Plant Physiology and Biochemistry 107 (2016) 237–247

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Protein synthesis is the most sensitive process when potassium is substituted by sodium in the nutrition of sugar beet (*Beta vulgaris*)

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ARTICLE INFO

Article history:
Received 2 April 2016
Received in revised form
4 June 2016
Accepted 6 June 2016
Available online 7 June 2016

Keywords:
Amino acids
Growth
Peptidyl transferase
Sugars
Translation
Transpiration

ABSTRACT

Potassium ions (K^+) and sodium ions (Na^+) share many physical and chemical similarities. However, their interchangeability in plant nutrition is restricted. Substitution studies showed that K^+ can be replaced by Na^+ to a large extent in the nutrition of *Beta vulgaris* L. However, the extent of substitution without negative impacts is not unlimited. The aim of the present study was to identify the process which is most sensitive during the substitution of K^+ by Na^+ in nutrition of young sugar beet plants. We focused on transpiration, growth, and net protein synthesis. Plants were grown under controlled environmental conditions. With transfer of seedlings into nutrient solution, plants were cultivated in different substitution treatments. For all treatments the sum of K^+ and Na^+ (applied as chloride) was fixed to 4 mM. The extent of substitution of K^+ by Na^+ in the nutrient solution was varied from low (0.25% substitution: 3.99 mM K^+, 0.01 mM Na^+) to almost complete substitution (99.75% substitution: 0.01 mM K^+, 3.99 mM Na^+). The supply of 3.99 mM K^+ in 0.25% substitution treatment guaranteed the absence of K^+ deficiency. Transpiration was not affected by the substitution. Growth was inhibited at a substitution level of 99.75%. Net protein synthesis was already affected at a substitution level of 97.50% (0.10 mM K^+, 3.90 mM Na^+). Hence, net protein synthesis was most sensitive to the substitution and limited the extent of substitution of K^+ by Na^+ in the nutrition of young sugar beet plants.

1. Introduction

Potassium ions (K^+) and sodium ions (Na^+) are present at high concentrations in the earth crust. Although these two monovalent inorganic cations share many similarities, their exchangeability in plant nutrition is restricted (Benito et al., 2014). Key functions of K^+ in plants are charge balance, enzyme activation, and osmotic functions. These specific functions of K^+ cannot be completely fulfilled by Na^+ due to its lager hydration shell compared to K^+ (Schubert, 2015). The hydrated Na^+ is not able to pass cation channels which often possess a high specificity for K^+, and therefore the permeability of Na^+ is too low to accomplish charge balance. Activation of many enzymes by K^+ is based on the sorption of K^+ to enzymes, which leads to conformational changes allowing stimulation of enzyme activity. Due to its larger radius of the hydrated Na^+, binding energy to enzymes is much lower compared to that of K^+ and does not allow conformational changes required for optimal enzyme activity. Although unspecific osmotic functions of K^+ can be taken over by Na^+, the low membrane permeability of Na^+ prevents that the regulation of the water economy can be fully guaranteed by Na^+ (Schubert, 2015). Due to the various key functions of K^+ the regulation of K^+ homoeostasis has been described as an essential process of plant adaptive responses to the environment (Anschütz et al., 2014).

The possible extent of substitution of K^+ by Na^+ varies strongly among plant species. It has been known for many years that K^+ can be replaced by Na^+ to a great extent in *Beta vulgaris*. These studies can be classified in three different approaches which focus on completely different aspects of the relationship between K^+ and Na^+ in the nutrition of *Beta vulgaris* under non-saline conditions (Fig. 1). Studies dealing exclusively with the response of *Beta vulgaris* to salt stress are not considered here because the interaction of K^+ and Na^+ could be masked by effects caused by osmotic stress or ion toxicity induced by salinity. Many authors studied the effect of adding Na^+ in the presence of adequate amounts of K^+ on growth and physiology of beets (e.g. Milford et al., 2008) (Fig. 1, Approach 1). In a second approach, it was investigated whether Na^+ can

Abbreviations: PM, plasma membrane; PPFD, photosynthetic photon flux density; DAS, days after sowing; DM, dry matter; SE, standard error.

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http://dx.doi.org/10.1016/j.plaphy.2016.06.009
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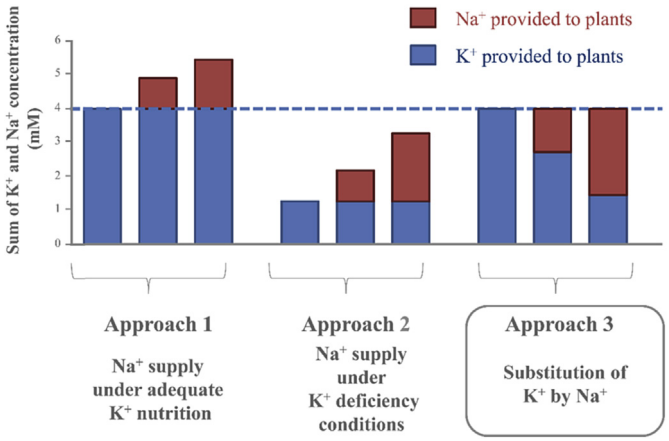

Fig. 1. Schematic illustration of experimental approaches dealing with the interaction of K^+ and Na^+ under non-saline conditions. Horizontal line indicates the level of adequate K^+ concentration.

restore growth, morphology, and physiology of beets deficient in K^+ (e.g. Subbarao et al., 2001) (Fig. 1, Approach 2). Although some experiments following the Approach 1 and/or Approach 2 were titled as substitution studies, we consider studies following the Approach 3 as substitution studies (e.g. Marschner et al., 1981) (Fig. 1, Approach 3). Only this third approach, in which the sum of supplied K^+ and Na^+ concentrations is kept constant equal at the level of adequate K^+ nutrition, can give reliable information about the replaceability of K^+ by Na^+ in the nutrition of beet.

Moreover, the onset of the substitution treatment has to be considered. Due to the fact that vacuoles can store K^+ up to 200 mM, a preceding cultivation with adequate K^+ supply can delay the conditions of real substitution (Wakeel et al., 2009). Moreover, the drastic change from K^+ supply to substitution conditions can distort the ionic homeostasis of the plants (Wakeel et al., 2009).

Therefore, in order to determine the potential of Na^+ to replace K^+, the substitution should be started with the beginning of plant cultivation. Studies following this substitution design showed that K^+ can be replaced by Na^+ to a large extent without negative impacts on shoot and root growth (Subbarao et al., 1999, 2003). However, these studies also demonstrated that increased substitution of K^+ by Na^+ inhibited growth of beets, reduced leaf area, and reduced photosynthetic rate (Pi et al., 2014; Subbarao et al., 1999). Unfortunately, up to now it has not been possible to identify the process which is most sensitively limited by the substitution of K^+ by Na^+. Therefore, the aim of the present study was to identify the process which is most sensitive to the substitution of K^+ by Na^+ and thus limits the extent of substitution in young sugar beet plants. We focused on transpiration, growth, and net protein synthesis:

1 K^+ plays a key role in stomatal regulation. Furthermore, it was shown for *Vicia faba* that Na^+ disrupted the ability of stomatal closure leading to unproductive water losses (Slabu et al., 2009). Hence, it is hypothesized that transpiration is most sensitive to the substitution of K^+ by Na^+.
2 The key functions of K^+ ensure numerous processes which are required for plant growth (Hawkesford et al., 2012). We expect that some of the key functions of K^+ cannot be fulfilled by Na^+ and that a variety of processes can be inhibited resulting in poor plant growth due to the substitution of K^+ by Na^+. Therefore, it is hypothesized that the growth of young sugar beet plants is sensitively inhibited due to the substitution of K^+ by Na^+.
3 The requirement of K^+ in protein synthesis was demonstrated in vivo (Besford, 1975) and in vitro (Smith et al., 1982). We assume that the substitution of K^+ by Na^+ could induce K^+ deficiency. Hence, it is hypothesized that protein synthesis is most sensitive to the substitution of K^+ by Na^+.

2. Materials and methods

2.1. Experiment I

2.1.1. Plant material and cultivation

Sugar beet seeds (*Beta vulgaris* L. cv. Felicita), obtained from KWS (Germany), were sown in quartz sand in a growth chamber. The standardized environmental conditions in the climate chamber were 22 °C (16 h)/20 °C (8 h) and 70% relative humidity. The seeds were watered with 1 mM $CaSO_4$ solution which contained 20 μM boric acid. Germination took place in the dark. After germination, the light intensity was increased stepwise until after 4 d a photosynthetic photon flux density (PPFD) of 556 μmol m^{-2} s^{-1} (FieldScout® Quantum Light Meter 3415FSE, Spectrum Technologies, USA) was reached. Eight days after sowing (DAS), the seedlings were transferred into pots (2.8 L) with ¼ strength nutrient solution. With the transfer of the plants into the nutrient solution five treatments were established. The five treatments differed in the extent of substitution of K^+ by Na^+ (Fig. 2). There were four plants in each pot and four replicates of pots for each treatment. The nutrient solution was aerated continuously. Nine DAS, the concentration of the nutrient solution was increased to ½ strength. Eleven DAS, plants were supplied with full-strength nutrient solution. The full-strength nutrient solution contained: 2.5 mM $Ca(NO_3)_2$, 0.3 mM $NH_4H_2PO_4$, 0.5 mM $MgSO_4$, 10 μM Fe-EDTA (sodium salt), 20 μM H_3BO_3, 0.5 μM $MnSO_4$, 0.1 μM $ZnSO_4$, 0.2 μM $CuSO_4$, and 0.01 μM $(NH_4)_6Mo_7O_{24}$. The treatments contained (at the level of full-strength nutrient solution) 3.99 mM K^+/0.01 mM Na^+ (for the 0.25% substitution), 3.00 mM K^+/1.00 mM Na^+(for the 25.00% substitution), 2.00 mM K^+/2.00 mM Na^+ (for the 50.00% substitution), 1.00 mM K^+/3.00 mM Na^+ (for the 75.00% substitution), and 0.01 mM K^+/3.99 mM Na^+ (for the 99.75% substitution). K^+ and Na^+ were supplied as KCl and NaCl. After reaching full-strength concentration, the nutrient solution was changed every 3 d. Twenty DAS, the roots were dipped into 0.03% (w/v) Benomyl (DuPont, USA) suspension for 1 min to prevent an infection of the plants by fungi.

2.1.2. Transpiration rate

The loss of water within 48 h was determined by weighing the pots (without plants and lids) 23 and 25 DAS. The transpiration rate was calculated as the loss of water per leaf area during this time period.

2.1.3. N-tester values and harvest of the plants

Twenty-five DAS, 30 random point measurements on the first leaf pairs of plants of each pot were made by means of the hand-held Yara N-Tester® (Yara, Norway). This non-destructive method allowed an estimation of the chlorophyll concentration of the leaves. After the determination of N-tester values the plants were harvested. Shoots and roots were separated by cutting at the transition of the hypocotyl to the primary root of the young beet. The fresh mass of shoots and roots was recorded. The shoots of three plants of each pot and the roots of all four plants of each pot were dried at 80 °C for 48 h. After drying the roots and shoots, the dry mass was recorded and the plant material was ground. The shoot of the fourth plant of each pot was frozen in liquid nitrogen and stored at −80 °C until analysis.