Journal of Plant Physiology 231 (2018) 1–8

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Calcium ameliorates the toxicity of sulfate salinity in *Brassica rapa*

Martin Reich[a,1], Tahereh A. Aghajanzadeh[b,1,*], Saroj Parmar[c], Malcolm J. Hawkesford[c], Luit J. De Kok[a]

[a] Laboratory of Plant Physiology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, P.O. Box 11103, 9700 CC Groningen, The Netherlands
[b] Department of Biology, Faculty of Basic Science, University of Mazandaran, Babolsar, Iran
[c] Plant Sciences Department, Rothamsted Research, Harpenden, Herts AL5 2JQ, UK

ARTICLE INFO

Keywords:
Brassica rapa
Calcium
Sulfate salinity
Sulfate transport
Vacuole

ABSTRACT

Salinity stress in Brassica, often only associated with osmotic effects and the toxicity of Na^+, was more severe when applied as Na_2SO_4 than as NaCl, indicating that SO_4^{2-} ions had toxic effects as well. Application of 10 mM calcium in the form of $CaCl_2$ in the growth medium of plants only slightly ameliorated growth impairment by NaCl and KCl, but almost completely prevented negative effects of Na_2SO_4 and K_2SO_4 on plant biomass production. This effect was calcium specific, as $MgCl_2$ ameliorated sulfate toxicity to a much lower extent. This sulfate toxicity coincided with a strong decrease in the plant content of calcium and manganese upon sulfate salinity. Application of $CaCl_2$ largely alleviated this decrease, however, it did not prevent the higher tissue concentration of sulfate. $CaCl_2$ prevented the increase in organic sulfur compounds presumably by reducing of relative gene expression of ATP-sulfurylase (ATPS) and adenosine 5′-phosphosulfate reductase (APR) indicating a possible regulation of sulfate assimilation by calcium. The upregulation of the genes encoding for Group 4 sulfate transporters (Sultr4;1 and 4;2) upon sulfate salinity, was absent in the presence of $CaCl_2$. Therefore, additional calcium may facilitate an increased vacuolar capacity for sulfate accumulation.

1. Introduction

Although salt stress is an increasing problem for cropproduction and although much research has been carried out on the phenomena of salt stress and tolerance in plants, progress in increasing crop salt tolerance via breeding remains rather limited. As an alternative to breeding salt tolerant crops, changes in agricultural practice and fertilization could ameliorate salt stress in cropping systems. The addition of a surplus of calcium was shown to ameliorate growth inhibition of crops by salt stress in the beginning of the last century (Kearney and Cameron, 1902; Kearney and Harter, 1901), and since then a number of studies on different crop species have shown similar results and characterized the effect under more controlled conditions (LaHaye and Epstein, 1969; Cramer et al., 1990; Lopez and Satti, 1996; Kaya et al., 2003). Calcium is an essential macronutrient with many vital and beneficial functions in plants. Due to their common positive charge, a competition of sodium with calcium and potassium is widely proposed to be one of the main causes for salt stress. Amelioration of salt stress by calcium has, up to now, almost exclusively been related to sodium toxicity, due to the fact that sodium as a cation competes with calcium in cell walls and membranes and therefore disturbs their function as selective barriers (Cramer et al., 1985; Lynch et al., 1987; Rengel, 1992). This may cause a loss of potassium from roots, which may be partly prevented by addition of calcium (Shabala, 2000). The important role of calcium for potassium/sodium homeostasis under salt stress is widely accepted (Epstein, 1998; Volkmar et al., 1998).

Salt stress caused by NaCl prevails in most salt affected soils, however, plants often have to deal with other salts, such as Na_2SO_4 (Garcia and Hernandez, 1996) and many areas in the world are dominated by sulfate salts (Chang et al., 1983; Keller et al., 1986). Such an excess of sulfate salts may occur in volcanic soils, in marine soils (as sea water contains high amounts of sulfate), in agricultural soils irrigated with saline water or may be caused by anthropogenic inputs from industry or deposition of atmospheric sulfur gases (Moss, 1978; Nriagu, 1978; Freedman and Hutchinson, 1980; Chang et al., 1983). In many plant species sulfate salinity appeared to be more toxic than chloride salinity (Eaton, 1942; Paek et al., 1988; Bilski et al., 1988; Datta et al., 1995; Renault et al., 2001). Recently, Reich et al. (2017) observed both Na_2SO_4 and K_2SO_4 showed a higher toxicity in *Brassica rapa* than NaCl and KCl). The sulfate toxicity coincided with a stronger decrease of the

* Corresponding author.
E-mail address: T.aghajanzadeh@umz.ac.ir (T.A. Aghajanzadeh).
[1] The first two authors contributed equally to this work and are both considered as first authors.

https://doi.org/10.1016/j.jplph.2018.08.014
Received 2 May 2018; Received in revised form 13 August 2018; Accepted 28 August 2018
Available online 01 September 2018
0176-1617/ Crown Copyright © 2018 Published by Elsevier GmbH. All rights reserved.

tissue content of calcium, magnesium and manganese than upon compared to chloride salinity, however, this difference appeared to be too small to explain the higher toxicity of sulfate over chloride and rather; the upregulation of genes for the Group 4 sulfate transporters (Sultr4;1 and 4;2) was suggested to play a key role under sulfate toxicity (Reich et al., 2015, 2017).

Is has been observed that calcium may ameliorate salinity stress, though in these studies only NaCl and Na_2SO_4 salts were used (e.g. Cramer and Spurr, 1986; Bilski et al., 1988; Reginato et al., 2014). The experimental set-up described by Reich et al. (2017) was used in the present study to test the differences in the amelioration of NaCl, KCl, Na_2SO_4 and K_2SO_4 salinity by addition of calcium. The hypothesis was that if calcium amelioration was related only to sodium toxicity, it should be less effective under Na_2SO_4 than under NaCl toxicity, as the first is mainly caused by sulfate toxicity but the latter by sodium toxicity (Reich et al., 2017). Furthermore, if the amelioration was restricted to sodium toxicity, additional calcium should not have an effect on plants grown in K_2SO_4, which was also shown to cause severe growth inhibition in contrast to KCl. Growth and physiological parameters were used as indicators for the toxicity of the different salts. From the current study it was evident that calcium ameliorates the toxicity of sulfate salinity but not chloride salinity in *B. rapa*. The results provide new insights in the mechanisms of sulfate toxicity and in the specificity of calcium in amelioration of stress caused by different salts.

2. Material and methods

2.1. Plant material and growth conditions

Seeds of *B. rapa*, cv. Komatsuna (Van der Wal, Hoogeveen, The Netherlands) were germinated in vermiculite. Ten day-old seedlings were transferred into a 25% Hoagland nutrient solution (pH 5.9) consisting of 1.25 mM $Ca(NO_3)_2.4H_2O$, 1.25 mM KNO_3, 0.25 mM KH_2PO_4, 0.5 mM $MgSO_4.7H_2O$, 89 11.6 μM H_3BO_3, 2.4 μM $MnCl_2.4H_2O$, 0.24 μM $ZnSO_4.7H_2O$, 0.08 μM $CuSO_4.5H_2O$, 0.13 μM $Na_2MoO_4.2H_2O$ and 22.5 μM Fe^{3+} -EDTA) in 30l containers (20 sets per container, three plants per set; Supplementary Fig. 1) in a climate-controlled room. Relative humidity was 60–70 % and the photoperiod was 14 h at a photon fluence rate of 300 ± 20 μ mol m^{-2} s^{-1} (400–700 nm) at plant height, supplied by Philips GreenPower LED lamps (deep white/red 120). Day/night temperatures were 21/18 °C. Ten-day old seedlings were grown without additional salt for three days, and subsequently salt concentrations were gradually increased during the following three days. For NaCl and KCl the steps were 25, 50 and 100 mM, and for Na_2SO_4 and K_2SO_4 the steps were 12.5, 25 and 50 mM. For half of the plants an additional 10 mM $CaCl_2$ was added to the nutrient together with the first addition of salt. Seedlings were grown in the final concentrations for five more days and then harvested. Roots were separated from the shoots, weighed and stored at either −20 °C or −80 °C, depending on the requirements for further analysis. For determination of the mineral nutrient content, plant tissue was dried at 80 °C for 24 h and stored in a desiccator for further use.

2.2. Maximum quantum efficiency of photosystem II (Fv/Fm) and pigment content

Prior to harvest, *Fv/Fm* of leaves in dark adapted conditions was determined in the morning prior to the onset of the light period (PAM 2000, Walz, Effeltrich, Germany). For determination of pigment content, frozen shoots were homogenized in 100% acetone by using an Ultra Turrax (10 ml g^{-1} fresh weight) and centrifuged at 30,000*g* for 20 min. The chlorophyll a + b content in the supernatant was determined according to Lichtenthaler (1987).

2.3. Sulfate and free amino acids content

Sulfate was extracted from frozen plant material in water and determined refractometrically after separation by HPLC (Reich et al., 2017). From the same extracts, free amino acids were measured after deproteinization using a ninhydrin color reagent according to Rosen (1957) by colorimetric determination at 578 nm.

2.4. Water-soluble non-protein thiol content

For determination of thiols, fresh plant material was used on the day of harvest and homogenized in an extraction medium (10 ml g^{-1} fresh weight) containing 80 mM sulfosalicylic acid, 1 mM EDTA and 0.15% (w/v) ascorbic acid. Samples and extract were kept on ice and the extraction medium was bubbled with N_2 one hour prior extraction to remove oxygen. After filtering through one layer of Miracloth the extract was centrifuged at 30,000 *g* for 15 min at 0 °C. Thiol content in the supernatant was determined colorimetrically at 413 nm after addition of 5,5′-dithiobis[2-nitrobenzoic acid] (De Kok et al., 1988).

2.5. Mineral nutrient composition

For determination of mineral nutrient content, dried leaf tissues (0.2–0.5 g) were digested with 5 ml of nitric acid:perchloric acid (87:13, v/v; 70% concentration, trace analysis grade; Fisher Scientific; Zhao et al., 1994). The minerals in the digested samples were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES) analysis. Repeat samples were carried out every 10 samples; blanks and standard reference were used for quality control. The sample introduction system consisted of a micromist glass concentric nebulizer, quartz Scott-type double-pass spray chamber at 2 °C, and nickel sample (1 mm) and skimmer (0.4 mm cones). Operating parameters were optimized daily using a tune solution containing 1 μg l^{-1} cerium, lithium, tellurium, and yttrium. Other instrument conditions were radio-frequency forward power of 1,550, sample depth of 8.0 mm, carrier gas flow rate of 0.89l min^{-1}, reaction gas flow rate of 4 ml min^{-1} (H_2) or of 4.5 ml min^{-1} (helium). An internal standard (500 μg l^{-1} germanium) was used to correct for signal drift.

2.6. RNA isolation and expression of the genes encoding the Group 4 sulfate transporters and sulfur assimilatory enzymes

Total RNA was isolated by a modified hot phenol method (Verwoerd et al., 1989). Frozen ground plant material was extracted in hot (80 °C) phenol/extraction buffer (1:1, v/v), 1 g ml^{-1}. The extraction buffer contained 0.1 M Tris − HCl, 0.1 M LiCl, 1% SDS (w/v), 10 mM EDTA, pH 8.0). After mixing, 0.5 ml of chloroform–isoamyl alcohol (24:1, v/v) was added. After centrifugation (13,400 × *g*) for 5 min at 4 °C, the aqueous phases were transferred to new tube. After adding an equal volume of chloroform and isoamyl alcohol, the total RNA was precipitated by 4 M LiCl overnight at 4 °C. Total RNA was collected and washed with 70% ethanol. Possible genomic DNA contamination was removed with a DNAase treatment step (Promega, USA). Phenol–-chloroform–isoamyl alcohol and chloroform–isoamyl alcohol were used for further purification and total RNA was precipitated by ethanol and dissolved in diethylpyrocarbonate-treated water. The quantity and quality of RNA was checked using ThermoNanoDrop 2000 and RNA in each sample was adjusted to the same concentration. The integrity of RNA was checked by electrophoresis by loading 1 μg RNA on a 1% TAE-agarose gel.

DNA-free intact RNA (1 μg) was reverse transcribed into cDNA with oligo-dT primers using a first strand cDNA synthesis kit (Promega, USA) according to the manufacturer-supplied instructions. Subsequently, the cDNA was used as a template in real-time PCR experiments with gene-specific primers. To design primers for the genes of the Sultr4;1, Sultr 4;2 and sulfur assimilatory enzymes such as ATP-sulfurylase (ATPS; EC

Download English Version:

https://daneshyari.com/en/article/8962061

Download Persian Version:

https://daneshyari.com/article/8962061

Daneshyari.com