the following parameters were studied:

- Plant growth and beet yield
- Cation concentration in leaf and beet
- Beet quality parameters
- White sugar yield (WSY)

3.1.1.1. Plant growth and beet yield

Application of K fertilizer improved sugar beet growth. There was a clear increase in shoot fresh weight and beet fresh weight. However, improvement in beet growth was higher in soil Kleinlinden by K fertilization. Plant growth was affected by replacing K with Na in sugar beet nutrition. Leaf fresh weight was significantly reduced at soils Kleinlinden and Giessen, whilst there was no significant effect of K substitution by Na at soil Trebur. Nonetheless, Na application stimulated the plant growth and shoot fresh weight in soils Giessen and Kleinlinden compared with the control (Fig. 3.1). There was no effect of K substitution by Na on beet fresh weight at soils Giessen and Trebur, whereas at soil Kleinlinden it was significantly reduced relative to the K treatment. Nevertheless, beet growth was significantly higher in the Na treatment than in the control. However, leaf dry weight remained unaffected, whilst beet dry weight showed a similar response as for beet fresh weight (Fig. 3.2). The highest beet dry weight (200 g $beet^{-1}$) was obtained at soil Kleinlinden when K was applied according to the K-fixing capacity of the soil and the lowest beet dry weight (~100 g $beet^{-1}$) was observed for soils Kleinlinden and Giessen when neither K nor Na was applied.

In soils Kleinlinden and Giessen severe K deficiency symptoms (especially at older leaves) were observed when plants were not supplied with K and Na fertilizer

(control). Surprisingly the plants, fertilized with Na, did not show the K deficiency symptoms (Fig. 3.3).

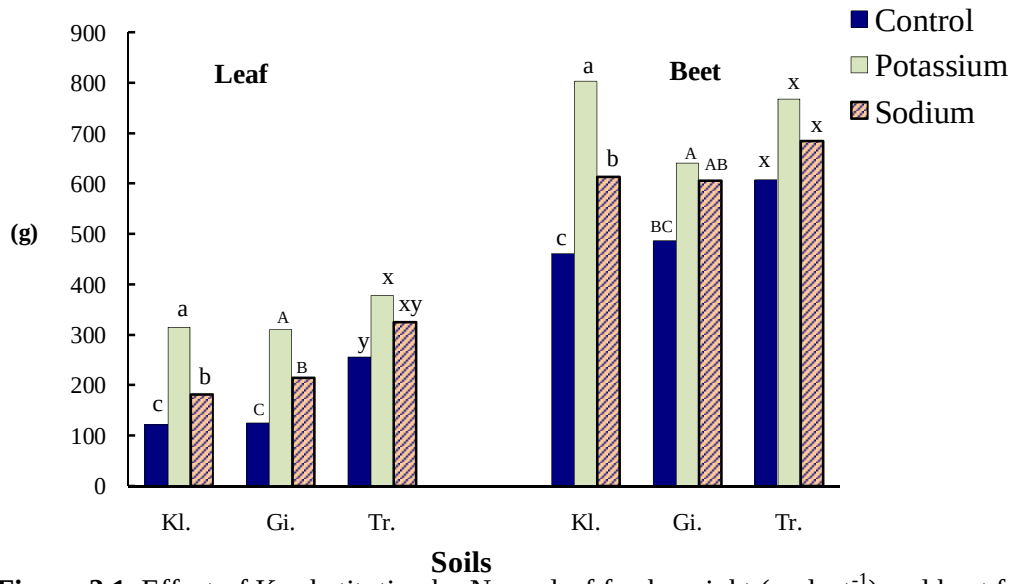


Figure 3.1. Effect of K substitution by Na on leaf fresh weight (g plant^{-1}) and beet fresh weight (g plant^{-1}) of sugar beet grown in three soils (Kl. = Kleinlinden, Gi. = Giessen and Tr. = Trebur) harvested at maturity. Means followed by the same letters at the same soil are not significantly different according to LSD test at 5% level of probability.

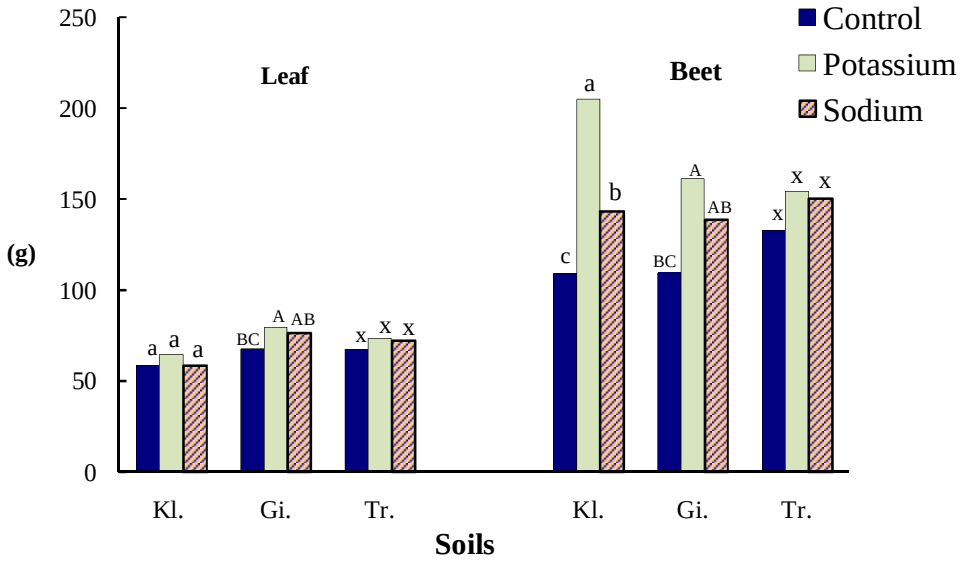


Figure 3.2. Effect of K substitution by Na on leaf dry weight (g plant^{-1}) and beet dry weight (g plant^{-1}) of sugar beet in three different soils (Kl. = Kleinlinden, Gi. = Giessen and Tr. = Trebur) harvested at maturity. Means followed by the same letters at the same soil are not significantly different according to LSD test at 5% level of probability.

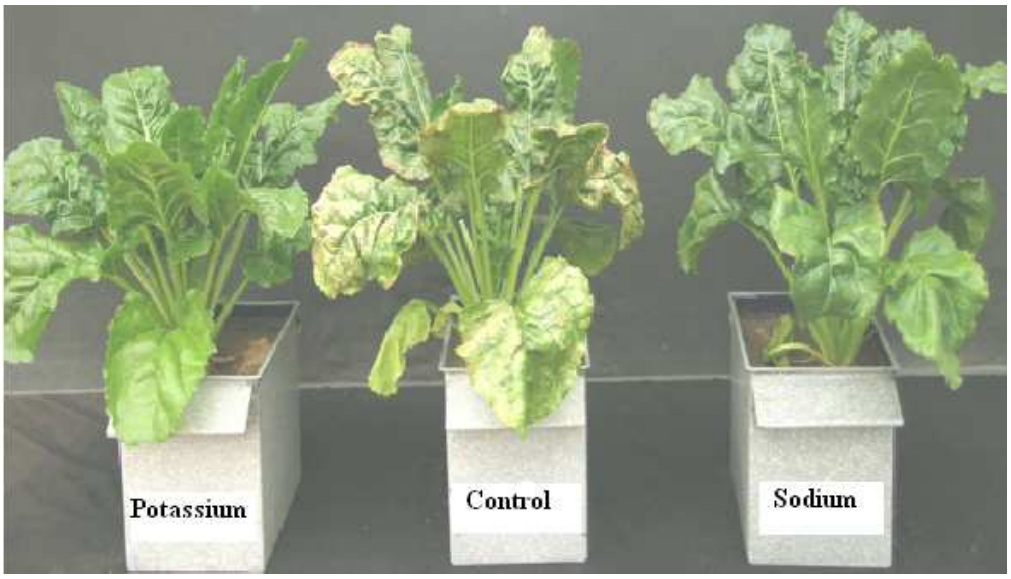


Figure 3.3. Photo was taken two months after sowing. It shows potassium deficiency symptoms (control) which appeared on sugar beet plants when K fertilizer was not applied and when K was substituted by Na.

3.1.1.2. Cation concentration in leaf and beet

Sodium and K treatments influenced the concentrations of cations both in leaf and beet (Tab. 3.1). Substitution of K by Na not only disturbed the concentrations of K and Na in leaf and beet but also affected the concentrations of Ca and Mg in the plant tissue. Sodium concentration was increased in leaf and beet by the application of Na fertilizer; however data showed that Na was accumulated more in leaf than in beet (Tab. 3.1 and Fig. 3.4). In beet, the concentration of Na was < 1 mg Na g^{-1} in contrast to a concentration of about 6 mg Na g^{-1} in leaf. Moreover, Na also showed an antagonistic effect on the concentration of Ca especially in young leaves of plants grown in soil Kleinlinden. The Ca concentration was higher in K-treated plants grown in soil Kleinlinden compared with control. It seems that K had a synergistic effect on Ca concentration. Magnesium concentration was not affected due to substitution of K by Na. Similar to concentration results, Na and K uptake were also increased in Na and K treatment, respectively. The Ca and Mg uptake was increased following the increase in dry matter yield by application of K or Na.

Table 3.1. Cation concentrations in young and old leaves and total cation uptake by leaves of sugar beet grown in Ahr pots with 15 kg soil pot^{-1}.

Treatment	Soils	Cations in old leaves (mg per g dry weight)				Cations in young leaves (mg per g dry weight)				Cations uptake per plant (mg)			
		Na	K	Ca	Mg	Na	K	Ca	Mg	Na	K	Ca	Mg
Control	Kl.	1.88 b	4.61 b	12.91 b	10.43 b	3.61 a	16.05 b	6.22 ab	5.26 a	175 b	804 b	1354 b	1215 a
Potassium	Kl.	1.78 b	26.48 a	19.51 a	14.48 a	1.33 b	27.05 a	8.43 a	5.99 a	174.5 b	3118 a	1519 a	1244 a
Sodium	Kl.	5.31a	4.5 b	15.87 b	11.92 b	5.96 a	11.5 b	4.7 b	4.23 a	419.7 a	913 b	1458 a	1163 a
Control	Gi.	2.72 b	4.27 b	19.36 a	13.7 a	5.42 a	7.41 b	8.25 a	7.23 a	279 b	858 b	1988 b	1377 b
Potassium	Gi.	1.88 c	14.06 a	20.62 a	13.82 a	1.72 b	24.02 a	5.84 a	4.46 a	223 b	2309 a	2254 a	1690 a
Sodium	Gi.	4.8 a	2.42 b	16.32 a	12.05 a	3.37 a	8.88 b	6.1 a	6.08 a	500 a	741 b	1982 a	1750 a
Control	Tr.	2.27 b	13.01 a	19 ab	7.25 b	2.33 ab	25.25 a	6.37 a	2.59 a	250 b	1430 b	1918 a	889 a
Potassium	Tr.	2.37 b	12.83 a	19.9 a	9.02 a	1.79 b	27.89 a	4.45 b	2.15 ab	255 b	1910 a	2011 b	918 a
Sodium	Tr.	4.62 a	10.47 a	15.9 b	6 c	3.79 a	23.04 a	4.24 b	1.88 b	416 a	1397 b	1848 c	836 a

Values with the same letters in a column do not differ significantly according to SPSS at 5 % significant level.

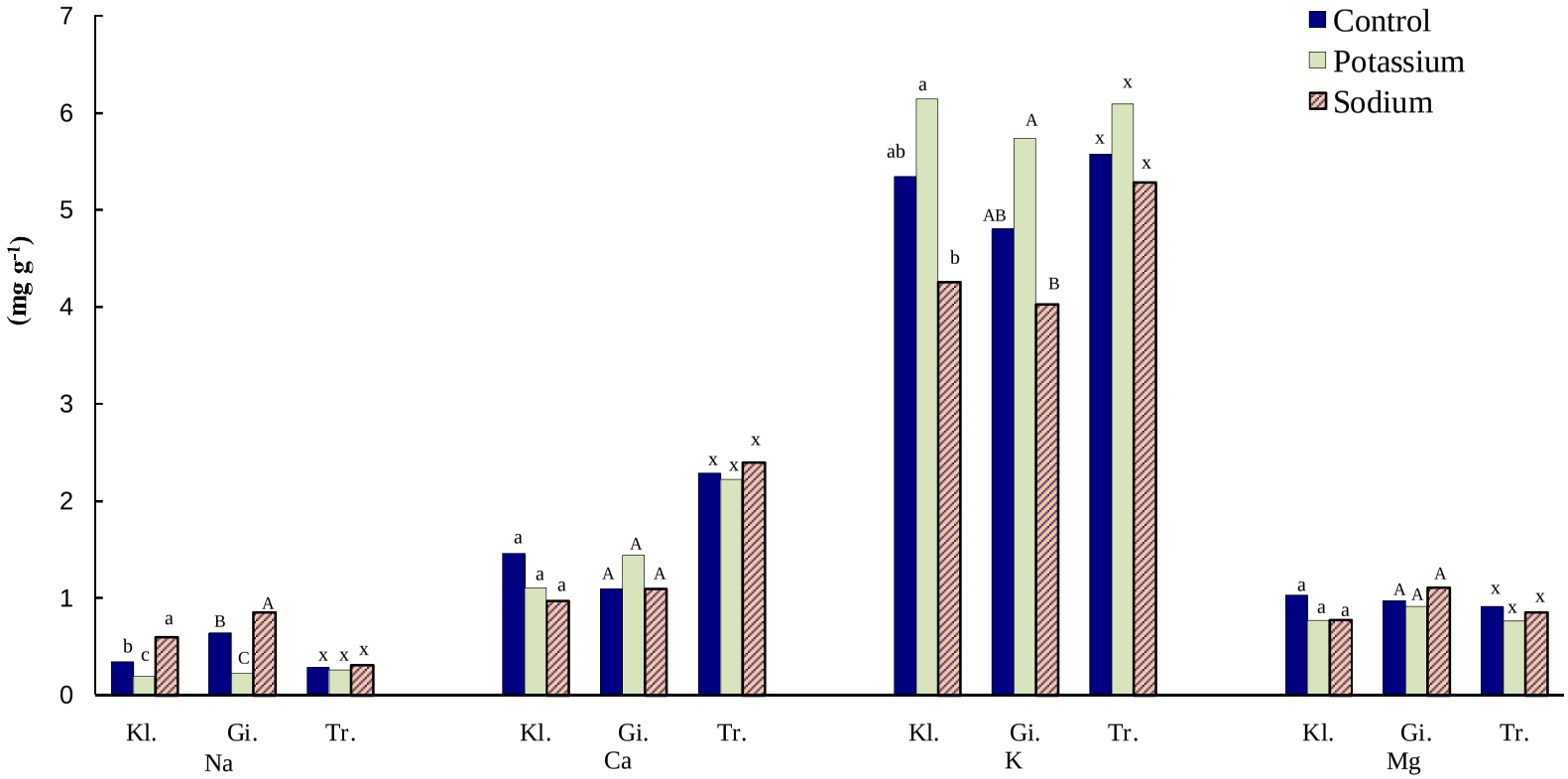


Figure 3.4. Effect of K substitution by Na on the cation concentrations in beet dry weights of sugar beet grown in three different soils (Kl. = Kleinlinden, Gi. = Giessen and Tr. = Trebur) and harvested at maturity. Means followed by the same letters in the same soil are not significantly different according to LSD test at 5% level of probability.

3.1.1.3. Beet quality parameters

Beet quality is an important factor for farmer's income in Germany. Sugar beet quality is determined by three parameters:

a) Sucrose concentration
b) Na + K concentration and
c) α-amino N

White sugar yield is calculated with the New Brunswick Formula being adapted by sugar industry in Germany and some other countries to check the quality of sugar beet before purchase

a) Sucrose concentration

Recently, the only purpose to cultivate sugar beet is the production of a maximum amount of white sugar. The sucrose concentration in the beet is the major factor affecting white sugar yield. In soils Kleinlinden and Giessen, K or Na fertilization increased the sucrose concentration in the beet compared with control (Fig. 3.5). Substitution of K by Na slightly decreased the sucrose concentration in the beet. In the soil Trebur, all treatments showed similar results regarding the beet sucrose concentration.

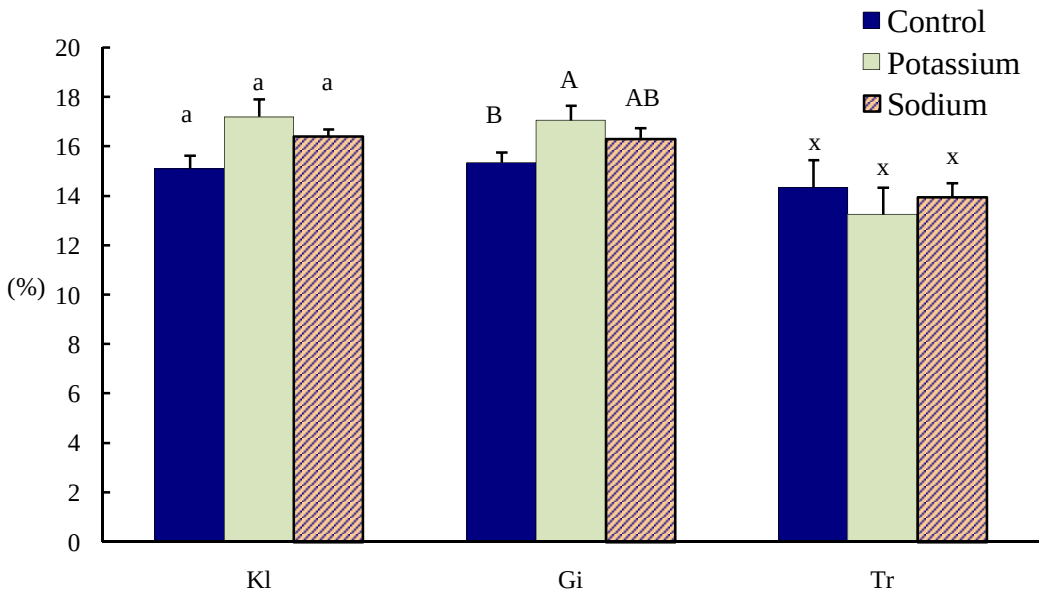


Figure 3.5. Effect of K substitution by Na on sucrose concentration (% beet fresh weight) in beets of sugar beet grown in three different soils (Kl. = Kleinlinden, Gi. = Giessen and Tr. = Trebur). Values in a column represent means + SE of four replications. Means followed by the same letters are not significantly different according to LSD test at 5% level of probability.

Concentrations of Na and K in the beet are important factors, which influence the production of white sugar yield. Actually, Na and K concentrations have a molassegenic effect and disturb the sucrose extraction from beet. The highest Na + K concentrations in beets were determined for plants fertilized with K. For each soil, their concentrations in the beet were about 4 mmol $100g^{-1}$ BFW. However, the lowest concentrations were in Na treated plants i.e. ~ 3 mmol $100g^{-1}$ BFW (Fig. 3.6).

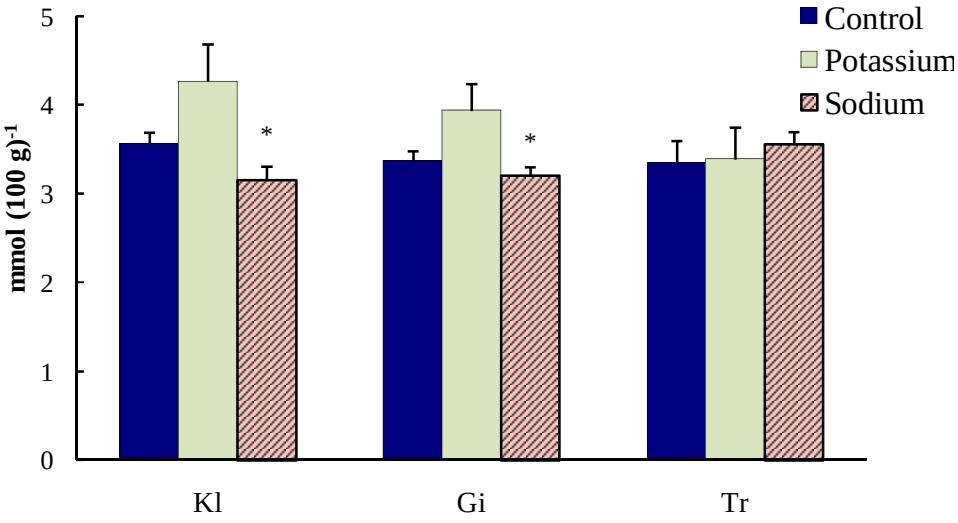


Figure 3.6. Effect of K substitution by Na on Na + K in beets of sugar beet grown in three different soils (Kl. = Kleinlinden, Gi. = Giessen and Tr. = Trebur). Values in a column represent means + SE of four replications.
* = significantly different according to LSD test at 5% level of probability

c) α-amino N

Alpha-amino N is also an important attribute, which affects the white sugar production from sugar beet. It has also a molassegenic effect like Na and K. There was no effect of treatments on α-amino N concentration in the beet (Fig. 3.7).

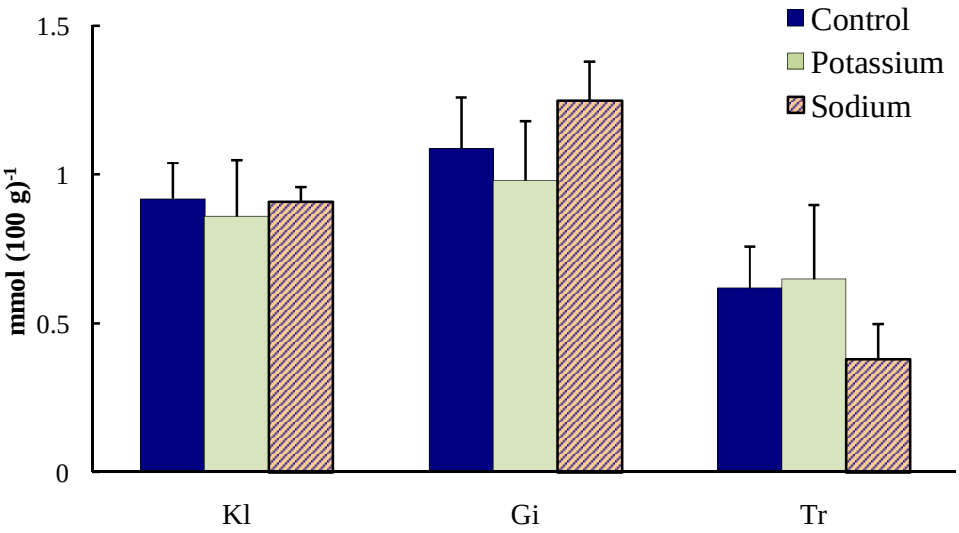


Figure 3.7. Effect of K substitution by Na on α-amino N in beets of sugar beet grown in three different soils (Kl. = Kleinlinden, Gi. = Giessen and Tr. = Trebur). Values in a column represent means + SE of four replications.
No treatment was significantly different according to LSD test at 5% level of probability.

3.1.1.4. White sugar yield

Considering sucrose concentration, Na + K concentration, and α-amino nitrogen, white sugar yield was calculated from beet fresh weight including other factors used in the New Brunswick Formula. The highest white sugar yield (120 g white sugar beet^{-1}) was observed for soil Kleinlinden in K-treated plants against the lowest from control (~ 60 g white sugar beet^{-1}), which was similar in all the soils. White sugar yield was not affected by the substitution of K by Na fertilizer in soils Giessen and Trebur. However, it was decreased in soil Kleinlinden due to this substitution (Fig. 3.8). There was an increase in white sugar yield by both Na and K treatments in soils Kleinlinden and Giessen compared with control, whilst there was no difference amongst the treatments in soil Trebur.

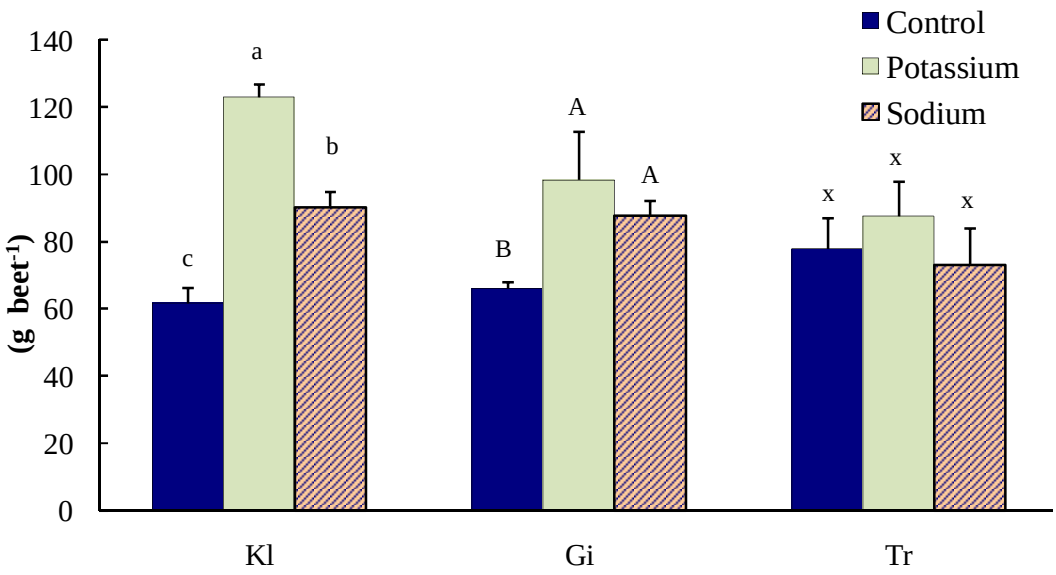


Figure 3.8. Effect of K substitution by Na on white sugar yield of sugar beet grown in three different soils (Kl. = Kleinlinden, Gi. = Giessen and Tr. = Trebur). Values in a column represent means + SE of four replications. Means followed by the same letters are not significantly different according to LSD test at 5% level of probability.

3.1.2. Container experiment

3.1.2.1. Plant growth

Similar to the Ahr pot experiment, plant growth was stimulated by application of K and Na. Plant growth and beet development were similar in Na and K treatments (Fig. 3.9). Maximum leaf fresh weight (> 800 g plant^{-1}) and beet yield (> 1400 g beet^{-1}) were obtained in the Na treatment (Fig. 3.10). This indicates that substitution of K by Na did not reduce the plant growth and beet yield.

Figure 3.9. Similar shoot growth was observed in the K treatment and when K fertilizer was substituted with equivalent amount of Na. On the other hand, plants of both treatments showed better growth than those in control where neither K nor Na fertilizer was applied.

Similar to the Ahr pot experiment, plants fertilized with Na also did not show K deficiency symptoms. Deficiency symptoms were observed when neither K nor Na was applied. In the container experiment K fertilizer was applied at the rate of 415 kg K ha^{-1}, which is almost 2-3 times less than that applied in the Ahr pot experiment.

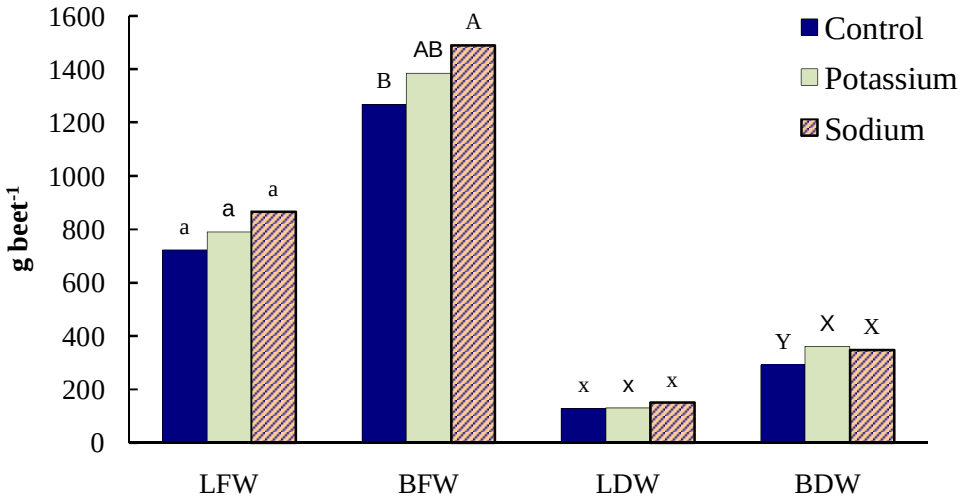


Figure 3.10. Effect of K substitution by Na on fresh and dry weights of leaf and beet of sugar beet grown in containers containing 169 kg Kleinlinden soil. Means followed by same letter are not significantly different according to LSD test at 5% level of probability.

3.1.2.2. Cation concentration and uptake by leaf

Cations are not equally distributed in all plant parts. Therefore, the young and the old leaves were analyzed separately. In young leaves, K concentration was much higher than that in old leaves. The highest K concentration was 48.6 mg K g^{-1} in young leaves, while the lowest K concentration was recorded in old leaves i.e. 20 mg K g^{-1}. Potassium concentration differences between old and young leaves were more in control and Na-treated plants than in K-treated plants. Potassium is highly mobile in plants, thus plants not supplied with K showed deficiency symptoms in old leaves except the plants fertilized with Na. Potassium was translocated from old leaves to young leaves to fulfill the growth requirements in growing parts of the plant. Thus the K concentrations in old leaves were extremely low compared with young leaves which

led to the appearance of deficiency symptoms. In the Na treatment there were no K deficiency symptoms but the K concentration was extremely low.

A large amount of K was translocated from old to young leaves. Even in the treatments without K application or substitution of K by Na there was a sufficient concentration of K in young leaves required for optimum plant growth i.e. 3.5% (Tab. 3.2; Bergmann, 1992). Calcium and Mg concentration were decreased in old leaves when K was substituted by Na and the Ca concentration was same in control and Na treated plants. Uptake of Ca and Mg was same in control and Na-treatment and was significantly decreased in the K treatment. Sodium uptake was higher both in old and young leaves of plants substituted by Na as compared with other treatments. It showed the ability of *Beta vulgaris* to include Na in their leaf cells without affecting the plant growth and utilizing it as a beneficial element.

Table 3.2. Cation concentrations in old and young leaves and total cation uptake by leaves in response to substitution of potassium by sodium in sugar beet plants grown in containers with 169 kg soil container^{-1}.

Treatment	Cations in old leaves (mg g^{-1} DW)				Cations in young leaves (mg g^{-1} DW)				Cations uptake per plant (g)			
	Na	***K***	***Ca***	***Mg***	***Na***	***K***	***Ca***	***Mg***	***Na***	***K***	***Ca***	***Mg***
Control	6.9 b	25.5 b	9.9 b	15.5 b	1.7 b	41.7 ab	0.76 c	1.8 b	0.89 b	0.34 b	0.13 b	0.20 b
Potassium	6.4 b	40.7 a	13.9 a	19.0 a	1.5 b	48.6 a	1.97 a	2.4 a	0.90 b	0.58a	0.20 a	0.27 a
Sodium	20.9 a	19.9 c	9.6 b	12.4 c	4.3 a	38.4 b	1.73 ab	2.3 ab	0.31 a	0.30 b	0.14ab	0.18b

Values with the same letters in a column do not differ significantly according to SPSS at 5 % significant level.

3.1.2.3. Cation concentration and uptake by beet

Due to the substitution of K by Na, the only change in cation concentration was the higher concentration of Na and significant reduction in K concentration in the beet. Potassium-treated plants had significantly higher K concentration (> 7 mg K g^{-1} DM) in beets. The increase in Na concentration was smaller than the decrease in K concentration. Calcium and Mg concentrations were not altered due to K substitution by Na, however, their concentration and uptake were decrease in control plants (Fig. 3.11; 3.12).

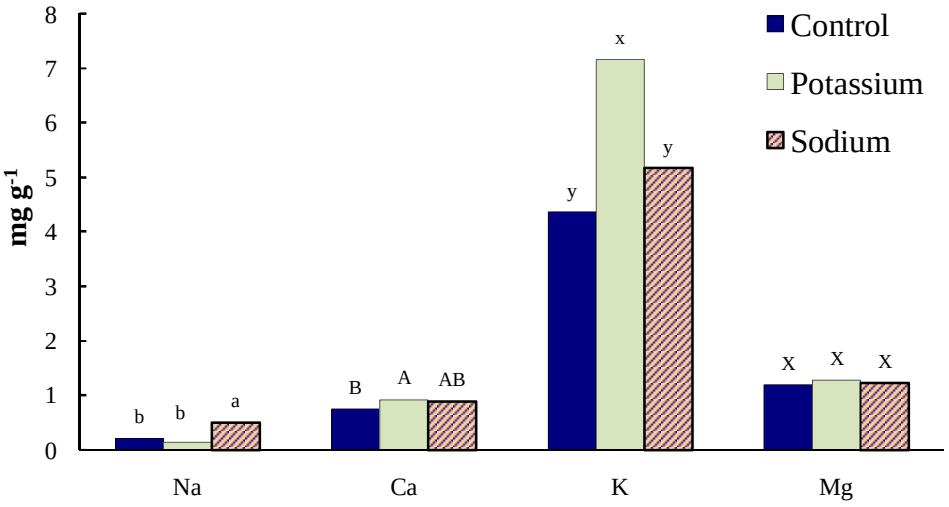


Figure 3.11. Effect of K substitution by Na on cation concentrations in beet dry weight of sugar beet grown in container containing 169 kg Kleinlinden soil. Means followed by the same letters are not significantly different according to LSD test at 5% level of probability.

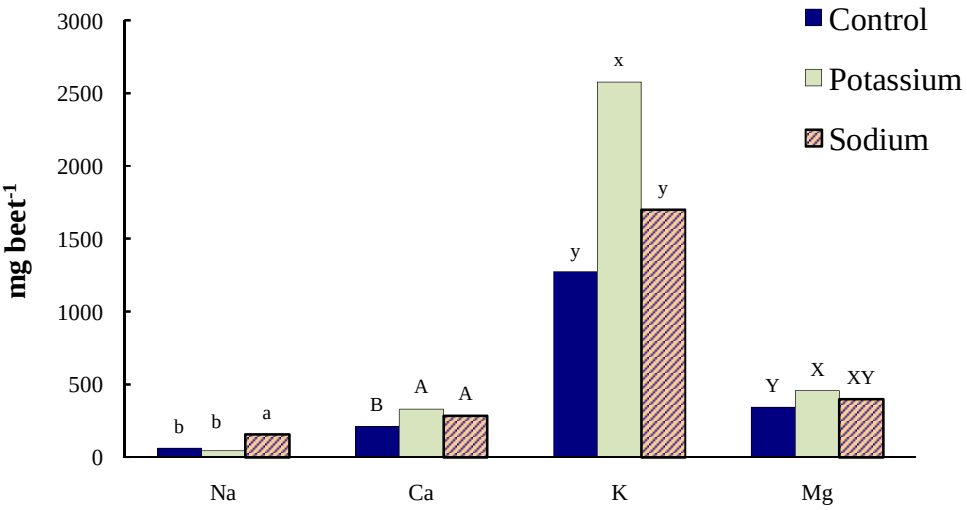


Figure 3.12. Effect of K substitution by Na on cation uptake (mg beet^{-1}) by beets of sugar beet in container containing 169 kg soil. Means followed by the same letters are not significantly different according to LSD test at 5% level of probability.

3.1.2.4. Beet quality parameters

a) Sucrose concentration

Similar to the Ahr pot experiment, sucrose concentration in beets was not affected due to the application of both fertilizers. Even plants grown without any fertilizer also showed the similar concentration of sucrose. The sucrose concentration in all treatments was about 15% of fresh beet matter (Tab. 3.3).

b) Na + K concentration and α-amino nitrogen

Sodium and K concentrations in the beet were similar to that in the Ahr pot experiment. The results of the container experiment showed that substitution of K by Na neither increased the concentration of Na + K nor affected the concentration of α-amino N. However, Na + K concentration in the beet was rather decreased in Na-treated plants (Tab. 3.3) because of drastic decrease in K concentration.

3.1.2.5. White sugar yield

White sugar yield calculated with the New Brunswick Formula, showed that both Na and K-treated plants had higher white sugar yield i.e. 200 and 191 g beet^{-1}, compared with control (161 g beet^{-1}). Nevertheless, beet yield was maximum when K was substituted by Na (Tab. 3.3).

Table 3.3: White sugar yield and beet quality parameters in response to substitution of K by Na. Values with the same letters in a column do not differ significantly at 5 % significant level. Concentrations of α-amino N, Na + K and sucrose are given for the beet fresh weight.

Treatment	α- amino N mmol kg^{-1}	Na + K mmol kg^{-1}	Sucrose %	White sugar yield g beet^{-1}
Control	4.3 A	27.7 z	14.23 X	161.5 b
Potassium	4.5 A	49.0 x	15.61 X	190.9 a
Sodium	4.5 A	36.3 y	15.51 X	200.5 a

3.1.3. Field experiment

3.1.3.1. Plant growth and beet yield

In the field experiment conducted at the site Trebur, application of K and Na fertilizers did not show a positive effect on sugar beet growth. The results indicated (Fig. 3.12) that plant growth was the same in all treatments. Potassium fertilization did not improve the plant growth even at higher K fertilization. Likewise, other yield parameters (beet fresh and dry weights) were not responsive to K and Na fertilization.

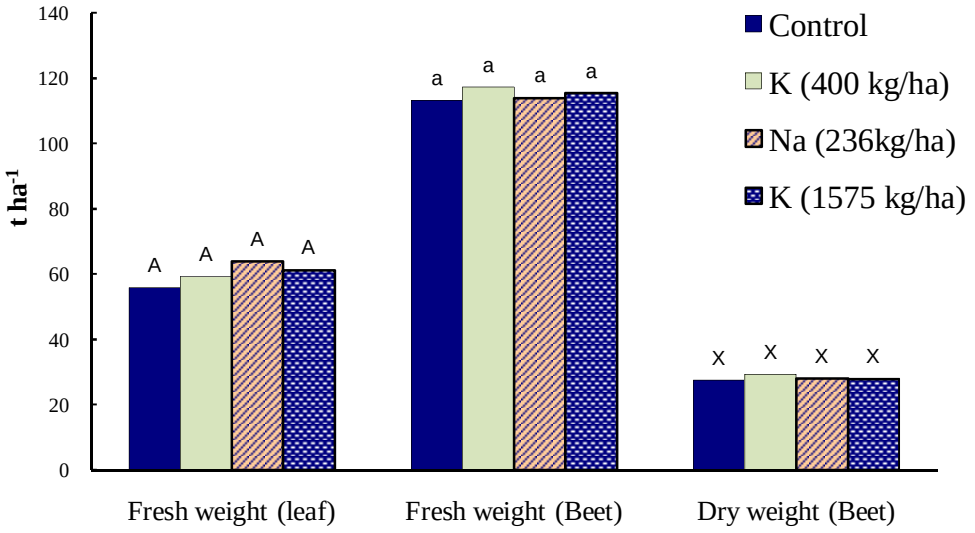


Figure 3.13. Effect of K substitution by Na on plant growth of sugar beet cultivated in the field at Trebur. Means followed by the same letters are not significantly different according to LSD test at 5% level of probability.

3.1.3.2. Cation concentrations in beet

Cation concentrations in the beets are presented in Fig. 3.14, which shows that Na, Ca and Mg concentrations were unchanged in all four treatments except slightly higher concentration of K in potassium supplied treatment. Surprisingly, there was no increase in Na concentration in the beet by Na application.

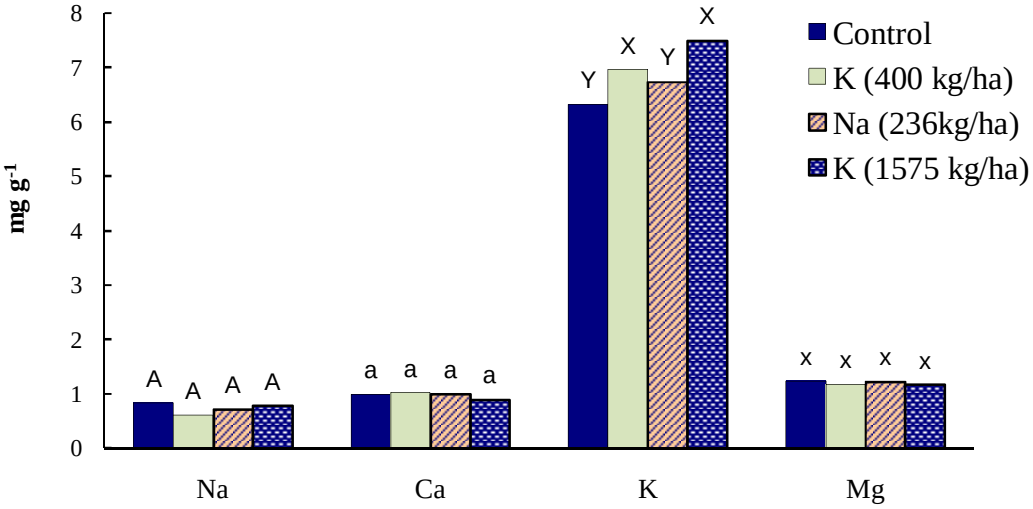


Figure 3.14. Effect of K substitution by Na on cation concentrations in the dry weight of sugar beets cultivated in the field at Trebur. Means followed by the same letters are not significantly different according to LSD test at 5% level of probability.

3.1.3.3. Beet quality parameters

Data of the field experiment showed that sucrose, Na + K (Fig. 3.15) and α-amino N concentrations (Tab. 3.4) were similar in all the treatments. Results revealed that the quality of beet not affected by the treatments. These results are very similar to previous experiments conducted in Ahr pots and containers.

Table 3.4. Alpha -amino nitrogen concentration in sugar beet cultivated under field conditions and harvested at maturity.

Treatments	α-amino N (mmol kg^{-1} fresh weight)
Control	**1.67 a**
K (400 kg/ha)	**1.67 a**
Na (236 kg/ha)	**1.65 a**
K (1575 kg/ha)	**1.61 a**

Values with the same letters in a column do not differ significantly according to *t*-test at 5 % significant level.

3.1.3.4. White sugar yield

White sugar yield was calculated with the New Brunswick Formula. Like the growth and quality parameters, white sugar yield was not affected by any of the treatments (Fig. 3.15). White sugar yield was in the range from 16.5 to 17.8 kg per 10 m^2, which is equal to the production of 16.5 - 17.8 t ha^{-1}. as it was done manually, secondly plants were hoed during the growth period, row to row and plant to plant distance was maintained. Moreover, plants were irrigated and well supplied with fertilizers.

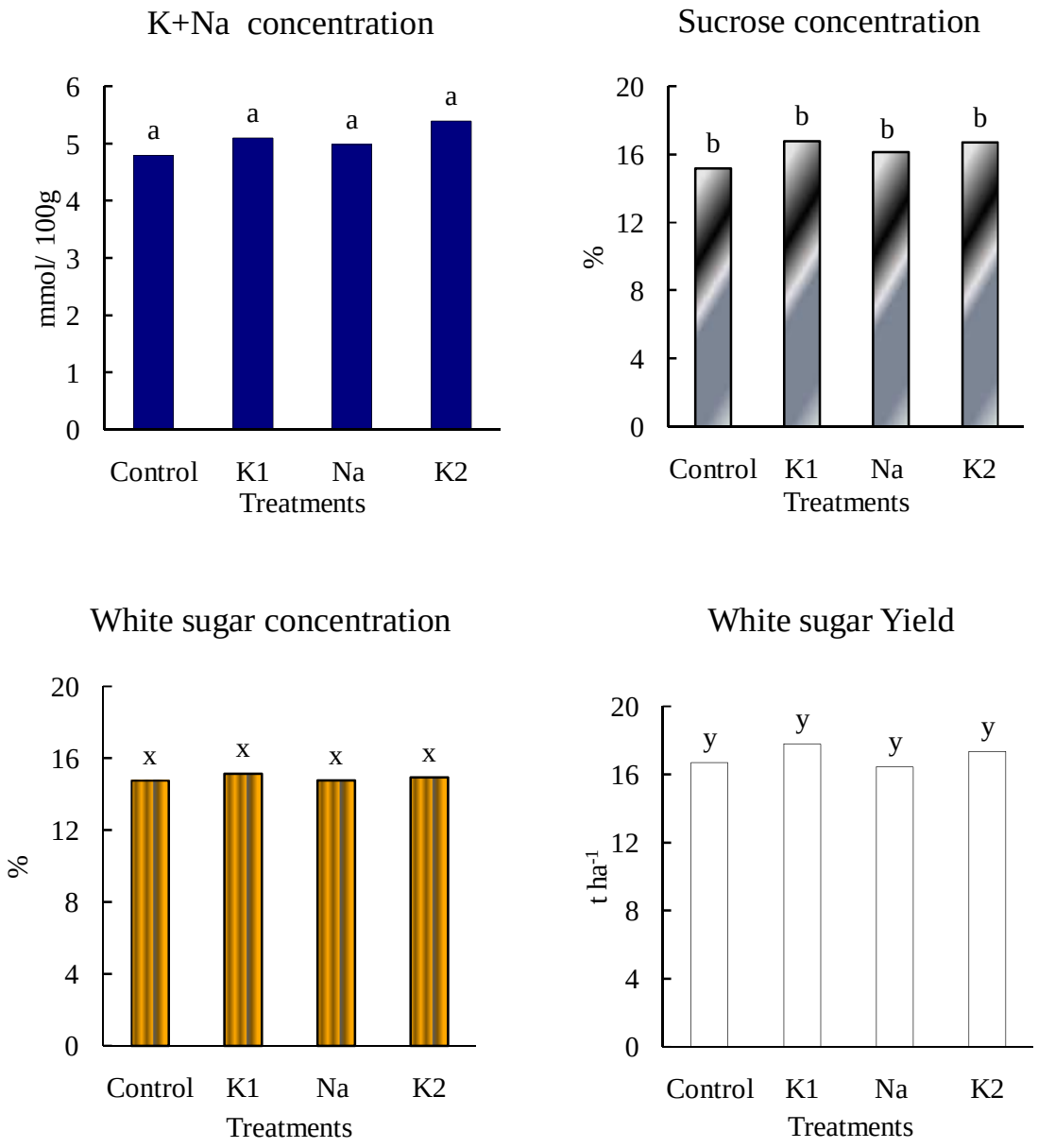


Figure 3.15. Effect of K substitution by Na on quality parameters and white sugar yield of sugar beet cultivated in the field at Trebur. Means followed by the same letters do not differ significantly according to LSD test at 5% level of probability. Control = no K or Na fertilizer, K1 = 400 kg K ha^{-1}, Na = 236 kg Na ha^{-1} (equivalent to K1) and K2 = 1575 kg K ha^{-1}

3.2. Nutrient solution experiments

In the soil culture experiments, substitution of K by Na showed similar plant growth and beet yield without affecting the quality of the beet. However in some cases, the Ca concentration in the shoot was decreased, especially in the younger leaves. Reduced Ca concentration was further investigated by conducting nutrient solution experiments. It was hypothesized that Na might decrease the Ca uptake or its translocation from root to shoot. Nutrient solution experiments were conducted to investigate the uptake of Ca by plant roots and its translocation to shoot in response to K substitution by Na in sugar beet nutrition. Moreover, B translocation and uptake were also determined. Results of both experiments are presented in the following.

3.2.1. Plant growth

Plant growth was stimulated by the application of Na. Shoot fresh weight was significantly higher when 4 mM K was substituted by 4 mM Na in the first nutrient solution experiment. The plants were harvested 3 d after treatment application. No difference was observed in plant growth when the plants were harvested 7 d after treatment application in the second experiment. However, the shoot dry weight and root dry weight were not changed in the first experiment, which revealed that relative water contents in the shoot were higher due to Na application (Fig. 3.17). Phenotypically, plants were looking similar in both treatments in both experiments. However, leaves of the plants receiving Na were broad and thick. Surprisingly no potassium deficiency symptoms were noticed when substituting K with Na but burning of the young leaf tips were observed for some plants (Figure 3.16). Such kinds of symptoms are typical Ca deficiency symptoms. However, sometimes plants show similar symptoms under B deficiency. Therefore both Ca and B were studied in these experiments.

Figure 3.16. Calcium deficiency symptoms in sugar beet when the K was substituted by Na in sugar beet nutrition.

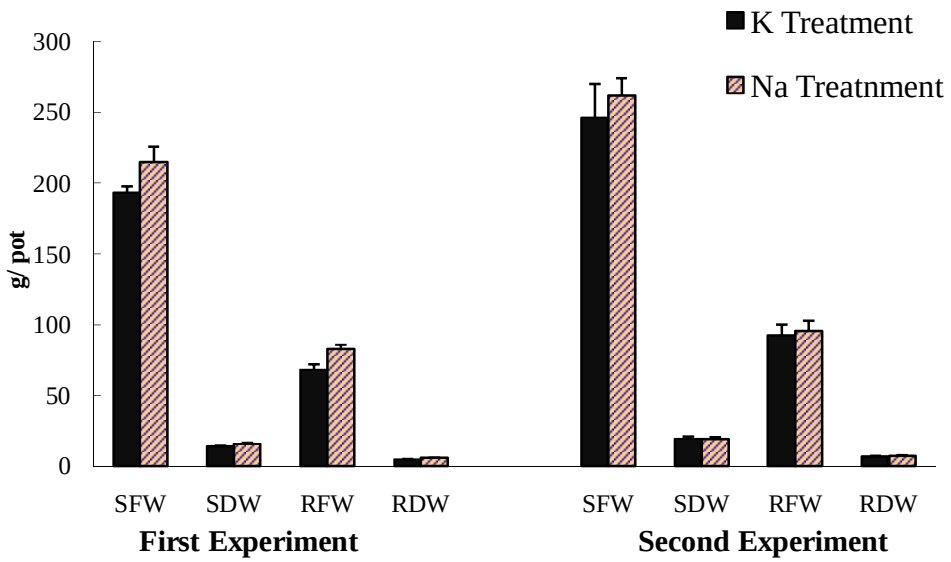


Figure 3.17. Effect of K substitution by equivalent amount of Na in sugar beet nutrition on fresh and dry weights of shoot and root. Experiments were conducted in nutrient solution under standard climatic conditions in a growth chamber.
(SFW= Shoot Fresh weight, SDW= Shoot Dry weight, RFW= Root Fresh weight, RDW= Root Dry weight). Value in a column represents mean + SE of four replications. In the first experiment plants were harvest 3 d after Na treatment application while in the second experiment plants were harvested 7 d after treatment application.

3.2.2. Ion concentration

Calcium and B concentrations along with other cations were determined in roots, old leaves and young leaves. In the first experiment decrease in K concentration from 97 mg g^{-1} to 69 mg g^{-1} shoot dry weight in old leaves and 59 mg g^{-1} to 39 mg g^{-1} shoot dry weight in young leaves were found in response to K substitution by Na (Fig. 3.18 and 3.19).

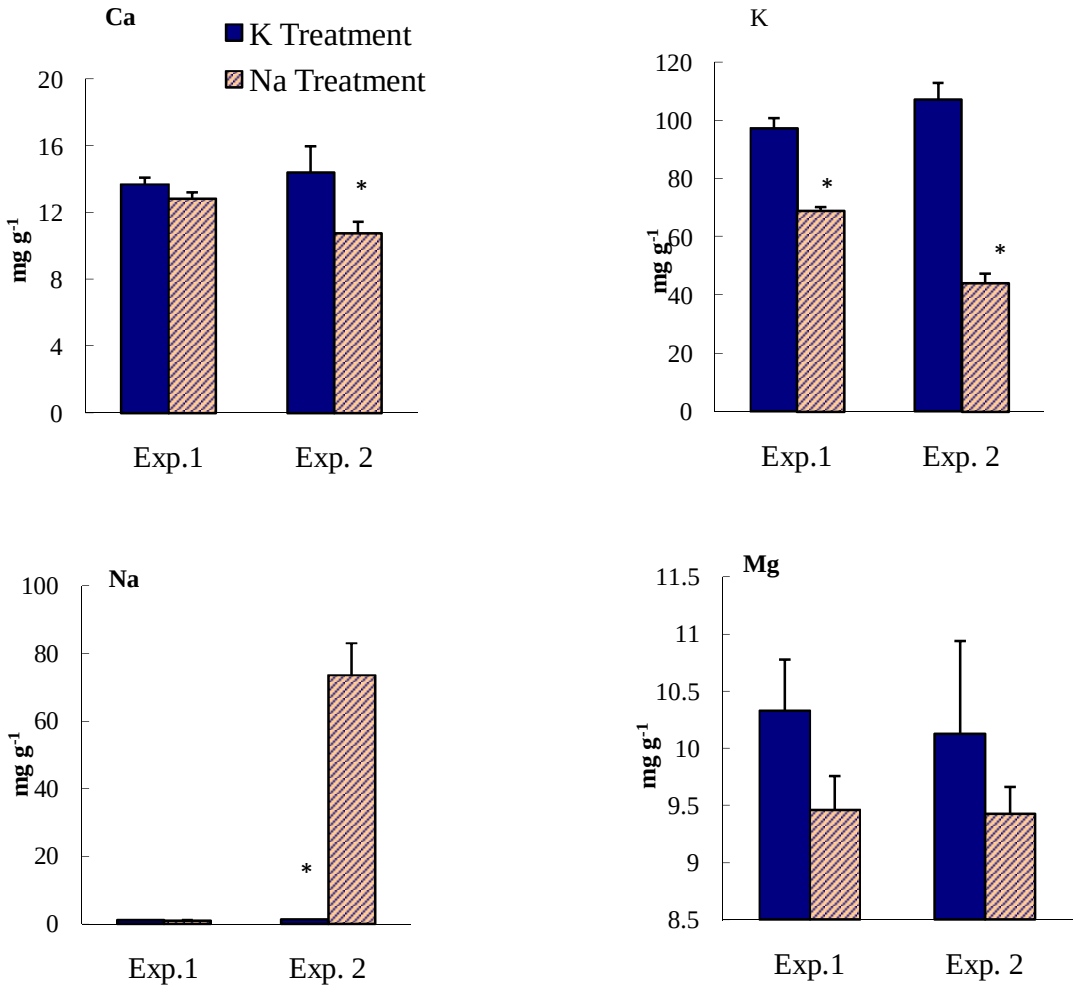


Figure 3.18. Effect of K substitution by equivalent amount of Na in sugar beet nutrition on cation concentrations in old leaves. Experiments were conducted in nutrient solution under standard climatic conditions in a growth chamber. Values in columns represents means + SE of four replications. * = significantly different according to LSD test at 5% level of probability. In the first experiment plants were harvest 3 d after Na treatment application while in the second experiment plants were harvested 7 d after treatment application.

However, Na concentration in old leaves was unaltered but in young leaves it was much higher i.e. 10.6 mg g^{-1}. Relatively lower Ca concentration was shown in old and young leaves while Mg concentration was similar in both treatments.

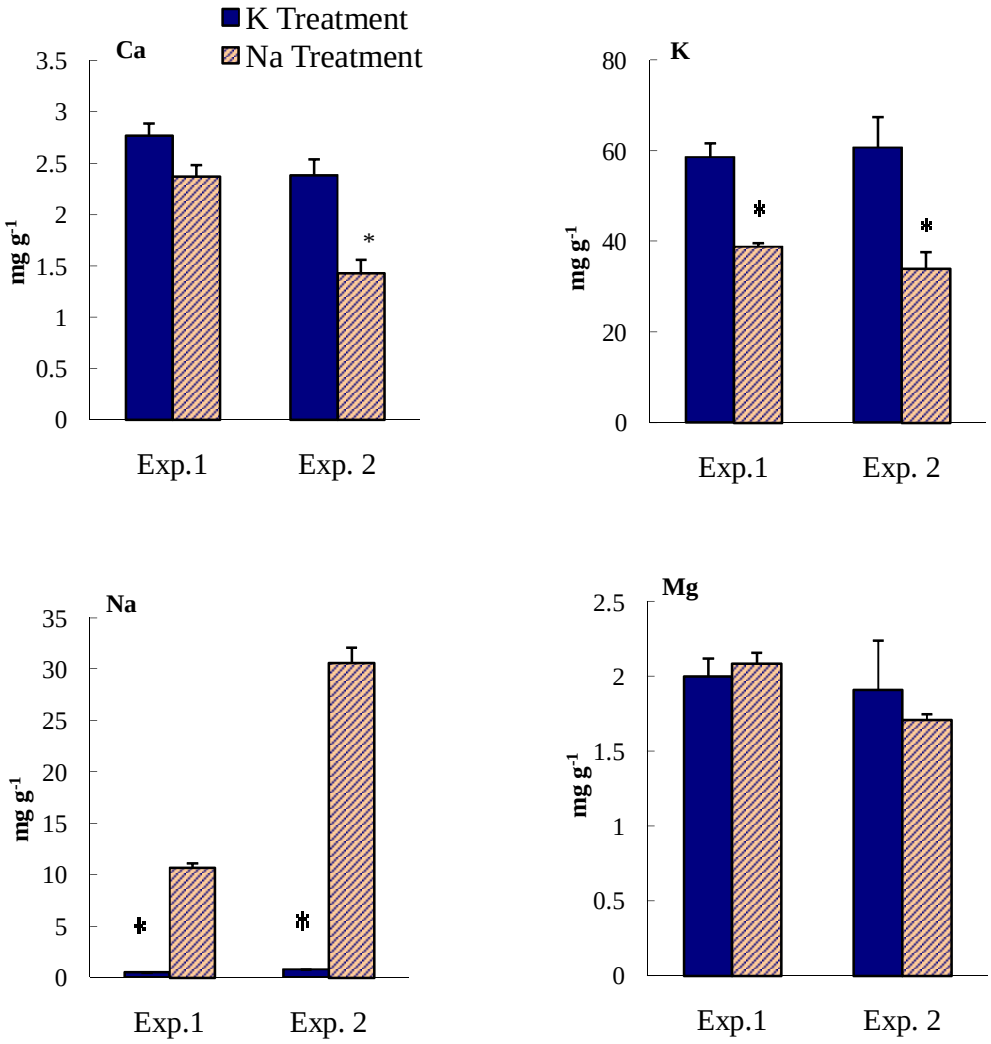


Figure 3.19. Effect of K substitution by equivalent amount of Na in sugar beet nutrition on cation concentrations in young leaves. Experiments were conducted in nutrient solution under standard climatic conditions in a growth chamber. Values in columns represent means + SE of four replications.* = significantly different according to LSD test at 5% level of probability. In the first experiment plants were harvest 3 d after Na treatment application while in the second experiment plants were harvested 7 d after treatment application.

Table 3.5. Cation concentrations in sugar beet roots under Na and K treatment (mg g^{-1} root dry weight). Values represent mean of four replications ± standard error.

Cation	After 3 d treatment		After 7 d treatment	
	4 mM K	4 mM Na	4 mM K	4 mM Na
K	53.1 ± 1.66	33.54 ± 1.3	50.24 ± 5.6	19.0 ± 0.76
Na	0.4 ± 0.06	9.45 ± 0.46	0.79 ± 0.05	27.04 ± 2.24
Ca	4.26 ± 0.2	4.01 ± 0.09	2.83 ± 0.19	2.89 ± 0.30
Mg	1.15 ± 0.08	1.51 ± 0.06	1.74 ± 0.09	2.63 ± 0.09

In the second experiment, plants were harvested 7 d after treatment application and it was observed that alterations in ionic concentrations as compared to the first experiment were more pronounced. Significant reductions in Ca concentrations were 35 - 40% with respect to those in controls, whereas K concentrations were decreased by 60 and 45% in old and young leaves, respectively. Sodium concentrations were about 74 mg g^{-1} and 31 mg g^{-1} in older and younger leaves, respectively. Similar to the first experiment, Ca concentrations in roots were unaffected (Tab. 3.5). Boron concentrations in the roots, old and young leaves also remained unaffected due to substitution of K with Na (Tab. 3.6).

Table 3.6. Effect of K or Na nutrition on the B concentrations in the dry matter of shoots and roots. The difference between K and Na treatment was not significant at 5 % significant level. (YL = young leaves, OL = old leaves, DW= dry weight).

Treatment	First Experiment			Second Experiment		
	µg B g^{-1} (OL)	µg B g^{-1} (YL)	µg B g^{-1} (Root)	µg B g^{-1} (OL)	µg B g^{-1} (YL)	µg B g^{-1} (Root)
4 mM K	53.9	53.5	37.9	57.60	45.96	37.63
4 mM Na	49.0	54.2	37.6	52.58	42.03	37.91

35 – 100 µg g^{-1} is the critical value given by Bergmann

3.2.3. Ca uptake and translocation

Calcium translocation was measured by collecting and analyzing the xylem sap. In the first experiment Ca concentration in the xylem sap was not reduced due to K substitution by Na (Fig. 3.20 A). The volume of xylem sap was higher in Na-treated plants (Fig. 3.20 B) which improved the Ca translocation from root to shoot (Fig. 3.20C). Unchanged concentrations of Ca in xylem sap were found after 3 d for Na treatment. In the second experiment, when the plants were given Na in place of K for 7 d decreased Ca concentration in the xylem sap (4.87 mM in control and 2.43 mM in Na treatment).Despite relatively higher volume of xylem sap in Na-treated plants, Ca translocation was significantly reduced (Fig. 3.20).

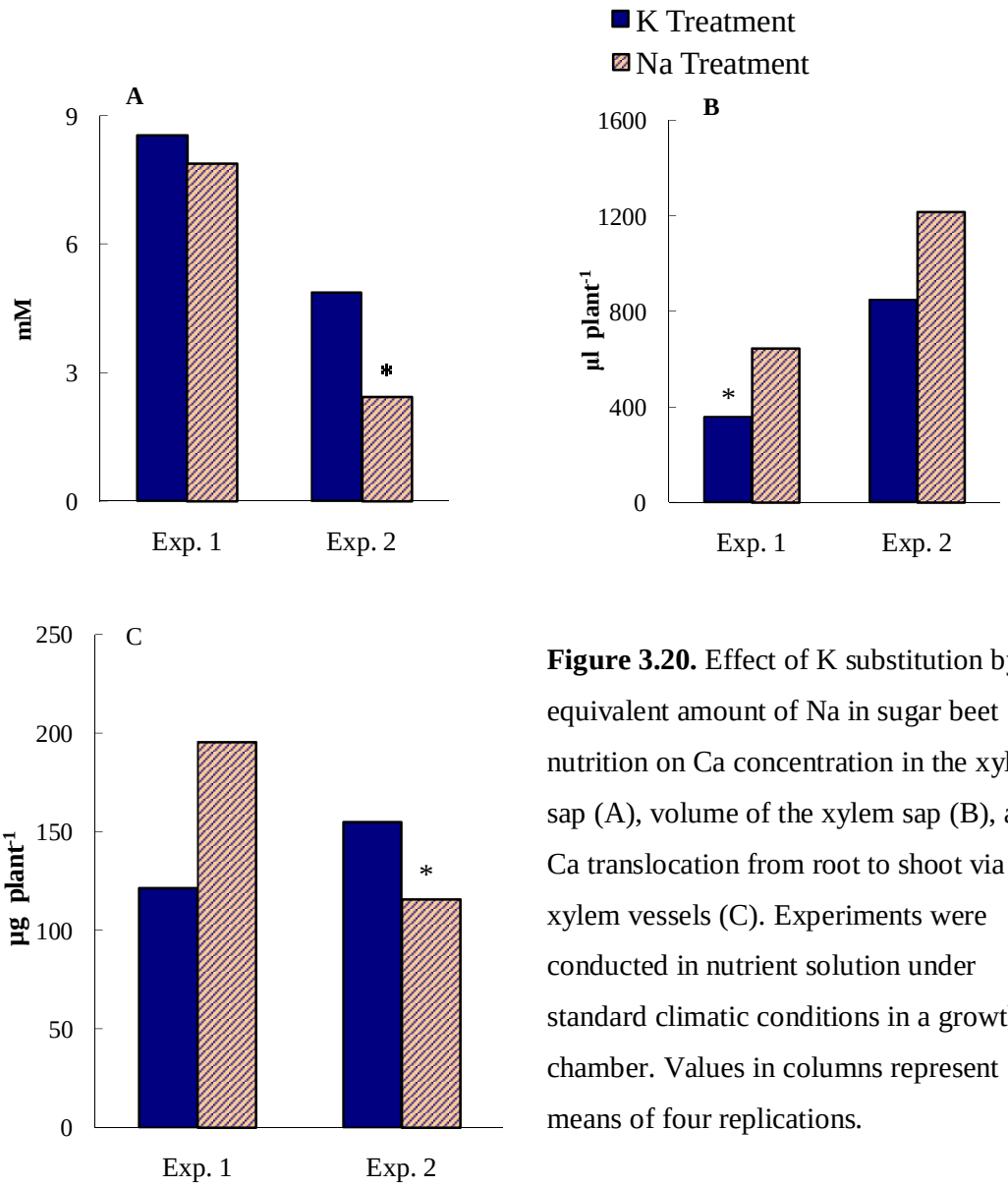


Figure 3.20. Effect of K substitution by equivalent amount of Na in sugar beet nutrition on Ca concentration in the xylem sap (A), volume of the xylem sap (B), and Ca translocation from root to shoot via xylem vessels (C). Experiments were conducted in nutrient solution under standard climatic conditions in a growth chamber. Values in columns represent means of four replications.

* = significantly different according to LSD test at 5% level of probability. In the first experiment plants were harvest 3 d after Na treatment application while in the second experiment plants were harvested 7 d after treatment application.

3.2.4 Boron translocation

Boron concentration in the xylem sap was not affected due to K substitution by Na in sugar beet nutrition. A similar B translocation rate was found in the both treatments (Tab. 3.7).

Table 3.7. Boron concentration in xylem sap and its translocation from root to shoot. Values represent means of four replications ± standard error

Treatment	**B concentration (mM)**		**B translocation (µg B plant^{-1} h^{-1})**	
	Exp. 1 (3 d)	**Exp. 2 (7 d)**	**Exp.1 (3 d)**	**Exp.2 (7 d)**
K Treatment	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.65 ± 0.19
Na Treatment	0.06 ± 0.01	0.05 ± 0.01	0.10 ± 0.01	0.62 ± 0.18

4 DISCUSSION

4.1. Sodium nutrition and plant growth

The research area concerning sodium (Na) nutrition in some plant species is of vital importance due to its significant role in plant metabolism. Nevertheless, the phenomenon of Na nutrition has remained an elusive topic despite several decades of intensive research efforts, particularly during the 1960s and 1970s. Epstein (1965) has given a modified definition of an essential element based on Arnon and Stout's (1939) definition for a plant nutrient. According to him "an element is essential if it fulfills either one or both of two criteria: (1) an element is part of a molecule that is an intrinsic component of the structure or metabolism of a plant (2) the plant can be so severely deprived of the element that it exhibits abnormalities in its growth, development or reproduction - that is its performance in comparison with plants not so deprived.

Taking into account the above criteria of an essential element, the role of Na in plants and its status as an essential element is still debated and Na has still not been shown to meet the criteria to be an essential element for all the higher plants. However, low concentrations of this element may be required for normal growth and development of some species particularly halophytes, which are known to tolerate high salt concentrations (Subbarao *et al*. 2003; Slama *et al*. 2007). In addition, it is possible that some elements such as Na and Si may promote maximal biomass production without meeting the preceding requirement for essentiality because all metabolic functions do not require a unique nutrient to function. Many essential metabolic processes can function equally well with a number of different but chemically and physically similar elements. According to Subbarao *et al*. (2003), it is possible for similar elements such as Na and K to replace each other fully in certain non-specific metabolic functions. Thus, even though an element may function completely in an essential function (may be even more effectual than any other element), it would not be considered an essential nutrient unless it has a unique function that it alone can meet. It could be argued, at least from agronomic considerations, that additional levels of essentiality should be

differentiated to denote nutrients that may be required for maximal yield or are able to replace other nutrients in certain essential metabolic functions reducing the critical level of an essential element.

Despite the fact that Na is not essential for many species, application of Na to the growth medium has been shown to stimulate the growth of asparagus, barley, carrot, chicory, cotton, millet, oat, pea, tomato, vetch, wheat, cabbage, horseradish, kohlrabi, mustard, radish, rape, marigold, sugar beet, red beet, Swiss chard, and turnip (Harmer and Benne, 1945; Larson and Pierre, 1953; Lehr, 1953; Montasir *et al.,* 1966; Subbarao *et al.,* 2003). Lehr (1953) indicated that it appears that visual leaf symptoms of low Na on sugar beet, marigold and red beet appear as a dull dark green color, rapid wilting in drought and a tendency for leaves to grow out horizontally from the crown. Moreover, in some cases marginal inter-venal scorch may develop, similar to that of K deficiency. In the same line in our experiment on sugar beet, peculiar effects of Na application were observed on plant growth of sugar beet in the Ahr pot experiment and Na was able to eliminate the K deficiency symptoms on sugar beet leaves (Fig. 3.1 and 3.3). Subbarao *et al*. (2003) in a study on red beet (*Beta vulgaris* L.), found that Na stimulated the growth of red beet when K was substituted by Na. They concluded that Na can replace K for vacuolar functions for which 95% of total acquired K is required.

Our studies also indicated that Na can be a competitive alternative for K in sugar beet nutrition. Similar plant growth was observed when K was substituted by Na in sugar beet nutrition and sugar beet yield was also not affected (Fig. 3.1). Our results are consistent with the findings of Hylton *et al.*, (1967) and Amin and Joham, (1968) who found growth improvement by application of Na to some glycophytic plants when K supply was limited. Similarly, Nunes *et al.,* (1983) found that growth of halophytic plants was stimulated and was mainly caused by Na effect on cell expansion.

Increase in shoot fresh weight with no change in shoot dry weight may be due to increased succulence in halophytic plants owing to Na accumulation in plant shoot (Flowers and Yeo, 1986). Water balance of plants and growth responses of halophytes

to Na are due to better osmotic adjustment (Flower and Läuchli, 1983). Indeed, Na can do better osmotic regulation than K (Eshel, 1985). Slama *et al*. (2007) found that presence of NaCl in the nutrient solution culture increased the level of Na and proline in the leaves for osmotic adjustment, which is coupled with an improvement in the photosynthetic activity, but had no affect on leaf soluble sugar content of the halophytic species *Sesuvium portulacastrum.* They further found a striking recovery in relative water content and growth of seedling in the presence of NaCl under water stress. Earlier, Grof *et al*. (1989) found a decrease in the amount of granal stacking in Na-deficient mesophyll chloroplasts of the C_4 plants *Amaranthus tricolor* L. and *Kochia childsii*. They found that PSII activity was markedly lower in mesophyll thylakoids extracted from Na-deficient plants, however bundle sheath thylakoids were relatively unaffected by Na deficiency.

In all the experiments on sugar beet including the container experiment, where the conditions were very similar to the field, it was clear that Na has a stimulating effect on beet yield relative to the control, however K and Na treatments showed similar yield (Fig. 3.9). Application of Na fertilizer significantly improved the sugar beet growth compared to control. Milford *et al.* (1977) also demonstrated similar results and assumed that growth improvement was possibly due to the stimulation of leaf area growth and water status in the plant.

Similarly, the effects of Na and K treatments were also comparable in most of the cases in our experiments. However, as an exception soil Kleinlinden in the Ahr pot experiment showed decreased plant growth and beet yield when K was substituted by Na (Fig. 3.1). Higher K concentration in the leaves of plants grown in Kleinlinden soil revealed that the growth response due to K fertilization was higher in K treatment as compared to the other soils. In fact soil Kleinlinden is dominant in illite clay minerals with less cation exchange capacity and low K-fixing capacity. Relatively higher response to K fertilization was due to low K-fixing capacity of the soil Kleinlinden, which is about 40 mg K $(kg\ soil)^{-1}$ less than of Giessen soil used in the same experiment (Table. 2.3). In soil Trebur, K and Na both did not show a significant

effect on leaf and beet yield in comparison to control. This may be due to the presence of smectite clay minerals in this soil, which are able to release interlayer K, when K concentration in the soil solution decreases. Giessen soil showed non-significant difference in plant growth and beet yield, whereas maximum plant growth and beet yield were observed in the K treatment. Numerous researchers have reported the stimulatory effect of Na on plant growth (See Hylton *et al.*, 1967; Takahashi and Maejima, 1998). Jafarzadeh and Aliasgharzad (2007) argued that this stimulation is particularly apparent in the plants of the family Chenopodiaceace. Application of NaCl improved the seed germination and root length of sugar beet. Moreover, a positive response of tomato to additional Na had also been reported by Woolley (1957).

Before we compare soil Kleinlinden in Ahr pot and container experiments, one point is worth mentioning. In the Ahr pot experiment the amount of applied potassium fertilizer was much higher (1000 kg K ha^{-1}) than the generally recommended fertilization (250 kg K ha^{-1}) employed in the container experiment. For soil Kleinlinden in the container and the Ahr pot experiments, it is clear from the results that the significant difference between K and Na treatment in the Ahr pot experiment was only due to higher amount of K-fertilizer, which is seldom applied under field conditions. Because in the container experiment, the K-fixing capacity of soil was almost similar to in the Ahr pot experiment, but K was applied at the rate of 250 kg K ha^{-1}, which could not show a similar growth response as in Ahr pot experiment. The results of the container and the Ahr pot experiments revealed that Na can substitute K in sugar beet nutrition and this substitution is more effective in the soils with higher K-fixing capacity. In soil Giessen, the K-fixing capacity was higher and substitution was more effective, even when Na was less than equivalent to K fertilization. Nevertheless, for the container experiment Na showed stimulatory effects on plant growth and beet yield in soil Kleinlinden and the difference between K and Na treatment was non-significant.

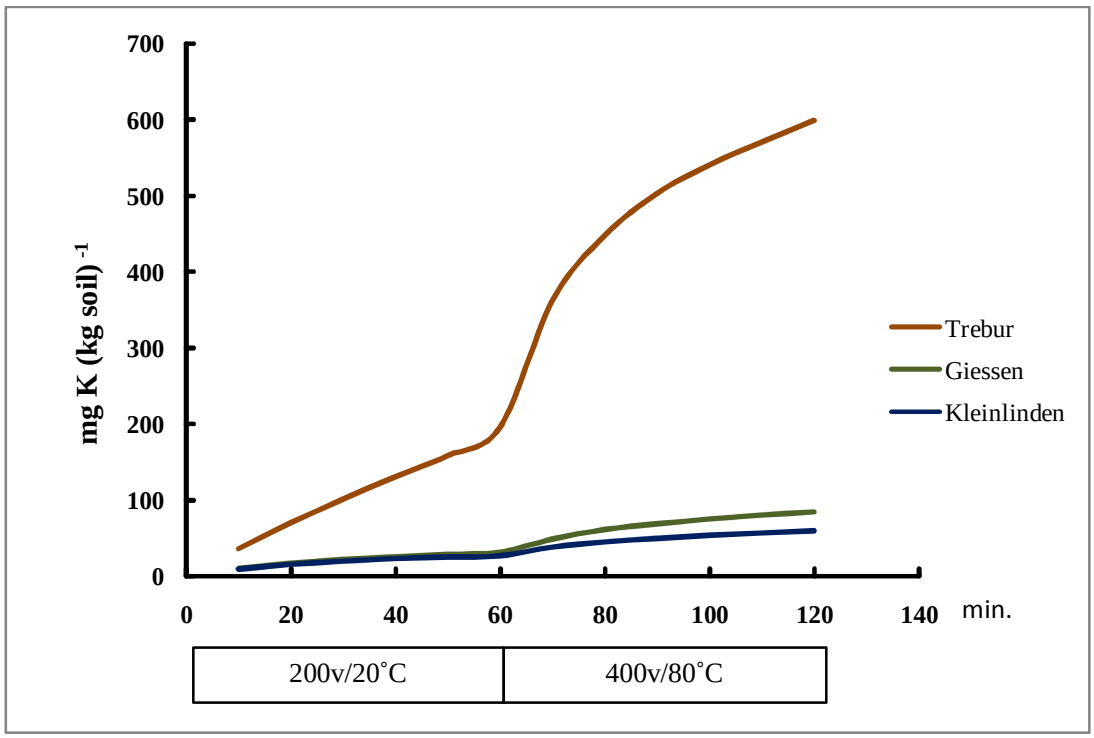


Figure 4.1. Cumulative curve of K dynamics in three different soils determined by electro-ultra filtration (EUF). Soil extract was taken after every 5 min. Potassium released during the first 60 min at 200v/20˚C is considered as easily available for plants and K release in next 60 min at 400v/80˚C is known to be slowly available for plants.

It is known that Na can substitute K to a large extent in many plant species (barley, wheat, red beet etc.), but in practical agriculture Na is not used as substituting nutrient in the nutrition of the crops. Our hypothesis emphasized that in K-fixing soils it may be possible to adjust the problem of K-fixing by substituting K with Na fertilizers. Doll and Lucas (1973) found that soils with vermiculite clay minerals in Michigan state have the severe problem of K fixation. We proposed that application of Na fertilizer in such soils may eliminate or diminish this problem. The original soils used in our studies were highly K-fixing but not deficient in available K. To prepare K-deficient soils for our studies, the soils were mixed with sand that reduced the K-fixing capacity. Moreover, only Giessen soil has a reasonable amount of vermiculite clay minerals and the other two soils i.e. Kleinlinden and Trebur have a huge amount of illite and smectite clay minerals, respectively (Paul, 1989). Soil Trebur and Giessen showed similar growth in K and Na treatments. Smectite clay minerals have low

charge (0.1 - 0.4/ half unit cell and large interlayer space 1.0 - 2.0 nm, Bohn *et al.*, 2001) due to which they are able to release K from the huge amount of total K i.e. 16 g K (kg soil)$^{-1}$ (Schubert *et al.*, 1989), when its concentration decreases in the soil solution. This may be the reason that the Ahr pots with soil Trebur and the field experiment conducted at Trebur showed similar yield in all treatments including control. This soil was deficient in available K (CAL K 65.9 mg (kg soil)$^{-1}$, Table, 2.2), but due to presence of a huge amount of smectite clay minerals, fixed K was released from the clay minerals and was available to the plants for optimum plant growth in all treatments.

The cumulative K release from the soils including soil Trebur is plotted in Fig. 4.1. It shows that soil Trebur is able to release a huge amount of K from the clay minerals when the K is depleted from the soil solution. Extreme weather conditions during the growth period may have influenced the results of the field experiment (Fig. 2.5). In the early growth period weather was dry and warm and then continuous rainfall may have positively affected the K release from the soil. Our studies showed that highly K-fixing soils with vermiculite clay minerals, such as in soil Giessen, might have a potential and a practical importance for this possible substitution.

4.2. Beet quality

Sugar beet is grown world-wide for the production of white sugar from its beets, which contain 17-18% sucrose on fresh weight basis. Parameters to measure the quality of beet have been defined in the New Brunswick Formula (subtitle 2.3.4.), which includes sucrose concentration, α–amino N and K + Na concentration in the beet. White sugar production is directly proportional to sucrose concentration in the beet. Alpha-amino N, K, and Na have a molassegenic effect, which hinders the extraction of sugar from beet and reduces white sugar yield. As a result, a lot of sugar is wasted in molasses because of low extractability of sucrose from sugar beet in the presence of higher concentration of α–amino N, K, and Na. Application of Na fertilizer could decrease the quality of the sugar beet if its major accumulation occurred in the beets. Wang *et al.* (2007) found that most of the halophytic plants

accumulated a huge amount of Na in their shoots. For *Suaeda maritima*, a halophytic plant, growing in 150 mM NaCl, he found that 95% of the total Na was accumulated in plant shoot. There was evidence that little or no Na is re-translocated from the shoots (Yeo, 1981). This suggests that most of the Na that enters the roots is transported to the shoots where it is accumulated. Most of the halophytic plants have succulent leaves with enlarged cell volume due to accumulation of a huge amount of Na (Waisel, 1972; Hajibagheri *et al.*, 1984).

Sugar beet experiments in our studies showed similar results, when K was substituted by Na. More than 90% of the total Na content of the plant were accumulated in shoot and the rest was in beet (Fig. 4.2). Due to small accumulation of Na in the beet, K + Na concentration in the beet was significantly decreased from 49 mmol kg^{-1} to 36 mmol kg^{-1} which definitely improved the beet quality as indicated by white sugar yield (Table, 3.3). In fact, K was low in the Na treatment because we did not apply K in that case. On the other hand, Na accumulation in the beet was negligible because the plants accumulated Na in the shoots. Sucrose and α–amino N concentration in the beet were totally unaffected by the substitution. Calculation of white sugar yield with the New Brunswick formula showed that substitution of K by Na did not negatively affect the quality of the sugar beet (Table, 3.3). The results obtained for white sugar yield were similar to those obtained for beet yield.

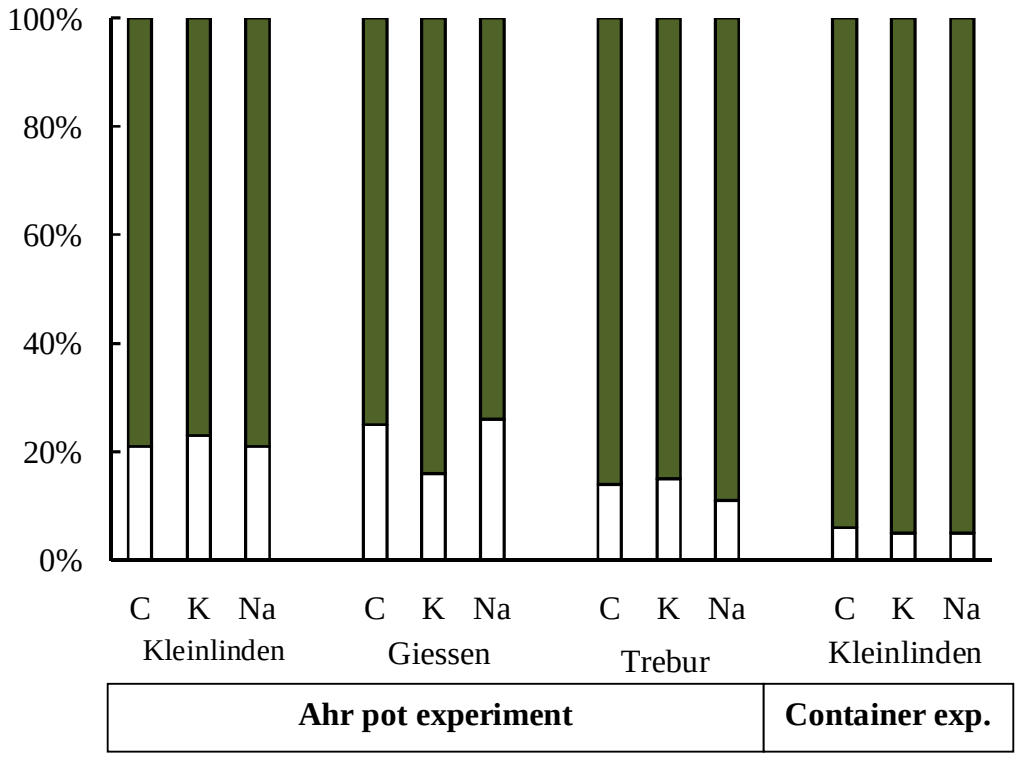


Figure 4.2. Effect of K substitution by Na on accumulation of Na in shoot and beet. C = control, K = potassium treatment and Na = sodium treatment. Kleinlinden, Giessen and Trebur were the soils used for the experiments. White portion of column shows Na concentration in beet and green portion shows Na concentration in shoot.

4.3. Sodium nutrition and ionic homeostasis

Before discussing the ionic homeostasis in response to K substitution by Na, it seems important to explain the mechanisms of K and Na uptake by plant cells. Epstein and co-workers established that at low external concentrations (under 1 mM), the unidirectional influx kinetics of K can be mathematically analyzed using a Michaelis–Menten model (Epstein *et al.*, 1963). Initially it was referred to as 'Mechanism 1' and later as 'High-affinity transport system' for potassium. At external concentrations higher than 1 mM, K ion transport patterns were dominated by a kinetically distinct system. This linear component of K transport was first termed 'Mechanism 2' by Epstein and later the 'Low-affinity transport system' (Fig. 4.3). In the high-affinity transport system, K ion enters the cell via symport with H^+, with a proposed 1:1 stoichiometry (Kochian *et al.*, 1989; Maathuis and Sanders 1994; Maathuis *et al.*, 1997) in an energy-dependent process involving the trans-membrane proton motive

force. The electrochemical H^+ gradient maintained by membrane-bound ATP-hydrolyzing transporters that pump H^+ out of the cytosol into the external medium (Cheeseman *et al*., 1980; Kochian *et al*., 1989; Maathuis and Sanders, 1994; Palmgren 2001; Pardo *et al*., 2006). The release of H^+ ion by roots of intact maize plants is an active process driven by plasmalemma located ATPase (Mengel and Schubert, 1985). In the low-affinity transport system K ion is thought to be transported via K-specific as well as non-selective cation channels, which can facilitate thermodynamically downhill fluxes that are at least three orders of magnitude higher than those transported by pumps and carriers (Tester, 1990).

In the recently devised transporter classification system, class 1 (channel/pore type) includes low-affinity transport systems and class 2 (electrochemical potential-driven transporter type) high-affinity transport systems (Busch and Saier, 2002). Potassium acquisition from low external concentrations is usually considered to be an energy-demanding process, while that from high concentrations is energetically passive. This view is supported by analyses of the electrochemical potential gradient for K ion transport into plant cells, which is primarily defined by the differences in K concentration and electrical potential on either side of the plasma membrane (Cheeseman and Hanson, 1980; Szczerba *et al*., 2006). Potassium is usually the most abundant cation in the cytosol, with concentrations typically ranging from 40 to 200 mM (Kronzucker *et al*., 2003; Leigh and Jones, 1984; Walker *et al*., 1996). In very dilute solutions, the electrical potential is insufficient to drive K influx and an active transport mechanism is thus postulated under such conditions (Cheeseman and Hanson, 1980; Maathuis and Sanders, 1994). Responses to plant K status also distinguish high-affinity transport systems from low-affinity transport systems (Britto and Kronzucker, 2008) (Fig. 4.3).

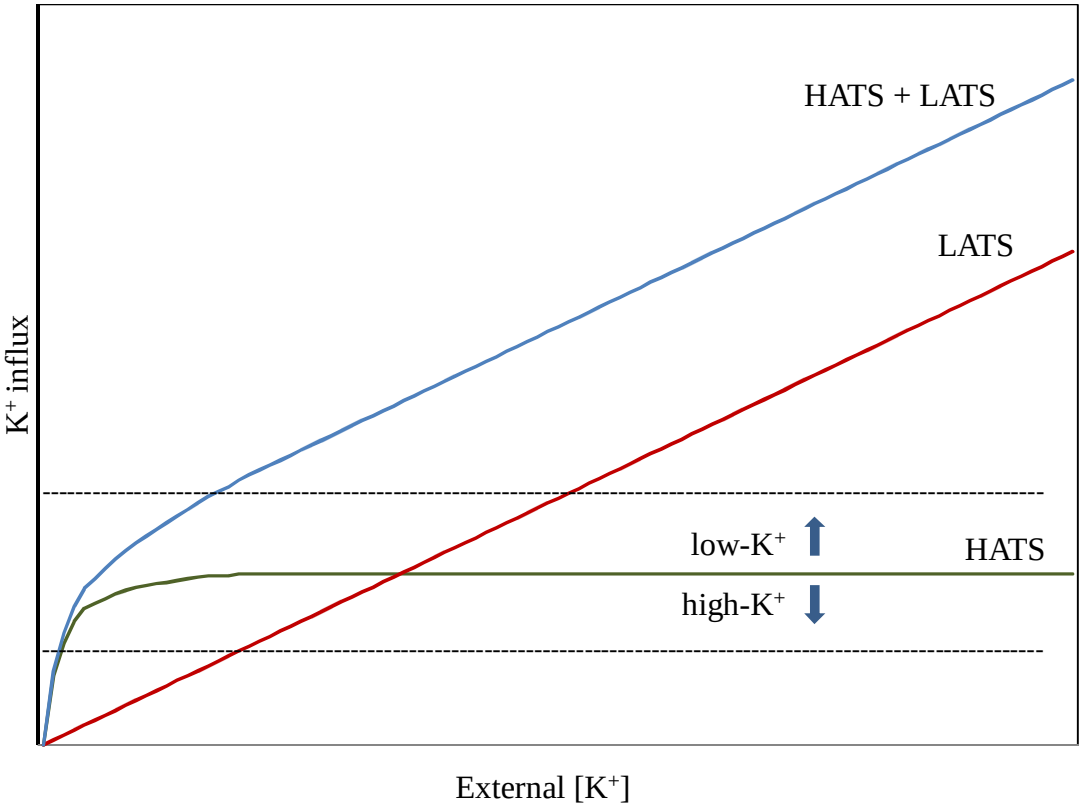


Figure 4.3. Potassium flux kinetics in plant roots. Isotherms for high-affinity transport systems (green line) and red line shows isotherm for low-affinity transport systems. Combine flux is represented by the blue line. Arrows and dashed lines indicate up- and down regulation of high-affinity transport systems, in response to plant K status (Britto and Kronzucker, 2008).

The high-affinity transport system was strongly down-regulated under K supply and was up-regulated under K starvation; however, by contrast, the low-affinity transport system appeared to be insensitive to plant K status (Glass, 1978; Kochian and Lucas, 1982).

Although the mechanisms for Na influx across the plasma membrane have not yet been well established, it is evident that non-selective cation channels appear to form a significant pathway for Na influx from the soil solution into roots. These channels have been studied with respect to salinity resistance and have been measured in several species, including rye (White, 1996; White and Ridout, 1995), maize (Robert and Tester, 1997b), wheat (Buschmann *et al.*, 2000; Davenport and Tester, 2000; Tyerman,

et al., 1997) and *Arabidopsis* **(**Demidchik and Tester, 2002). Non-selective cation channels in plasma membrane have relatively high Na/K selectivity and provide a pathway for the entry of Na into plant cells (Maathuis and Amtmann, 1999). Sodium can also enter the cell through several low- and high-affinity K transporters. Among these, AtHKT1 from *Arabidopsis* has been shown to function as a selective Na transporter (Uozumi *et al.,* 2000; Rus *et al.,* 2001). Similar results were shown by Horie *et al.* (2007) for rice (*Oryza sativa*). Recent evidence showed that AtHKT1 is a determinant of the accumulation in the root and retrieval of Na from the xylem (Davenport *et al.,* 2007). Rodriguez-Navarro and Rubio (2006) suggested that HKT1 transporter mediate high-affinity Na uptake but also function in low-affinity Na transport. It has been shown that 10 mM Ca did not significantly affect whole-plant Na content of *Suaeda maritima,* a halophyte, under either 25 mM or 150 mM NaCl treatment (Wang *et al*., 2007). They suggested that low-affinity cation transporters are unlikely to be the major pathway of Na influx in *Suaeda maritima*. Hirschi (2004) also showed similar findings. According to Wang *et al.* (2007) under low external NaCl concentrations (25 mM) Na uptake is mediated by high-affinity potassium transporters. However, under higher external salt concentrations (150 mM NaCl) other pathways mediate Na entry into the plant through inward-rectifying potassium channels in *Suaeda maritima.* These high-affinity transporters of K and/or Na are important under K-deficient conditions where they may take up Na and thereby promote plant growth. Some of them are specifically expressed in plasma membrane of cells in the epidermis and cortex of roots (Huang *et al.,* 2008).

Balanced ion concentrations in the plant cell are of great importance for optimum plant growth. Homeostasis of ions is a very important feature of natrophilic plants such as sugar beet. The discussion about mechanisms of ion uptake in the previous paragraphs concluded that Ca, K, and Na ion concentrations in the plants are interdependent and the concentration of an ion affects the concentration of the other. Ionic concentration in the plant cell depends upon many factors. Calcium, K and Na compete with each other to be taken up by the plants. Large reduction in Na/K + Ca ratios in salt-stressed roots of seedlings grown with supplemental Ca may have a significant effect on

metabolic functions of the plant (Kent and Läuchli, 1985). Higher concentration of Ca in the nutrient solution has reduced the Na uptake by plants and has an ameliorating effect on plant grown under salinity (Cramer, 2002; Lazof and Bernstein, 1999). Likewise, Ca concentrations and uptake by plant were decreased when external Na concentrations were increased (Rengel, 1992; Cramer, 1997). These findings suggest that Na and Ca may compete for influx through non-selective cation channels. In barley, Na/Ca ratios in expanding leaf tissue increased with increasing salinity, while leaf growth was reduced (Lynch *et al*., 1988). In our studies, instead of wide Na/Ca ratio (Fig. 4.4) in sugar beet shoot under Na treatment, plant growth was not affected (Fig. 3.9). Halophytic and Na-tolerant plants such as sugar beet have a different behavior due to their ability to sequester a huge amount of Na into the cell vacuoles without affecting cytosolic metabolism. Low cytosolic Na concentration is maintained by tonoplast Na^+/H^+ antiporters (Aps *et al*., 1999; Hamada *et al*., 2001; Ma *et al*., 2004; Saqib *et al*., 2005) and Na extrusion from the cell occurs through SOS1 (Martinez-Atienza *et al*., 2007).

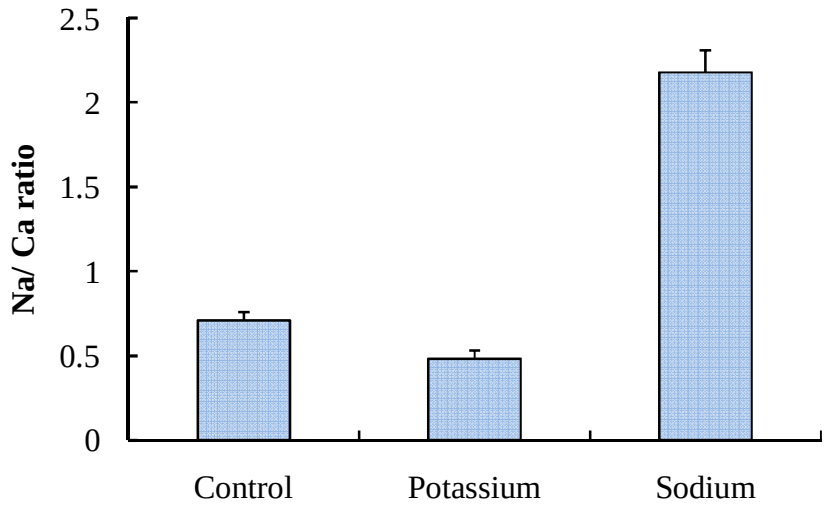


Figure 4.4. Sodium to Ca ration in sugar beet shoot grown in containers containing 169 kg Kleinlinden soil. Values in a column represent means + SE of four replications. In control no K and Na fertilizer, in potassium treatment K was applied at the rate of 415 kg K ha^{-1} and in sodium treatment Na was applied as NaCl equivalent to K fertilization.

Most of the previous work showed negative effect of wide Na : Ca ratios under saline conditions. However, we applied a smaller amount of Na as compared to salinity and Na concentration in cytosol was easily maintained by the plant to protect the cytosolic metabolism.

Despite the toxic effect of high Na concentrations, plants have transporters that allow the uptake of Na from the soil (Mueller-Roeber and Dreyer, 2007). Sodium competes with K to pass through the high affinity K channels (HKT1), especially when the K concentration in the nutrient solution is low (Rus *et al.*, 2001; Platten *et al*., 2006). Non-selective cation channels in the plasmalemma are permeable both for Na and K but these have high affinity for Na (Yamaguchi and Blumwald, 2005). Davenport and Tester (2000) also demonstrated the existence of such types of cation channels that reside in the plasma membrane of plant cells. The similarity of the hydrated ionic radii of Na and K makes it difficult for non-selective cation channels to discriminate between them.

In vitro protein synthesis requires physiological K concentrations (100 – 150 mM) and is inhibited by Na concentrations above 100 mM (Jones and Pollard, 1983) owing to competition by Na for K-binding sites. Studies showed that cytosolic enzymes of halophytes are not adapted to high salt levels and express the same sensitivity to salt as enzymes from glycophytes (Flowers *et al*., 1977). Similarly, application of Na to sugar beet could be responsible for intracellular K/Na homeostasis, which is crucial for cell metabolism (Tester and Davenport, 2003; Chen *et al*., 2007). Substitution of K by Na in sugar beet nutrition had a drastic decrease in K concentration in the shoot (Tables. 3.1 and 3.2). However, plant growth was not affected (Fig. 3.9). Moreover, Na-treated plants did not show K deficiency symptoms while in contrast K deficiency symptoms were observed for control (Fig. 3.3). Many plants require K to fulfill the osmotic functions (Subbarao *et al*., 2003) for which it is accumulated in cell vacuoles and can be transported to cytosol to maintain the K concentration.

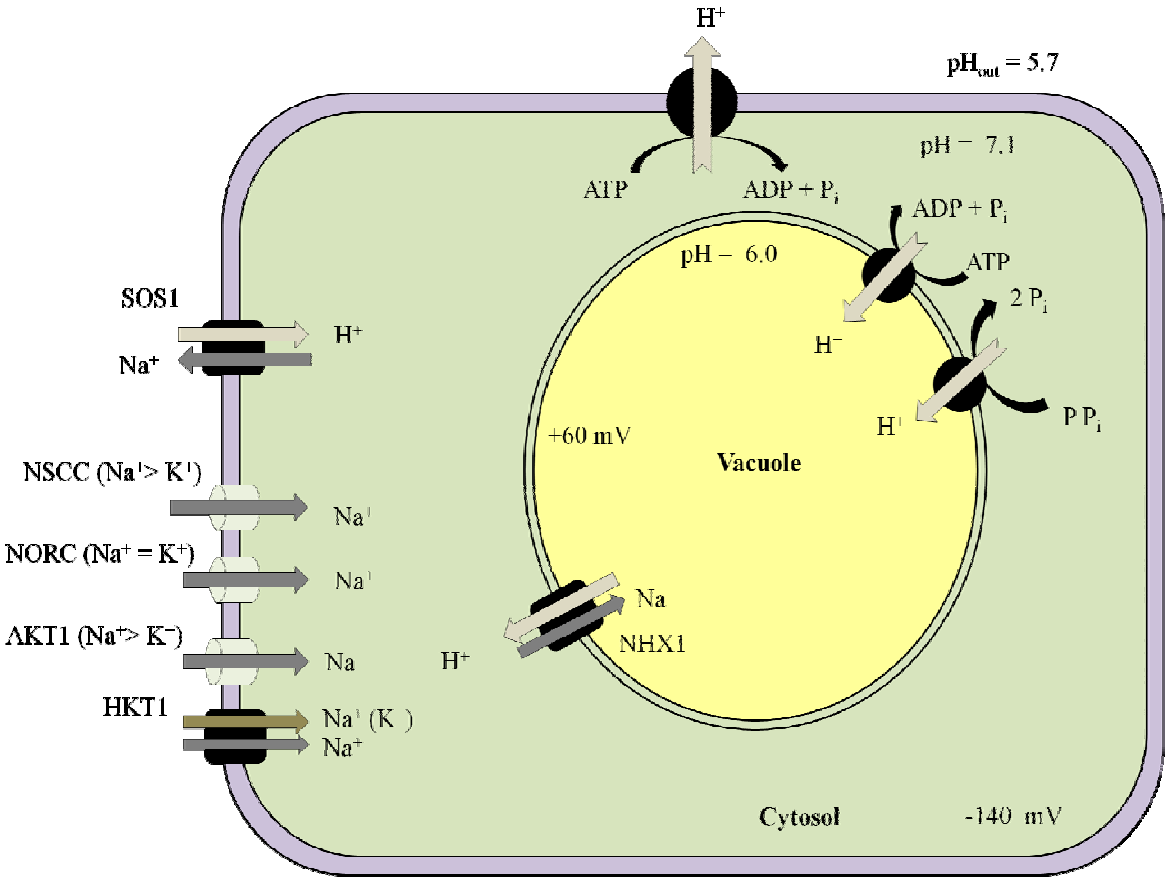


Figure 4.5. Schematic representation of Na^+ transport in plant cells. Electrogenic H^+ transport (H^+-ATPase in the plasma membrane and vacuoler membrane, H^+-PP_iase in the vacuoler membrane) generates gradients of pH and electrical potential differance across the cell and vacuoler membrane. Na ions enter the cell via various channels (AKT1, NORC, NSCC) or carriers (HKT1) and can be translocated out of the cell or into the vacuole by the action of a plasma membrane Na^+/H^+ antiporters (SOS1) or a vacuoler Na^+/H^+ antiporter (NHX1), respectively (after Yamaguchi and Blumwald, 2005).

From our results, we propose that K was substituted by Na in vacuoles and was maintained in the cytosol. The Na transport scheme is presented in Fig. 4.5.

Sodium resistant plants have mechanism of active Na extrusion from the cytosol into the external medium (Shabala *et al.*, 2005; Schubert and Zörb, 2005) and vacuolar compartmentation of Na via tonoplast-located Na^+/H^+ antiporters (Blumwald, 2000; Neubert *et al.*, 2005; Saqib *et al.*, 2005). Zörb *et al.* (2005) found linear response of ZmNHX to NaCl concentration in root medium ranging from 1 to 100 mM for the root tissue of salt resistance maize inbred lines. In our experiments, we had applied a much

lower amount of Na, which may have taken over the osmotic functions of K in vacuoles without disturbing K concentrations in the cytosol (Jeschke, 1977). Therefore, the plant growth was maintained. Under saline conditions, K uptake is inhibited due to direct competition of Na and also reduced electrochemical gradient for passive K uptake (Chen *et al.*, 2007). However, this was not the case for our study. We observed that by application of Na, K uptake by plant was not reduced because K concentration in the tissue was the same as in the control treatment (Table. 3.1 and 3.2).

Calcium concentration in the plant tissues, on the other hand, was disturbed by substituting K with Na. Reduced Ca concentration was also observed by Rengel (1992) when external Na concentration was increased. In our study, a comparatively low Na concentration was applied but Ca concentration was reduced, especially in young leaves. Moreover, Ca deficiency symptoms were also observed in some plants for a short period of time. Sodium may have an antagonistic effect on Ca uptake or its translocation from root to shoot.

4.4. Sodium nutrition and Ca uptake and translocation

Surprisingly, a decrease in Ca concentration and Ca deficiency symptoms in some of the plants in soil culture experiments were observed when K was substituted by Na. Similarly, Ca deficiency symptoms were also observed in maize under NaCl salinity. Fortmeier and Schubert (1995) concluded that in the first phase of salt stress, Ca transport to youngest leaves of maize was impaired, which may have affected cell expansion because Ca plays an important role in cell walls. Our investigation in nutrient solution culture also confirmed that Na had a negative effect on Ca concentration in the plant leaves without affecting its concentration in plant roots. Calcium is taken up by plants via apoplast and Ca-permeable ion channels in the plasma membrane (Cramer and Jones, 1996; Foreman *et al.*, 2003). Miedema *et al.* (2008) described that patch-clamp electrophysiological studies revealed the existence of three main types of Ca channels in plasma membrane of *Arobidopsis thaliana* root cells. The hyperpolarization-activated calcium channels (Demidchik *et al.*, 2002, 2007;

Foreman *et al*., 2003), non-selective cation channels (Demidchik *et al*., 2002) and depolarization-activated calcium channels (Kiegel *et al*., 2000). Significantly reduced Ca concentration in the old and young leaves (Fig. 3.17 and 3.18) may be due to Na inhibition of Ca uptake through non-selective cation channels in the plasmalemma of root epidermal cells (White, 1998; White, 1994) or NaCl interference with active Ca release from the root endodermal cells into xylem vessels (Halperin *et al*., 1997).

For the findings on the Ca uptake per unit root fresh weight as depicted in Fig. 4.6, we suppose that non-selective cation channels of root epidermal cells could be responsible. Among several types of cation channels that reside in the plasma membrane of plant cells, it has been proposed that non-selective cation channels greatly contribute to Na entry (Amtmann and Sanders, 1999; Davenport and Tester, 2000; Tyerman *et al*., 1997). In an experiment by Lynch *et al*. (1988) Ca content of barley leaves was reduced when 1 – 40 mM NaCl were applied in the nutrient solution. In addition to the role of Na, Halperin *et al*. (1997) indicated that NaCl can reduce Ca transport in the apoplast and the symplast, however the symplastic pathway should be affected more severely because Na exposure inhibits membrane-mediated symplastic pathway. An ameliorating effect of Ca on Na toxicity in plants by decreasing Na influx through non-selective cation channels (Shabala *et al*., 2006; Ebert *et al*., 2002) also confirms the competition between Na and Ca for non-selective cation channels. We found that Ca concentration in roots was unchanged (Table. 3.5). However, in shoots it was decreased. No doubt, the Ca uptake through epidermal cells was affected by Na. However, we propose that non-selective cation channels may not be responsible for it and reduced translocation from root to shoot might be responsible for decreased Ca uptake. The authors who concluded that Na may block non-selective cation channels of plasmalemma of root epidermal cells worked under saline conditions, where Na concentration in the root zone was much higher and definitely could compete with Ca for uptake through non-selective cation channels. We carried out our studies under very low concentration (4 mM) of Na with no K in the nutrient solution. High-affinity potassium transporters (HKT1) might be available for Na transport (Rus *et al.*, 2001; Platten *et al*., 2006). However, competition between Na and Ca cannot be denied for

transport through non-selective cation channels. Calcium concentration in the roots of the halophyte *Cochlearia anglica* was not affected when exposed to a NaCl concentration of 170.8 mM (Le Saos, 1976, cited by Lynch and Läuchli, 1985). In a symplastic pathway for Ca uptake, Na may inhibit xylem loading of Ca from xylem parenchyma cells. Calcium is exported from cells via the plasma membrane Ca^{2+}-ATPase and this enzyme play a regulatory role in modulation of cytosolic Ca levels. Halperin *et al.* (1997) suggested that NaCl-stressed endodermal cells could affect the radial transport of Ca by affecting the Ca^{2+}-ATPase. However, it is still not clear how Na interacts with Ca loading into xylem vessels. Outward-rectifying cation channels, discovered in maize root stelar cells (Roberts and Tester, 1997a) can also be responsible for interaction between Na and Ca during xylem loading. In our studies on sugar beet, calcium translocation via xylem sap was reduced due to lower Ca concentration in the xylem sap of Na-treated plants (Fig. 3.19). The decreased ratio of Ca content in shoot/root for Na treatment (Fig. 4.6) also indicates that Ca translocation from root to shoot was reduced. These findings agree with those of Lynch and Läuchli (1985) who proposed that NaCl inhibited Ca transport from root to shoot by interfering with the release of Ca into the root xylem, possibly via an effect on the active loading of Ca into the xylem vessels. Hu and Schmidhalter (1997) found that saline conditions significantly reduced the Ca ion accumulation in wheat leaves.

The non-significant effect of Na on Ca translocation in the first nutrient solution experiment was probably due to the short treatment period and because Na may have accumulated in the vacuoles of root cells to replace K. The higher Na concentration and the lower K concentration in the root (Table. 3.5) in the second experiment as compared to the first experiment reveals that sugar beet root had a capacity to accumulate more Na by replacing K. Thus, the apparent decrease in the Ca concentration and the translocation via xylem sap were observed after 7 d. Secondly, NaCl stress was initially less inhibitive of Ca translocation, probably owing to cation exchange occurring during radial and xylem transport, which released wall-bound Ca for xylem transport (Halperin *et al.*, 1997).

Interestingly, the volume of xylem sap was higher for Na treatment in both experiments. The increase in the volume of xylem sap may be due to higher root pressure as a result of relatively more root fresh weight. Application of NaCl in nutrient solution also decreased the root and leaf water potential (Ψ_w) in *Sesuvium portulacastrum* (Slama *et al.*, 2007). It may increase the water movement from soil to root due to larger water-potential gradient. Moreover, the most noticeable feature of halophytes - correlation between uptake of alkali ions and whole plant succulence – can also be responsible for higher volume of xylem sap (Waisel, 1972).

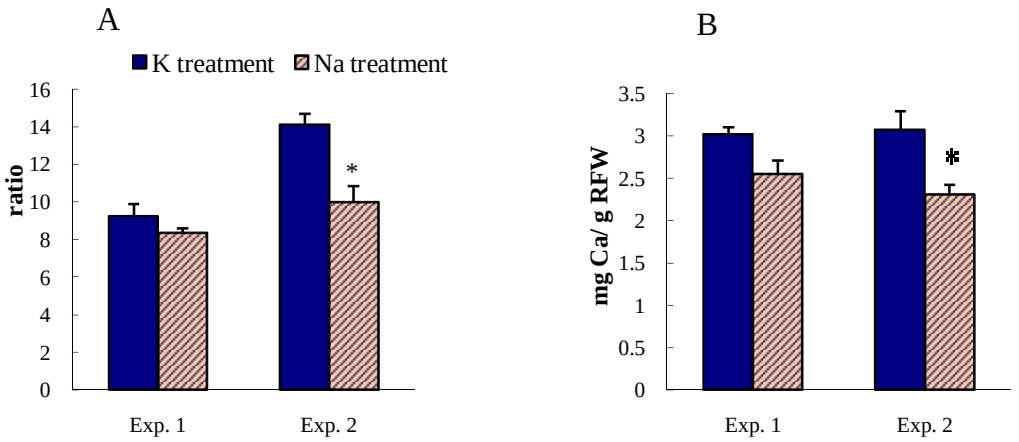


Figure 4.6. Effect of K substitution by equivalent amount of Na in sugar beet nutrition on Ca translocation from root to shoot (A) and Ca uptake per unit root fresh weight (RFW) (B). Experiments were conducted in nutrient solution under standard climatic conditions in a growth chamber. In the first experiment, plants were harvested 3 d after Na treatment while in the second experiment plants were harvested 7 d after treatment. Values in columns represent means + SE of four replications.* = significantly different according to LSD test at 5% level of probability.

4.5. Concluding remarks

Based on the observations and findings in our experiments on sugar beet we conclude that Na is able to substitute K to a large extent in sugar beet nutrition. Substitution of K by Na gave similar beet yield without affecting their quality, and hence white sugar yield was not affected. Moreover, Na was able to eliminate the K deficiency symptoms in the plant leaves. Nevertheless, application of Na fertilizer decreased the Ca concentration in the expanding leaves, which was most probably due to reduced Ca

translocation from root to shoot. However, despite of decreased Ca concentration in young leaves plant growth was not affected. It is well known that sugar beet plants preferably accumulate Na in the vacuoles of the plants cell (Jeschke, 1977). Thus it is proposed that osmotic functions of Ca may be substituted by Na in the vacuoles. Furthermore, under field conditions, Ca concentration is very high which can also improve Ca uptake via the apoplast of younger roots. Our results revealed that sugar beet plants grown in a container experiment did not show reduced Ca concentration in young leaves owing to a huge volume of soil which might increase the availability of Ca in the soil solution and improved its uptake.

Economically, the soils with higher K-fixing capacity (especially with vermiculite clay minerals) have more potential for the substitution of K by Na and application of a huge amount of expensive K-fertilizer may be replaced with sufficient amount of cheaper Na fertilizer.

5 SUMMARY

Plant growth does not response to the application of generally recommended levels of potassium (K) fertilizer in the soils with expandable three layer clay minerals. In the soils rich in illite and vermiculite clay minerals with high cation exchange capacity, a major part of applied K is fixed and becomes unavailable to plants immediately.

It is known that several members of the family Chenopodiaceae such as sugar beet, spinach, red beet *etc*. are capable to use sodium (Na) as an osmoticum for which 95% of total acquired K is required. We hypothesized that Na is able to substitute K to a large extent in sugar beet nutrition without affecting the plant growth and beet quality. We assumed that in K-fixing soils it would be possible to replace a huge amount of K fertilizer with adequate amount of Na fertilizer, which may lead to the development of an interesting fertilizer strategy for sugar beet in K-fixing soils. In this study, an attempt was made to identify the limiting processes when K was substituted by Na in K-fixing soils.

Three soil and two nutrient solution experiments were conducted to test the above-mentioned hypothesis. In the first soil experiment, soils from three locations (i.e. Kleinlinden, Giessen and Trebur) with different K-fixing capacity were tested and sugar beet plants were grown in Ahr pots under natural climatic conditions (April 2005 to September 2005, Giessen, Germany) and harvested at maturity. Three treatments were used i.e. control (no K and Na fertilizer), potassium treatment (K_2SO_4 fertilizer was applied according to K-fixing capacity of soil) and in third treatment NaCl was applied equivalent to regular K fertilization. Soil Kleinlinden was tested again in another experiment where sugar beet plants were grown in containers (169 kg soil container^{-1}) with the same treatments as in the previous Ahr pot experiment, except potassium treatment. Potassium was not applied according to K-fixing capacity of soil, rather according to regular K fertilization. A field experiment was conducted on the Trebur soil (similar to the soil used in the Ahr pot experiment). The treatments were the same as in the container experiment; nevertheless an extra treatment was used where a huge amount (i.e. equivalent to half K-fixing capacity of the soil) of K was applied. The results of all the experiments revealed that application of Na fertilizer significantly improved the plant growth relative to

the control. However, white sugar yield in Na treatment was similar to that in K treatment. Moreover, Na eliminated the K deficiency symptoms in the plant leaves, but application of Na fertilizer decreased the calcium (Ca) concentration in the expanding leaves due to reduced Ca uptake and Ca translocation from root to shoot, which was investigated in nutrient solution experiments with Na and K treatments.

Despite many fruitful findings from the earlier studies on Na nutrition of plants, in practical agriculture Na is not used as a nutrient. We conclude that Na may substitute K to a large extent in sugar beet nutrition without affecting the plant growth and beet quality, and soils with higher K-fixing capacity and illite and/or vermiculite clay minerals are more favorable for this substitution.

6 ZUSAMMENFASSUNG

Es wurde beobachtet, dass die allgemein empfohlene Applikation von K-Düngemittel das Pflanzenwachstum in Böden mit aufgeweiteten Dreischicht-Tonmineralen nicht beeinflusst. Die Böden, reich an den Tonmineralen Illit und Vermiculit haben eine hohe CEC und der größte Teil des gedüngten K wurde fixiert, sodass es für die Pflanzen nicht verfügbar war.

Es ist bekannt, dass viele Arten der Familie der Chenopodiaceae, wie Zuckerrübe, Spinat, Rote Beete, etc. in der Lage sind, Na als Osmoticum zu verwenden hierfür werden ansonsten 95% des aufgenommenen K benötigt. Ziel unserer Untersuchungen war es herauszufinden, ob Na in der Lage ist, K in der Ernährung von Zuckerrüben zu ersetzen, ohne das Pflanzenwachstum oder die Rübenqualität zu beeinflussen. Des Weiteren wurde überlegt, ob in K-fixierenden Böden ein großer Anteil des K-Düngers durch Na-Dünger ersetzt werden kann, was zu einer interessanten Düngerstrategie für Zuckerrüben führen könnte. Zunächst wurden die limitierenden Einflüsse erforscht, die auftreten, wenn K durch Na ersetzt wird.

Drei Boden- und zwei Nährlösungsversuche wurden zur Überprüfung der Hypothese durchgeführt. Für den ersten Bodenversuch wurden drei verschiedene Böden (Klein Linden, Gießen und Trebur) mit unterschiedlicher K-Fixierungskapazität verwendet. Die Zuckerrüben wuchsen in Ahrgefäßen bei natürlicher Witterung (April 2005 bis September 2005, Gießen, Deutschland) und wurden zur Reife geerntet. Drei Varianten wurden getestet, eine Kontrollvariante (kein K- und Na-Dünger), eine Kaliumvariante (K_2SO_4-Applikation in Übereinstimmung mit der K-Fixierungskapazität) und eine Natriumvariante (NaCl entsprechend einer normalen K-Düngung). Ein weiteres Experiment wurde mit Kleinlindener Boden angelegt. Hier wurden die Zuckerrüben in Containern (169 kg Boden Container^{-1}) unter den gleichen Bedingungen und Varianten mit Ausnahme der Kaliumvariante angezogen. Hierzu wurde K in praxisüblicher Menge dazugegeben. Ebenso wurde ein Feldversuch in Trebur auf dem gleichen Boden wie in den Ahrgefäßen durchgeführt. Die Varianten waren die gleichen wie im Containerversuch, allerdings mit einer zusätzlichen Variante, die eine enorme Menge

an K gedüngt bekam (entsprechend der halben K-Fixierungskapazität). Die Ergebnisse von aller Versuchen zeigen, dass eine Na-Düngung das Pflanzenwachstum relativ zu-Kontrolle signifikant erhöht, jedoch der Zuckerertrag der gleiche ist, wie in der Kaliumvariante. Darüber hinaus konnte Na die K-Mangelsymptome an Blättern verhindern; dennoch wurde die Ca-Konzentration vor allem in wachsenden Blättern beeinflusst. Daher wurde, ein Nährlösungsversuch in einer Klimakammer mit kontrollierten Bedingungen angelegt. Es zeigte sich, dass durch das Ersetzen von K durch Na die Ca-Konzentration im Spross signifikant verringert wurde ohne das Wachstum der Pflanzen zu stören. Die Ca-Aufnahme und Ca-Translokation im Xylemsaft waren als Folge der Substitution ebenfalls reduziert.

Bisher wird in der praktischen Landwirtschaft Na nicht als Pflanzennährstoff verwendet. Abschließend stellen wir fest, dass Na fähig ist, K in der Ernährung von Zuckerrüben zu ersetzen, ohne dass das Wachstum der Pflanze oder die Qualität beeinflusst wird. Böden mit einer hohen K-Fixierungskapazität und einem hohen Anteil an Illit bzw. Vermiculit eignen sich besser für diese Substitution.

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Erklärung

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Gießen, July 31, 2008 **ABDUL WAKEEL**

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